UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

# Polish Journal of Natural Sciences

20
(1/2006)

PUBLISHER UWM

OLSZTYN 2006

The Polish Journal of Natural Sciences is indexed and abstracted
in Biological Abstracts and Biosis Previews

PL ISSN 1643-9953



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PUBLISHER UWM OLSZTYN

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Ark. wyd. 35,7; ark. druk. 29,0
Druk – Zakład Poligraficzny UWM w Olsztynie
zam. nr 486

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 9-21, Y. 2006

# CORRELATIONS BETWEEN NPK CONCENTRATION IN DRY GRASS MASS AND LIGHT SOIL FERTILIZED WITH SEWAGE SLUDGE

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Key words: nitrogen, phosphorus, potassium, sewage sludge, soil materials, correlation, regression, plant material, light soil.

Abstract

Sewage sludge is good known for soil-forming and fertilizing properties due to high concentrations of nutrients available to both plants and soil fauna. The aim of the present experiment was to determine the effect of rates of sewage sludge and NPK fertilizer on the improvement in the properties of nutrient-poor soils. Sewage sludge caused an increase in the concentrations of N, P and K in the soil. Correlation and regression analyses showed significant relationships between total and available forms of N, P and K contained in fertilized soil and plant material.

## ZALEŻNOŚCI MIĘDZY ZAWARTOŚCIĄ NPK W MASIE TRAW I GLEBACH LEKKICH UŻYŹNIANYCH OSADEM ŚCIEKOWYM

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Słowa kluczowe: azot, fosfor, potas, osady ściekowe, utwory glebowe, korelacja, regresja, materiał roślinny, gleby lekkie.

Abstrakt

Osady ściekowe, ze względu na zawartość składników potrzebnych do wzrostu i rozwoju roślin i fauny glebowej, mają właściwości glebotwórcze i nawozowe. W doświadczeniu badano wpływ dawki osadu ściekowego oraz nawożenia NPK na poprawę właściwości gleb ubogich w składniki pokarmowe. Dodatek osadu ściekowego wpłynął na zwiększenie zawartości N, P i K w badanych utworach glebowych. Analiza korelacji i regresji wykazała istotne zależności między wartościami ogólnymi i przyswajalnymi N, P i K zawartych w użyźnianych utworach glebowych i materiale roślinnym.

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## Introduction

Biologically treated sewage sludge is rich in organic matter, micro-and macronutrients indispensable for the growth and development of plants and soil fauna. It is also good known for soil-forming and fertilizing properties. The manurial and nutritive value of sewage sludge, as well as its soil-forming properties and suitability for organic fertilizer production, depend on the concentrations of organic matter, nitrogen, phosphorus, calcium, magnesium and potassium in its dry matter (GAMBUŚ, WIECZOREK 1999, PARKPIAN et al. 2000, SCHAECKE et al. 2002). The contained of nitrogen contained in sewage sludge most often occurs in the form of organic compounds, unavailable to plants. Fresh sludge contains small amounts of ammonium nitrogen, which disappears during storage. Most of the phosphorus contained in municipal wastes comes from soluble phosphates present in the form of detergents in washing agents (GAMBUŚ 2001). According to MAĆKOWIAK (1999), the mean annual phosphorus concentration in sewage sludge from numerous treatment plants ranged between 2.2 and 4.7%, whereas SIUTA and WASIAK (2000) reported that the P ($P_2O_5$) content of sludge is usually 1.5 – 6% d.m. Sewage sludge is very poor in potassium, which concentration ranges between 0.05 and 0.6% d.m. (SIUTA, WASIAK 2000). KALEMBASA et al. (2001) reported that the K content of sewage sludge from some treatment plants in the region of Siedlce ranged from 0.26 to 0.39%. Sludge can improve the physicochemical properties of the soil and contribute to increasing plant yield, especially in light soils (PIOTROWSKA, GAŁCZYŃSKA 1990, BARAN et al. 1998). According to KOBUS et al. (1990), sewage sludge can be used for the fertilization degraded soil, and it helps to stimulate the biological activity of its surface layer.

Taking into account the need to determine the interrelations between N, P and K contained in soil materials, fertilized with sewage sludge, and the yield, the objective of the present study was to estimate the significance of correlations and regressions between the parameters.

## Materials and Methods

The experimental materials used for preparing soil materials were are follows: overlayer from gravel pit and barren land, fertilized with sewage sludge form the municipal sewage treatment plant. The overlayer showed soil texture of light loamy sand (clay = 14%, Ø < 0.02 mm) and the barren land soil texture of weakly loamy sand (clay = 7.1%, Ø < 0.02 mm). The pots contained 10 kg of soil materials, they were filled with overlayer, barren land and sewage sludge in different proportions: $^1/_2$ + $^1/_2$ and $^1/_4$ + $^3/_4$. The overlayer was taken

from the Gravel-Pit in Żabi Róg, barren land from Tomaszkowo (the Experimental Station) and sewage sludge from the municipal sewage treatment plant at Olsztyn. The treatment combinations are presented in Table 1. There were four replications in each combination.

The following NPK rates were applied in combinations: N – 1 g, P – 0.2 g, K – 1.25 g, i.e. 2.2 g urea + 1.1 g triple superphosphate + 2.5 g potassium salt per pot. Fertilizers were applied in the first, second and third year of the experiment. A grass mixture in the amount of 40 kg · $ha^{-1}$ was grown in pots on soil materials. The mixture was composed of red fescue (*Festuca rubra* L.) – 40%, perennial ryegrass (*Lolium perenne* L.) – 30%, meadow bluegrass (*Poa pratensis* L.) – 30%. The mixture of grasses applied in the study is used for soil amendment and restoration of barren lands. Plant material was harvested four times during the vegetation season, every six weeks. It was then dried, finely ground and subjected to chemical analyses.

The concentrations of total N, P and K, and available P and K, were determined in soil materials, and the contents of N, P and K – in plant material. The determinations were made by generally accepted methods, according to the Polish Standards, modified by the Chemical and Agricultural Station. Nitrogen concentration in soil and plant materials was determined by distillation; the concentrations of phosphorus and potassium were estimated by colorimetry and flame photometry respectively.

The results were analyzed statistically by analysis of variance. The significance of differences was verified with the Duncan test, at a significance level $p = 0.01$. The results of post-hoc tests are presented as homogenous groups, denoted by letters ($a$, $b$, ..., $m$) to compare the type of soil material and interactions, and letters ($x$, $y$) to compare fertilized and unfertilized objects. The following experimental factors were considered: factor I – kind of soil material, according to the design given in Table 1 (fixed factor), factor II – fertilization (fixed factor), factor III – growing season (2001 and 2002).

The relationships between selected properties of fertilized soil materials were determined by two-variable linear Pearson correlation. The significance of correlation coefficients r was verified at significance levels $p < 0.01$ and $p < 0.05$. The correlation levels proposed by STANISZ (1998) were adopted: $r = 0$ variables are not correlated, $0 < r < 0.1$ very low correlation, $0.1 \leq r < 0.3$ low correlation, $0.3 \leq r < 0.5$ average correlation, $0.5 \leq r < 0.7$ high correlation, $0.7 \leq r < 0.9$ very high correlation, $0.9 \leq r < 1$ almost full correlation.

Table 1

The total concent of N, P and K in the soil materials in objects with and without fertilization ($g \cdot kg^{-1}$ d.m.)

| Soil material | $N_{og.}$ | | | | $P_{og.}$ | | | | $K_{og.}$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2001 | | 2002 | | 2001 | | 2002 | | 2001 | | 2002 | |
| | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization |
| Sewage sludge (100%) | $5.500^{d}$ | $6.260^{d}$ | $5.950^{d}$ | $6.910^{d}$ | $3.477^{f}$ | $4.266^{g}$ | $4.285^{h}$ | $4.444^{h}$ | $1.426^{d}$ | $1.548^{ef}$ | $1.940^{bc}$ | $1.935^{bc}$ |
| Barren land (100%) | $0.140^{a}$ | $0.130^{a}$ | $0.130^{a}$ | $0.140^{a}$ | $0.647^{a}$ | $0.511^{a}$ | $0.876^{b}$ | $0.877^{b}$ | $1.954^{g}$ | $1.929^{g}$ | $2.314^{d}$ | $2.315^{d}$ |
| Overlayer (100%) | $0.350^{a}$ | $0.410^{a}$ | $0.430^{a}$ | $0.620^{a}$ | $0.652^{a}$ | $0.653^{a}$ | $0.656^{ab}$ | $0.874^{b}$ | $1.148^{b}$ | $1.314^{c}$ | $1.651^{a}$ | $1.650^{a}$ |
| Sewage sludge + barren land ($^1/_2$ + $^1/_2$) | $1.710^{b}$ | $1.860^{b}$ | $1.260^{b}$ | $1.590^{b}$ | $1.439^{bc}$ | $1.703^{cd}$ | $2.136^{e}$ | $2.349^{ef}$ | $1.467^{de}$ | $1.607^{f}$ | $2.138^{c}$ | $1.969^{c}$ |
| Sewage sludge + overlayer ($^1/_2$ + $^1/_2$) | $2.840^{c}$ | $3.490^{c}$ | $2.980^{c}$ | $3.280^{c}$ | $1.994^{de}$ | $2.221^{e}$ | $2.964^{g}$ | $2.554^{f}$ | $1.228^{bc}$ | $0.967^{a}$ | $1.945^{ab}$ | $1.633^{ab}$ |
| Sewage sludge + barren land ($^1/_4$ + $^3/_4$) | $0.840^{a}$ | $0.530^{a}$ | $0.590^{a}$ | $0.680^{a}$ | $0.652^{a}$ | $0.749^{a}$ | $1.531^{cd}$ | $1.311^{c}$ | $1.968^{g}$ | $0.989^{a}$ | $1.980^{bc}$ | $1.979^{bc}$ |
| Sewage sludge + overlayer ($^1/_4$ + $^3/_4$) | $1.660^{b}$ | $1.430^{b}$ | $1.660^{b}$ | $1.520^{b}$ | $1.680^{c}$ | $1.216^{b}$ | $1.655^{d}$ | $1.743^{d}$ | $1.138^{b}$ | $0.983^{a}$ | $1.643^{a}$ | $1.644^{a}$ |
| **Average** | **1.860 *x*** | **2.000 *x*** | **1.860 *x*** | **2.110 *x*** | **1.506 *x*** | **1.617 *y*** | **2.015 *x*** | **2.022 *y*** | **1.475 *y*** | **1.334 *x*** | **1.945 *x*** | **1.875 *x*** |

The differences between values followed by different letters are statistically significant at $p = 0.01$; the values followed by the ($a$, $b$, $c$, ..., $m$) denote different kinds of soil materials and interactions, and the values followed by ($x$, $y$) denote objects with and without fertilization; these values belong to different homogenous groups (based on post-hoc tests).

## Results and Discussion

As regards the initial experimental materials, the highest concentrations of total N, P and K were recorded in sewage sludge, i.e. N – 5, K – 2.6, P – 3.3 g · · $kg^{-1}$ d.m. The concentrations of these macronutrients in the overlayer and barren land were much lower. N content was higher in the overlayer than in barren land (0.39 g · $kg^{-1}$). P content was at the same level in both cases (0.66 g · $kg^{-1}$), and K content was the highest in barren land (0.99 g · $kg^{-1}$). CZYŻ et al. (1999) also reported low content of the above elements.

In almost all cases the nutrient content of soil materials increased after the first year of experiment. Fertilization also resulted in higher concentrations of macronutrients. A combination of sewage sludge with the overlayer and barren land caused an increase in their levels, but there were some exceptions. The analysis showed that in the first year of experiment the concentrations of total P ($P_{og.}$) and total K ($K_{og.}$) in soil materials were considerably affected by the type of soil material, fertilization, and the interactions between these two experimental factors. In the second year total P content was significantly affected by the two factors and interactions, and total K content – by the type of soil material only (Table 2). The synthesis of results for both years indicated that the concentrations of total P and total K depended on all experimental factors. Only in the case of potassium there was no interaction between fertilization and the year of experiment. Total N ($N_{og.}$) content was significantly affected by the type of soil material only.

Table 2

Analysis of variance (F test) of macroelements in the soil materials

| Years | Factors | Empirical F (values and significance) for parameters: | | |
|---|---|---|---|---|
| | | $P_{og.}$ | $K_{og.}$ | $N_{og.}$ |
| 2001 | U | 563.99* | 436.76* | 138.76* |
| | N | 8.95* | 164.25* | 1.39 |
| | UxN | 15.53* | 189.52 | 1.43 |
| 2002 | U | 335.57* | 20.64* | 239.42* |
| | N | 322.51* | 3.05 | 5.37 |
| | UxN | 309.37* | 1.38 | 1.56 |
| Synthesis 2001-2002 | L | 49.45* | 598.64* | 0.25 |
| | U | 791.77* | 97.10* | 357.84* |
| | N | 86.70* | 26.16* | 5.70 |
| | LxU | 145.75* | 3.84* | 1.50 |
| | LxN | 192.66* | 3.06 | 0.30 |
| | UxN | 83.99* | 14.56* | 2.70 |
| | LxUxN | 192.09* | 15.18* | 0.27 |

U = soil materials, N = fertilization, L = years, LxU, LxN, UxN, LxUxN = interactions between factors

* Femp. > Ftab. — significant correlation at $p = 0.01$

The analysis of variance of total forms of macronutrients in plant material (Table 3) showed that P content depended on the type of soil material (from which plant material was harvested) in the first and second year of experiment, and on the year of experiment, type of soil material and their interaction in the synthesis of results for both years. All experimental factors had a significant effect on the levels of K and N. Their interactions affected K content and, in most cases, N content.

Table 3

Analysis of variance (F test) of macroelements in the plant material

| Years | Factors | Empirical F (values and significance) for parameters: | | |
|---|---|---|---|---|
| | | P | K | N |
| 2001 | U | 19.22* | 69.72* | 35.87* |
| | N | 7.72 | 493.01* | 19.62* |
| | UxN | 3.87 | 5.23* | 13.03* |
| 2002 | U | 10.23* | 133.44* | 283.30* |
| | N | 0.07 | 59.22* | 14.73* |
| | UxN | 1.43 | 7.78* | 7.49* |
| Synthesis 2001-2002 | L | 78.99* | 2178.64* | 931.35* |
| | U | 25.31* | 147.33* | 121.81* |
| | N | 2.85 | 543.70* | 7.49 |
| | LxU | 4.03* | 17.04* | 14.89 |
| | LxN | 4.30 | 272.58* | 30.46* |
| | UxN | 3.52 | 5.25* | 14.94* |
| | LxUxN | 1.57 | 6.21* | 9.66* |

Explanations as in Table 1

The analysis of variance concerning the results obtained in particular years (Table 4) indicated that the available P ($P_{przys.}$) content of soil materials depended on fertilization and the type of soil material, their interaction, and year of experiment. In 2001 significant correlations were recorded for both factors and their interaction, whereas in 2002 – for the type of soil material only. Fertilization had no considerable effect on the concentration of this macronutrient. In the case of available K ($K_{przys.}$) content, significant correlations were observed for the year of experiment, type of soil material and fertilization. The interactions between individual experimental factors were also significant. In 2001 the level of available K in soil materials was affected by fertilization, type of soil material and their interaction, and in 2002 – by fertilization and the type of soil material, whereas their interaction turned out to be not-significant.

Table 4

Analysis of variance (F test) of available macroelements in the soil materials

| Years | Factors | Empirical F (values and significance) for parameters: | |
|---|---|---|---|
| | | $P_{przys.}$ | $K_{przys.}$ |
| 2001 | U | 12285.68* | 220.78* |
| | N | 12256.01* | 515.64* |
| | UxN | 11369.30* | 156.97* |
| 2002 | U | 20.78* | 279.75* |
| | N | 2.93 | 10.01* |
| | UxN | 2.37 | 2.34 |
| Synthesis 2001-2002 | L | 17.25* | 141.51* |
| | U | 57.08* | 479.56* |
| | N | 22.17* | 199.60* |
| | LxU | 2.83 | 50.27* |
| | LxN | 1.64 | 74.89* |
| | UxN | 19.57* | 51.84* |
| | LxUxN | 1.82 | 30.65* |

Explanations as in Table 1

Table 4 presents the concentrations of N, P and K in soil materials as dependent upon the type of soil material and mineral fertilization in the first and second year of experiment. Mean total P content was higher in the second than the first year. The lowest amount of this element was found in the overlayer and barren land, both in fertilized and unfertilized objects. Average total K content was also higher in the second year of experiment. The lowest total K level was noted in the overlayer and soil materials containing this overlayer. Mean total N content was similar in both years of experiment. The lowest total N concentration was noted in the overlayer and barren land.

The analysis of the levels of N, P and K in plant material revealed the opposite tendencies (Table 5). They were lower in the second year of experiment. Similarly as in the case of soil materials, plant material harvested from the overlayer and barren land contained the lowest amounts of the above macronutrients. Nitrogen, in comparison with the other nutrients, has the strongest influence on plant growth. According to FALKOWSKI et al. (2000), the nitrogen concentration in plants varies greatly 2.5 – 6.2% d.m., depending on the species, plant part, growth stage, development stage, and fertilization. The N content of plant material determined in the present experiment remained within the above limits. Its concentration was significantly lower in the second year of experiment. FALKOWSKI et al. (2000) and NIEDŹWIEDZKI et al. (1999) reported the potassium content of grass dry matter to be 2%; its level exceeding 2.5% may be harmful to farm and domestic animals. In the experiment

Table 5

The concent of N, P and K in plant material from objects with and without fertilization (g · kg$^{-1}$ d.m.)

| Soil material | N | | | | P | | | | K | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2001 | | 2002 | | 2001 | | 2002 | | 2001 | | 2002 | |
| | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization |
| Sewage sludge (100%) | 33.72$^{bc}$ | 31.96$^{bc}$ | 28.50$^{g}$ | 26.21$^{g}$ | 4.23$^{cd}$ | 4.32$^{cd}$ | 3.57$^{c}$ | 3.76$^{c}$ | 16.06$^{a}$ | 22.00$^{b}$ | 7.05$^{a}$ | 9.64$^{bc}$ |
| Barren land (100%) | 14.69$^{a}$ | 27.77$^{b}$ | 9.42$^{ab}$ | 9.67$^{ab}$ | 3.48$^{a}$ | 2.26$^{a}$ | 2.73$^{a}$ | 2.46$^{a}$ | 24.38$^{bc}$ | 38.88$^{e}$ | 14.50$^{de}$ | 14.12$^{de}$ |
| Overlayer (100%) | 13.41$^{a}$ | 23.06$^{b}$ | 9.17$^{a}$ | 9.53$^{ab}$ | 3.97$^{bc}$ | 3.66$^{bc}$ | 3.06$^{ab}$ | 2.86$^{ab}$ | 26.63$^{c}$ | 35.16$^{d}$ | 17.49$^{f}$ | 18.29$^{f}$ |
| Sewage sludge + barren land ($^1/_2$ + $^1/_2$) | 34.80$^{bc}$ | 34.07$^{bc}$ | 21.28$^{ef}$ | 20.36$^{f}$ | 4.19$^{bcd}$ | 3.92$^{bcd}$ | 3.27$^{bc}$ | 3.54$^{bc}$ | 16.19$^{a}$ | 26.51$^{c}$ | 8.95$^{abc}$ | 9.56$^{bc}$ |
| Sewage sludge + overlayer ($^1/_2$ + $^1/_2$) | 33.05$^{bc}$ | 36.40$^{c}$ | 22.93$^{f}$ | 18.90$^{de}$ | 4.394$^{cd}$ | 4.33$^{cd}$ | 3.54$^{bc}$ | 3.14$^{bc}$ | 17.36$^{a}$ | 26.52$^{c}$ | 7.64$^{ab}$ | 8.86$^{abc}$ |
| Sewage sludge + barren land ($^1/_4$ + $^3/_4$) | 34.00$^{bc}$ | 34.78$^{c}$ | 17.32$^{cd}$ | 15.43$^{c}$ | 3.80$^{b}$ | 3.61$^{b}$ | 3.25$^{c}$ | 3.93$^{c}$ | 14.92$^{a}$ | 27.01$^{c}$ | 9.86$^{c}$ | 14.47$^{e}$ |
| Sewage sludge + overlayer ($^1/_4$ + $^3/_4$) | 33.86$^{bc}$ | 28.93$^{b}$ | 14.90$^{c}$ | 11.89$^{b}$ | 4.23$^{d}$ | 4.48$^{d}$ | 3.53$^{c}$ | 3.68$^{c}$ | 14.67$^{a}$ | 26.97$^{c}$ | 9.55$^{bc}$ | 12.55$^{d}$ |
| **Average** | **28.22 *x*** | **30.99 *y*** | **17.65 *y*** | **16.01 *x*** | **4.04 *x*** | **3.80 *x*** | **3.28 *x*** | **3.34 *x*** | **18.60 *x*** | **29.01 *y*** | **10.72 *x*** | **12.50 *y*** |

Explanations as in Table 4

Table 6

The available concent of P and K in the soil materials in objects with and without fertilization ($g \cdot kg^{-1}$ d.m.)

| Soil materials | $P_{przys.}$ | | | | $K_{przys.}$ | | | |
|---|---|---|---|---|---|---|---|---|
| | 2001 | | 2002 | | 2001 | | 2002 | |
| | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization | without fertilization | with fertilization |
| Sewage sludge (100%) | $0.217^{c}$ | $4.101^{d}$ | $3.636^{b}$ | $4.444^{b}$ | $0.057^{cd}$ | $0.201^{f}$ | $0.162^{c}$ | $0.185^{c}$ |
| Barren land (100%) | $0.0475^{a}$ | $0.056^{a}$ | $0.047^{a}$ | $0.058^{a}$ | $0.061^{de}$ | $0.068^{de}$ | $0.037^{b}$ | $0.054^{b}$ |
| Overlayer (100%) | $0.060^{a}$ | $0.080^{a}$ | $0.086^{a}$ | $0.105^{a}$ | $0.045^{abc}$ | $0.066^{de}$ | $0.021^{a}$ | $0.037^{a}$ |
| Sewage sludge + barren land ($^1/_2$ + $^1/_2$) | $0.193^{bc}$ | $0.199^{bc}$ | $0.595^{a}$ | $0.685^{a}$ | $0.044^{ab}$ | $0.048^{abc}$ | $0.021^{a}$ | $0.021^{a}$ |
| Sewage sludge + overlayer ($^1/_2$ + $^1/_2$) | $0.205^{bc}$ | $0.229^{c}$ | $0.786^{a}$ | $0.771^{a}$ | $0.042^{a}$ | $0.056^{bcd}$ | $0.020^{a}$ | $0.020^{a}$ |
| Sewage sludge + barren land ($^1/_4$ + $^3/_4$) | $0.168^{b}$ | $0.222^{c}$ | $0.302^{a}$ | $0.417^{a}$ | $0.041^{a}$ | $0.071^{e}$ | $0.021^{a}$ | $0.021^{a}$ |
| Sewage sludge + overlayer ($^1/_4$ + $^3/_4$) | $0.221^{c}$ | $0.237^{c}$ | $0.595^{a}$ | $0.567^{a}$ | $0.041^{a}$ | $0.057^{cd}$ | $0.021^{a}$ | $0.016^{a}$ |
| **Average** | **0.129 *x*** | **0.732 *y*** | **0.864 *x*** | **1.007 *x*** | **0.047 *x*** | **0.081 *y*** | **0.043 *x*** | **0.051 *y*** |

Explanations as in Table 1

performed by NIEDŹWIEDZKI et al. (1999), the K content of plant material ranged from 1.3 to 2.3% d.m. FALKOWSKI et al. (2000) claims that the mean potassium content of grasses varied from 0.6 do 8.3%. In our investigations the concentration of this element was more differentiated, and varied from 0.7 to 3.9%. According to PIOTROWSKA and GAŁCZYŃSKA (1990), its level decreases when the rate of sewage sludge is increased. This tendency was observed in the second year of our experiment. According to other reference data (PIOTROWSKA, GAŁCZYŃSKA 1990, FALKOWSKI et al. 2000), mean phosphorus content is 0.35%. In this experiment it ranged between 0.2 to 0.45%. Similar values were obtained by NIEDŹWIEDZKI et al. (1999). In the second year of experiment P content was significantly lower.

As concerns the concentration of available P in fertilized and unfertilized objects, in the first and second year of experiment, it was higher in fertilized soil materials (Table 6). However, the differences between means of objects were statistically significant in the year 2001 only. The highest available P level was recorded in NPK-fertilized sewage sludge in 2002, and the lowest – in unfertilized barren land, also in 2002. The analysis of mean available K concentration in the first and second year of experiment showed that it reached the highest level in 2001 in fertilized objects, and the lowest – in 2002 in unfertilized objects. In the first year its content was the highest in NPK-fertilized sewage sludge. Its twelve-fold lower concentration was noted in sewage sludge combined with overlayer (1/4 + 3/4) in objects fertilized with NPK.

The values of linear Pearson correlation between the concentrations of total N, P, and K, and available P and K in soil materials and plant material, in 2001 and 2002, are given in Tables 7 and 8. The correlations between the macronutrients examined have positive and negative values. In the first year of experiment high and very high correlations were found only between P in plant

Table 7
Coefficients of Pearson correlation between the concentrations of N, P and K in the soil and plant material – first year of the experiment

| Variables | | Plant material | | |
|---|---|---|---|---|
| | | N | P | K |
| Soil materials | $N_{og.}$ | 0.25 | 0.57* | 0.08 |
| | $P_{og.}$ | 0.23 | 0.60* | 0.07 |
| | $K_{og.}$ | 0.14 | -0.32 | -0.50 |
| | $P_{przys.}$ | -0.28 | 0.40 | 0.57* |
| | $K_{przys.}$ | -0.01 | -0.23 | 0.82** |

* simple correlation coefficients significant at $p = 0.05$
** simple correlation coefficients significant at $p = 0.01$

material and total N and total P in soil materials, as well as K in plant material and available K and available P in soil materials. In the second year of experiment – 2002 high and very high positive simple correlations were noted between N in plant material and total and available P and K in soil materials, as well as between K in plant material and total N, total P and available P in soil materials. In the first year of experiment K uptake by plants was proportional to its concentration in fertilized soil materials ($r = +0.82$), but there was no such a relationship in the second year. In 2002 the N content in plant material depended on the level of total P in soil materials ($r = +0.74$); in 2001 this correlation was not-significant.

Table 8

Coefficients of Pearson correlation between the concentrations of N, P and K in the soil and plant material – second year of the experiment

| Variables | | Plant material | | |
|---|---|---|---|---|
| | | N | P | K |
| Soil materials | $N_{og.}$ | 0.37 | -0.36 | -0.56* |
| | $P_{og.}$ | 0.74** | -0.43 | -0.73** |
| | $K_{og.}$ | 0.53* | 0.06 | -0.05 |
| | $P_{przys.}$ | 0.50* | -0.40 | -0.55* |
| | $K_{przys.}$ | 0.59* | -0.16 | -0.14 |

Explanations as in Table 7

Figures 1 and 2 show the graphical scheme of correlations between the concentrations of total and available N, P and K in soil materials and plant material, together with regression equations and 100 $R^2$ coefficients (only the correlations with the highest values of 100 $R^2$ are presented).

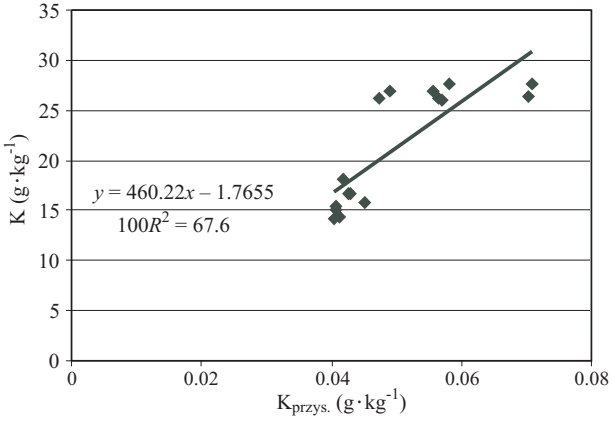


Fig. 1. Correlation between the concentration of K in plant material and available K in the soil materials (first year of the experiment)

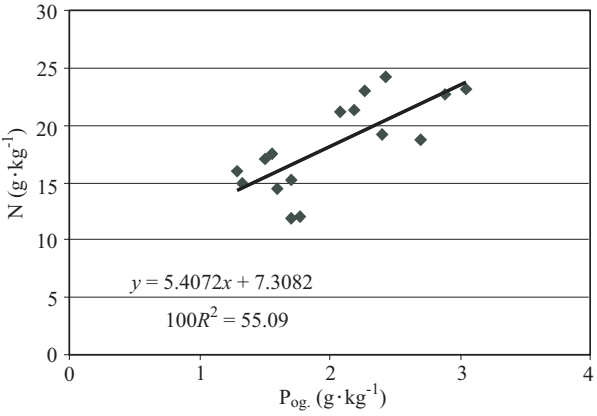


Fig. 2. Correlation between the concentration of N in plant material and total P in the soil materials (second year of the experiment)

## Conclusions

1. The concentrations of total N, P and K in soil materials and plant material are characterized by significant differences depending on the type of soil material, fertilization, and interactions between the experimental factors.

2. The mean N and K contents of plant material decreased significantly with time. Potassium concentration in fertilized objects was significantly higher than in unfertilized objects. No such differences were noted in the case of P.

3. The values of simple correlation coefficients varied with time. In the first year of experiment significant and positive correlations were noted between P and total N and total P, K and available P and available K. In the second year significant correlations were found between N (positive) and K (negative) in plant material, and selected macronutrients in soil materials.

4. The highest regression values, determined with the coefficient of determination, were noted for the K content of plant material as dependent upon the available K content of soil materials in the first year, and for N as dependent upon total P in the second year of experiment.

Translated by Aleksandra Poprawska

Accepted for print 9.02.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 23-31, Y. 2006

# FEEDING DETERRENT ACTIVITY OF $\alpha$-METHYLENELACTONES TO PEA APHID *ACYRTHOSIPHON PISUM* (HARRIS) AND GREEN PEACH APHID *MYZUS PERSICAE* (SULZER)

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K e y w o r d s: pea aphid, green peach aphid, semiochemicals, antifeedants, $\alpha$-methylenelactones.

A b s t r a c t

Deterrent properties of $\alpha$-methylenelactones derived from synthetic alkenes 2,4,4-trimethyl--pent-1-ene, 2-methyl-pent-1-ene, 1-tetradecene, and 1-methylcyclohexene and cyclic terpenes (±)-3-carene, and (±)camphene to the pea aphid *Acyrthosiphon pisum* and green peach aphid *Myzus persicae* were examined. The strongest deterrents to the pea aphid were 2,4,4-trimethyl-pent-1-ene, 2-methyl-pent-1-ene, and (±)camphene and their respective $\alpha$-methylenelactones: 5-(2,2-dimethyl-propyl)-5-methyl-3-methylene-dihydro-furan-2-one, 5-methyl-3-methylene-5-propyl-dihydro-furan--2-one, and (±)3,3-dimethyl-4'-methylenedihydro-5'H-spiro[bicyclo[2.2.1]heptan-2,2'-furan]-5'-one: their effect was observed immediately after aphids had had access to the plants and it lasted until the end of experiment, i.e. 24 hours after application. The green peach aphid was prevented from settling and feeding by tetradec-1-ene and (±)camphene.

## AKTYWNOŚĆ DETERENTNA $\alpha$-METYLENOLAKTONÓW DLA MSZYCY GROCHOWEJ *ACYRTHOSIPHON PISUM* (HARRIS) i MSZYCY BRZOSKWINIOWEJ *MYZUS PERSICAE* (SULZER)

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S ł o w a k l u c z o w e: mszyca grochowa, mszyca brzoskwiniowa, semiozwiązki, antyfidanty, $\alpha$-metylenolaktony.

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Abstrakt

Badano deterentne własności $\alpha$-metylenolaktonów, pochodzących od następujących substancji wyjściowych: syntetycznych alkenów: 2,4,4-trimetyl-pent-1-enu, 2-metyl-pent-1-enu, 1-tetradecenu i 1-metylcykloheksanu, oraz cyklicznych terpenów: (±)-3-karenu i (±)kamfenu dla mszycy grochowej *Acyrthosiphon pisum* i mszycy brzoskwiniowej. Najsilniejszymi deterentami dla mszycy grochowej były: 2,4,4-trimetyl-pent-1-en, 2-metyl-pent-1-en i (±)kamfen oraz odpowiadające im $\alpha$-metylenolaktony: 5-(2,2-dimetyl-propyl)-5-metyl-3-metyleno-dihydro-furan-2-on, 5-metyl-3-metyleno-5-propyl-dihydro-furan-2-on i (±)3,3-dimetyl-4'-metylenodihydro-5'H-spiro[bicyklo[2.2.1]heptan-2,2'-furan]-5'-on: ich wpływ widoczny był już od momentu kontaktu mszyc z tymi substancjami aż do końca eksperymentu, tzn. 24 h po zastosowaniu. W przypadku mszycy brzoskwiniowej, zasiedlanie roślin i żerowanie było hamowane przez tetradec-1-en i (±)kamfen.

## Introduction

The pea aphid *Acyrthosiphon pisum* (Harris) and green peach aphid *Myzus persicae* (Sulzer) are two aphid species of great economic importance. *M. persicae* is considered the most polyphagous aphid species with secondary hosts in over 40 different plant families. It is also the most important insect vector of over 100 plant viruses. *A. pisum*, although rather oligophagous (restricted mostly to leguminose plants) is also an important pest species able to transmit over 30 virus diseases (CAPINERA 2004). At present, aphid control depends mainly on the use of insecticides. Due to the repeating applications, many aphid species, especially the green peach aphid, have developed resistance to several chemical aphicides. Therefore, an alternative method of aphid control is needed. One of the possible approaches is the use of behaviour modyfing chemicals that would repell aphids or deter their feeding. The most known antifeedants belong to different chemical groups and come from natural sources (WAWRZYNIAK 1996, KLEIN GEBBINCK et al. 2002, WAWRZEŃCZYK et al. 2002). Compounds with the lactone moiety are commonly occurring natural products and frequently exhibit feeding deterrent properties against insects (PICMAN 1986).

In the present work we examine the deterrent properties of some synthetic $\alpha$-methylenelactones to *A. pisum* and *M. persicae*. We also compare the activity of the $\alpha$-methylenelactones to their respective non-lactone starting compounds.

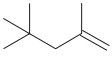
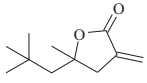
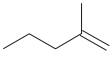
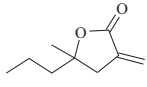
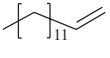
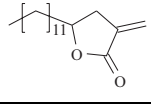
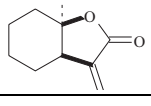
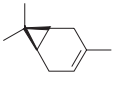
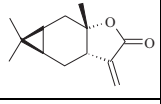
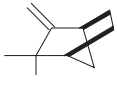
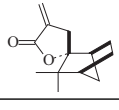
# Material and Methods

## Chemistry

The starting compounds for the syntheses of $\alpha$-methylenelactones, four synthetic alkenes: 2,4,4-trimethyl-pent-1-ene, 2-methyl-pent-1-ene, 1-tetradecene, and 1-methylcyclohexene as well as two cyclic terpenes: (±)-3-carene, and (±)camphene (Table 1), which were purchased from Aldrich. The $\alpha$--methylenelactones were synthesized from these alkenes in two-step synthesis (Figure 1). The first step was the addition reaction of the free radical formed from Meldrum's acid (a) to alkene or cycloalkene (b) mediated by cerium (IV) ammonium nitrate (V) (CAN). The products of these reactions, $\alpha$-carboxylactones (c), were subjected to decarboxylative methylenation with solution containing diethylamine, formaldehyde (30% aqueous solution), sodium acetate and acetic acid to give the final $\alpha$-methylenelactones. Crude products were purified by column chromatography on silica-gel (mesh 230-400) using mixture of hexan-diethyl ether (2:1) as eluent. Structures of final products were

Table 1

Structures of compounds tested for feeding deterrent activity to *Acyrthosiphon pisum* and *Myzus persicae*

| Starting compounds | | $\alpha$-methylenelactones | |
|---|---|---|---|
|  | 2,4,4-trimethyl-pent-1-ene |  | 5-(2,2-dimethyl-propyl)--5-methyl-3-methylene--dihydro-furan-2-one |
|  | 2-methyl-pent-1-ene |  | 5-methyl-3-methylene-5--propyl-dihydro-furan-2-one |
|  | tetradec-1-ene |  | 5-dodecyl-3-methylene--dihydro-furan-2-one |
|  | 1-methylcyclohexene |  | 1-methyl-7-metylene-9-oxabicyclo [4,3,0]nonan-8-one |
|  | (±)-3-carene |  | (1*S*, 3S, 5*R*, 7*S*) 1,4,4--trimethyl-8-metylene-10--oxatricyclo[4,3,1,03,5]decan--9-one |
|  | (±)camphene |  | (±) 3,3-dimethyl-4'--methylenedihydro-5' H-spiro[bicyclo[2.2.1] heptan-2,2'-furan]-5'-one |

established on the basis of data from $^1$H NMR, IR and EI-MS spectra. The yields of the synthesis of corresponding $\alpha$-methylenelactones and their physical and spectra data are given elsewhere (SZUMNY, WAWRZEŃCZYK 2006).

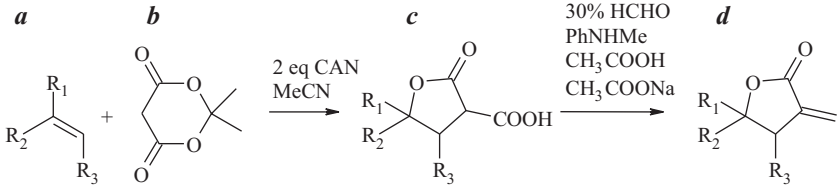



Fig. 1. Scheme of the synthesis of $\alpha$-methylenelactones

## Biological tests

Aphids (*Myzus persicae and Acyrthosiphon pisum*) and plants (Chinese cabbage *Brassica pekinensis* and *Pisum sativum* for the two aphid species, respectively) were reared in laboratory at 20°C, 65% r.h., and L16:8D photoperiod. All experiments were carried out under the same conditions.

The compounds were applied to adaxial surface of a leaf as 0.1% ethanolic solutions, 0.01 $ml \cdot cm^{-2}$ of the leaf according to a method described by POLONSKY et al. (1989). All biological tests were performed 1 hour after the application of the compounds to allow the evaporation of the solvent.

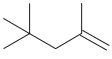
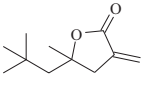
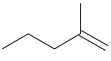
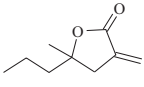
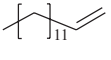
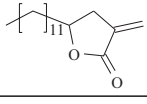
The feeding deterrence was assessed by means of a choice-test, in which the aphid settling on plants was observed. The compounds were applied on one half of the leaf; the other side of the midrib was treated with ethanol and acted as a control. Aphids had a choice between equal areas of treated and control surfaces. Aphids that settled, i.e. they did not move and the position of their antennae indicated feeding (HARDIE et al. 1992) on each side of the midrib were counted at 15', 30', 1 h, 2 h, and 24 h intervals after access to the leaf (8 replicates, 20 viviparous apterous females/replicate). The data were analyzed using one way ANOVA. If aphids showed clear preference to the half of the leaf treated with the tested compound ($P < 0.05$), the compound was described as having attractant properties. If aphids settled mainly on the control half of the leaf ($P < 0.05$), the compound tested in the respective choice-test was stated a feeding deterrent.

## Results and Discussion

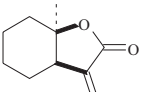
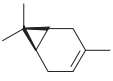
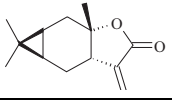
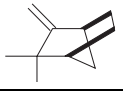
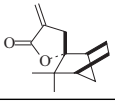
All tested synthetic alkenes and their corresponding $\alpha$-methylenelactones were feeding deterrents to *Acyrthosiphon pisum* (Table 2). The strongest deterrents were 2,4,4-trimethyl-pent-1-ene and 2-methyl-pent-1-ene: their effect was observed immediately after aphids had had access to the plants and it lasted until the end of experiment, i.e. 24 hours after application. The same was true for their respective lactones: 5-(2,2-dimethyl-propyl)-5-methyl-3-methylene-dihydro-furan-2-one and 5-methyl-3-methylene-5-propyl-dihydro-furan-2-one. The deterrent character of tetradec-1-ene and its lactone 5-dodecyl-3-methylene-dihydro-furan-2-one showed at the beginning of the experiment (until 30 minutes after application) and again after 24 hours. The deterrent activity of 1-methylcyclohexene and 1-methyl-7-metylene-9-oxabicyclo [4,3,0]nonan-8-one occurred until 1 or 2 hours after application, respectively. Of the cyclic terpenes, the most active was (±)camphene and its respective $\alpha$-methylenelactone. The activity of (±)-3-carene and (1*S*, 3S, 5*R*, 7*S*) 1,4,4-trimethyl-8-metylene-10-oxatricyclo[4,3,1,$0^{3,5}$]decan-9-one ceased after 15 minutes from the beginning of the experiment (Table 2).

Table 2

Deterrent activity of $\alpha$-methylenelactones to *Acyrthosiphon pisum*

| Compound | | Time after access to the plants | | | | |
|---|---|---|---|---|---|---|
| | | 15 min | 30 min | 1 h | 2 h | 24 h |
| 1 | | 2 | 3 | 4 | 5 | 6 |
|  | test | 3.3 | 3.8 | 2.9 | 3.1 | 1.9 |
| | control | 6.7 | 6.2 | 7.1 | 6.9 | 8.1 |
| | P | **0.0000** | **0.0060** | **0.0000** | **0.0000** | **0.0000** |
|  | test | 3.5 | 4.3 | 3.3 | 3.8 | 2.1 |
| | control | 6.5 | 5.7 | 6.1 | 6.2 | 7.9 |
| | P | **0.0002** | 0.0810 | **0.0062** | **0.0021** | **0.0000** |
|  | test | 3.3 | 4.3 | 3.3 | 2.5 | 2.8 |
| | control | 6.7 | 5.7 | 6.7 | 7.5 | 7.2 |
| | P | **0.0000** | 0.1457 | **0.0002** | **0.0000** | **0.0000** |
|  | test | 3.0 | 3.8 | 2.9 | 2.1 | 2.5 |
| | control | 7.0 | 6.2 | 7.1 | 7.9 | 7.5 |
| | P | **0.0000** | **0.0319** | **0.0001** | **0.0000** | **0.0000** |
|  | test | 3.5 | 3.9 | 4.4 | 4.3 | 3.7 |
| | control | 6.5 | 6.1 | 5.6 | 5.7 | 6.3 |
| | P | **0.0000** | **0.0063** | 0.1224 | 0.1420 | **0.0028** |
|  | test | 3.2 | 3.9 | 4.6 | 4.5 | 3.1 |
| | control | 6.8 | 6.1 | 5.4 | 5.5 | 7.3 |
| | P | **0.0000** | **0.0035** | 0.2635 | 0.2409 | **0.0000** |

cont. Table 2

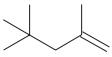
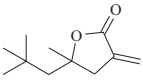
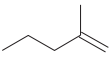
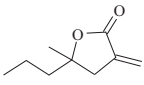
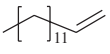
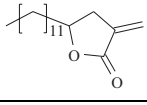
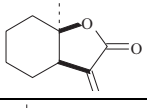
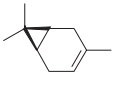
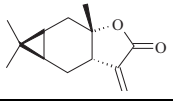
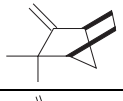
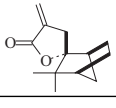
| 1 | | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
|  | test | 3.1 | 3.1 | 3.2 | 4.4 | 4.9 |
| | control | 6.9 | 6.9 | 6.5 | 5.6 | 5.1 |
| | P | **0.0000** | **0.0002** | **0.0009** | 0.0793 | 0.8189 |
|  | test | 2.9 | 2.9 | 2.7 | 4.2 | 4.4 |
| | control | 7.1 | 7.3 | 7.3 | 5.8 | 5.6 |
| | P | **0.0000** | **0.0000** | **0.0000** | **0.0059** | 0.1628 |
|  | test | 4.2 | 4.7 | 4.7 | 4.9 | 5.2 |
| | control | 5.9 | 5.3 | 5.3 | 5.1 | 4.8 |
| | P | **0.0061** | 0.1120 | 0.0920 | 0.5793 | 0.5434 |
|  | test | 3.3 | 4.6 | 4.7 | 4.5 | 4.9 |
| | control | 6.7 | 5.4 | 5.3 | 5.5 | 5.1 |
| | P | **0.0000** | 0.2032 | 0.4670 | 0.2012 | 0.8773 |
|  | test | 3.0 | 3.3 | 3.0 | 2.8 | 3.7 |
| | control | 7.0 | 6.9 | 7.0 | 7.2 | 6.3 |
| | P | **0.0000** | **0.0000** | **0.0001** | **0.0000** | **0.0062** |
|  | test | 2.9 | 3.5 | 3.1 | 2.7 | 4.0 |
| | control | 7.1 | 6.5 | 6.9 | 7.3 | 6.0 |
| | P | **0.0000** | **0.0000** | **0.0000** | **0.0000** | **0.0146** |

Numbers for "test" and "control" represent mean number of aphids settled on test or control half of the leaf (choice-test). *P* – significance level (ANOVA) – significant differences between number of aphids settled on either half of the leaf are underlined.

*Myzus persicae* was prevented from settling and feeding by few of the tested compounds (Table 3). Of the tested synthetic alkenes, only tetradec-1-ene was deterrent to the green peach aphid and as for the cyclic terpenes – only (±)camphene (its corresponding $\alpha$-methylenelactone was not active). Their activity however, was long term. The two compounds were active as long as 24 hours after application. Within the group of $\alpha$-methylenelactones, two compounds: 5-methyl-3-methylene-5-propyl-dihydro-furan-2-one (derived from the inactive 2-methyl-pent-1-ene) was feeding deterrent to *M. persicae*. (1*S*, 3S, 5*R*, 7*S*) 1,4,4-trimethyl-8-metylene-10-oxatricyclo[4,3,1,$0^{3,5}$]decan-9-one (derived from slightly active attractant (±)-3-carene) had attractant properties to the green peach aphid (Table 3).

Table 3

Deterrent activity of $\alpha$-methylenelactones to *Myzus persicae*

| Compound | | Time after access to the plants | | | | |
|---|---|---|---|---|---|---|
| | | 15 min | 30 min | 1 h | 2 h | 24 h |
|  | test | 5.9 | 6.1 | 7.4 | 7.6 | 8.8 |
| | control | 5.8 | 7.9 | 8.6 | 8.6 | 9.0 |
| | P | 0.9152 | 0.2624 | 0.3701 | 0.4249 | 0.8144 |
|  | test | 7.9 | 7.9 | 7.8 | 6.1 | 6.9 |
| | control | 7.8 | 8.3 | 9.0 | 10.0 | 9.9 |
| | P | 0.9347 | 0.7677 | 0.4735 | 0.0839 | 0.1741 |
|  | test | 5.8 | 6.9 | 7.0 | 7.3 | 6.6 |
| | control | 8.5 | 8.8 | 8.3 | 7.8 | 6.8 |
| | P | **0.0111** | 0.1621 | 0.3599 | 0.7403 | 0.8994 |
|  | test | 5.0 | 6.3 | 7.6 | 7.1 | 7.0 |
| | control | 9.5 | 9.5 | 8.9 | 10.0 | 10.9 |
| | P | **0.0004** | **0.0050** | 0.2573 | **0.0027** | **0.0003** |
|  | test | 6.3 | 6.6 | 6.0 | 7.6 | 6.9 |
| | control | 8.6 | 8.9 | 8.1 | 9.3 | 9.4 |
| | P | **0.0424** | **0.0551** | **0.0126** | **0.0538** | **0.0457** |
|  | test | 8.1 | 8.5 | 9.1 | 9.1 | 9.6 |
| | control | 6.3 | 7.1 | 7.1 | 7.3 | 7.3 |
| | P | 0.0730 | 0.1632 | **0.0288** | **0.0316** | **0.0406** |
|  | test | 5.9 | 9.3 | 7.4 | 6.5 | 7.8 |
| | control | 8.5 | 6.8 | 9.3 | 9.4 | 9.6 |
| | P | **0.0172** | 0.1117 | **0.0608** | **0.0134** | 0.0803 |
|  | test | 6.9 | 6.9 | 7.1 | 8.0 | 7.3 |
| | control | 7.9 | 8.9 | 7.9 | 8.1 | 8.1 |
| | P | 0.1075 | **0.0066** | 0.6062 | 0.9197 | 0.5924 |
|  | test | 8.0 | 7.9 | 9.3 | 9.3 | 10.3 |
| | control | 7.1 | 7.1 | 8.0 | 7.9 | 5.5 |
| | P | 0.5034 | 0.5792 | 0.3059 | 0.2922 | **0.0018** |
|  | test | 6.9 | 8.9 | 9.3 | 8.9 | 10.8 |
| | control | 5.4 | 6.0 | 6.4 | 7.0 | 7.3 |
| | P | 0.0929 | **0.0138** | **0.0118** | **0.0323** | **0.0058** |
|  | test | 5.0 | 3.3 | 3.6 | 3.1 | 2.5 |
| | control | 9.1 | 9.1 | 8.4 | 8.9 | 7.4 |
| | P | **0.0217** | **0.0000** | **0.0023** | **0.0009** | **0.0030** |
|  | test | 6.8 | 6.4 | 7.6 | 7.3 | 8.3 |
| | control | 7.4 | 8.9 | 8.8 | 10.1 | 9.9 |
| | P | 0.7221 | 0.1293 | 0.5549 | 0.1508 | 0.4330 |

Numbers for "test" and "control" represent mean number of aphids settled on test or control half of the leaf (choice-test). *P* – significance level (ANOVA) – significant differences between number of aphids settled on either half of the leaf are underlined.

The biological tests showed that the pea aphid was more sensitive to different studied compounds than the green peach aphid. *A. pisum* was deterred by these compounds just after it had had an access to the treated leaf. Usually, the negative aphid response was observed as long as for 24 hours. In the case of (±)-3-carene and its respective $\alpha$-methylenelactone, *A. pisum* was weakly deterred by them, while *M. persicae* was attracted by these compounds.

In our previous work with the green peach aphid, storage pest insects and Colorado potato beetle, we found that the deterrent effect of a variety of compounds, mainly of terpenoid character, was species-specific and instar-specific (WAWRZEŃCZYK et al. 2005). The difference in sensitivity to the tested compounds between the pea aphid and the green peach aphid may be a result of their different adaptations to herbivory in nature. Phytophagous insects use plant secondary metabolites as one of the cues for host plant selection. Specialized herbivores are usually attracted by the compounds characteristic of their host plants. *A. pisum* can live on plants restricted to one plant family, the Fabaceae. Therefore, it is bound to respond to semiochemicals characteristic of this group, i.e. mainly alkaloids. Other chemicals may be either neutral or deterrent to the pea aphid as they do not represent plants of its host range (DEL CAMPO et al. 2003). *A. pisum* refused to accept *Vicia faba* as its host plant when it was infiltrated with sinigrin, the chemical typical of Brassicaceae (GABRYS, TJALLINGII 2002). Generalist herbivores, such as *M. persicae*, select rather for the nutritional value of their host plants while being relatively insensitive to allelochemicals. This may be the reason that this aphid was rather unresponsive to the compounds tested in the present study.

In respect to the chemical modifications of alkenes and cyclic terpenes, the incorporation of $\alpha$-methylenelactone moiety did not have an effect on their actvity in the majority of cases. The $\alpha$-methylenelactones derived from 2-methyl-pent-1-ene and (±)-3-carene were deterrent to *M. persicae*, while their starting compounds were not. On the other hand, (±)camphene that was a strong deterrent to green peach aphid, lost the activity after incorporation of h-methylenelactone moiety.

Translated by JOLANTA IDŹKOWSKA

Accepted for print 4.04.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 33-44, Y. 2006

# CLASSIFICATION OF PERIODS OF ATMOSPHERIC DROUGHT ON THE GORZÓW PLAIN IN THE YEARS 1965-2004

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K e y w o r d s: atmospheric drought, an index of relative precipitation, hydrothermal index, Gorzów Plain.

A b s t r a c t

The aim of the study was to determine the frequency and degree of intensification of the periods of atmospheric drought on the Gorzów Plain and also to define the tendency of variability of this phenomenon. The material constituted average monthly air temperatures and monthly totals of atmospheric precipitation from the vegetation period (from April to September) gathered in 1965--2004 by the IMGW station in Gorzów Wielkopolski. The periods of drought were determined by two methods – on the basis of the values of the *RPI* index of relative precipitation (%) and on the basis of Sielianinow's hydrothermal index *K*. On the Gorzów Plain in 1965-2004 the deficiency of precipitation in relation to the norm was most often observed in July. The assessment of the hydrothermal conditions shows that the most frequent droughts are noticed in August and in September. Decreasing values of the *K* index in May observed in the years of the studies can indicate an increasing risk of occurrence of dry periods in this month on the Gorzów Plain.

## KLASYFIKACJA OKRESÓW SUSZY ATMOSFERYCZNEJ NA RÓWNINIE GORZOWSKIEJ W LATACH 1965-2004

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Katedra Meteorologii i Klimatologii
Akademia Rolnicza w Szczecinie

S ł o w a k l u c z o w e: susza atmosferyczna, wskaźnik względnego opadu, wskaźnik hydrotermiczny, Równina Gorzowska.

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Abstrakt

Celem pracy było wyznaczenie częstości występowania i stopnia nasilenia okresów suszy atmosferycznej na Równinie Gorzowskiej oraz określenie tendencji zmienności tego zjawiska. Materiał stanowiły średnie miesięczne temperatury powietrza oraz miesięczne sumy opadów atmosferycznych z sezonu wegetacyjnego (od kwietnia do września) z lat 1965-2004, ze stacji IMGW Gorzów Wlkp. Okresy suszy wyznaczono dwiema metodami: na podstawie wartości wskaźnika względnego opadu *RPI* (%) oraz wskaźnika hydrotermicznego Sielianinowa *K*. W latach 1965-2004 na Równinie Gorzowskiej niedobór opadów w stosunku do normy najczęściej występował w lipcu. Ocena warunków hydrotermicznych wskazuje na najczęstsze występowanie suszy w sierpniu i we wrześniu. Malejące w latach badań wartości wskaźnika *K* w maju mogą wskazywać na zwiększające się na Równinie Gorzowskiej ryzyko wystąpienia okresów suszy w tym miesiącu.

## Introduction

The location of western part of Pomerania in the neighbourhood of the Baltic Sea and the Szczecin Lagoon, large variability of weather conditions connected with a frequent movement of atmospheric fronts and a considerable ifferentiation of physiographic conditions result in a very unstable moisture balance of air and soil in this area. As a result of large instability of moisture balance there are late spring atmospheric droughts which lead to using up after-winter reserves of water in soil and to a considerable threat for vegetation and yields (CZARNECKA et al. 2004). In summer, instability of moisture conditions is greater and it means, on the one hand, the largest recorded precipitation in this region and on the other hand, the most frequent atmospheric droughts (KOŹMIŃSKI, KALBARCZYK 2004). South-western parts of Pomerania, the areas adjacent to the valley of the Warta river in particular, are characterized by the lowest totals of precipitation in spring and summer and by the lowest moisture reserves in all the periods of the year, compared to other parts of Pomerania (CZARNECKA et al. 2004). On the Gorzów Plain deficiency of precipitation may cause a decrease in the yields of spring crops by about 10% and those of potato by about 20% (KALBARCZYK et al. 2001, KOŹMIŃSKI et al. 2001). The time of occurrence and the intensification of drought are most frequently determined by means of indices based on the totals of atmospheric precipitation (KACZOROWSKA 1962, PRZEDPEŁSKA 1973), frequency and length of periods with no precipitation (PRAWDZIC, KOŹMIŃSKI 1966, SCHMUCK, KOŹMIŃSKI 1967) and on the totals of precipitation and at the same time air temperature or evaporation (BALLING 1996, BĄK, ŁABĘDZKI 2002, BRIFFA et al. 1994, KALBARCZYK 2003, QUIRING, PAPARYIAKOU 2003).

The aim of the work was to determine the frequency and intensification of the periods of droughts on the Gorzów Plain in the years 1965-2004 and also to define the tendency of variability of this phenomenon using an index of relative precipitation and a hydrothermal index.

## Material and Methods

Material for the analysis was based on the meteorological data including average monthly values of air temperature and monthly totals of atmospheric precipitation during the vegetation period (from April to September) in the years 1965-2004 gathred at the IMGW station in Gorzów Wielkopolski, published in Miesięczny Przegląd Agrometeorologiczny (1965-1999) and made available by IMGW in Warsaw.

The initial evaluation of the meteorological conditions in the vegetation period in individual years was carried out using a simultaneous classification of thermal conditions according to LORENC (2000) and precipitation conditions according to KACZOROWSKA (1962). The thermal classification was carried out on the basis of the air temperature deviation from the norm in the analyzed period and the formation of a distributive series with defined intervals of standard deviation (S). According to LORENC (2000), the following division was accepted: a normal month – the temperature deviation from the norm from -0.5 S to 0.5 S, slightly warm from 0.51 S to 1.0 S, warm from 1.01 S to 1.5 S, very warm from 1.51 S to 2.0 S, anomalously warm from 2.01 S to 2.5 S, extremely warm > 2.51 S, slightly coll from -0.5 S to -1.0 S, coll from -1.01 S to -1.5 S, cold from -1.51 S to -2.0 S, anomalously cold from -2.01 S do -2.5 S and extremely cold < -2.51 S. According to KACZOROWSKA (1962), the month in which the total of precipitation is lower than 25% of the total of a many year norm should be regarded as an extremely dry month, as a very dry month the month in which the total of precipitation varies from 25 to 49% of the norm, and as a dry month, the month in which the total of precipitation varies from 50 to 75% of the norm. The assumed criteria for longer periods were respectively: an extremely dry period – precipitation below 50% of the norm, very dry – 50-74%, dry – 75-89%, normal – 90-110% of a many year average of precipitation total.

Dry periods were determined by means of two methods – according to the values of an index of relative precipitation *RPI* (%) and on the basis of a hydrothermal index *K*.

On the basis of the value of an index *RPI* (BĄK, ŁABĘDZKI 2002), calculated according to the equation:

$$RPI = (P / \overline{P})\ 100\% \qquad (1)$$

where:

$P$ – precipitation total in a given period, mm;

$\overline{P}$ – a many year average value of precipitation in a given period, mm;

dry periods were determined using KACZOROWSKA'S criterion (1962). Then a Sielianinow hydrothermal index in the form of:

$$K = P / 0.1 \Sigma t, \quad (2)$$

where:

$P$ – atmospheric precipitation total, mm;

$\Sigma t$ – air temperature total >0°C;

made it possible to set apart three periods of different intensity of drought: an extremely dry period – $K \leq 0.4$, a very dry period – $0.4 < K \leq 0.7$, a dry period – $0.7 < K \leq 1.0$. This index was used in the periods in which average daily temperature amounted to at least 8°C (PRAWDZIC, KOŹMIŃSKI 1966).

## Results and Discussion

During the period of 1965-2004 an average total of precipitation on the Gorzów Plain from April to September amounted to 318 mm, the standard deviation – 68 mm, and the variability coefficient – 21%. In the course of the analyzed 40 years, the first and the last decades during the time of vegetation were characterized by precipitation above the average, the two middle decades had precipitation total lower than the average of the period of many years. The lowest average total of precipitation in the years 1985-1994, amounting to 302 mm was caused by exceptionally low precipitation in the years 1990-1994 (285 mm, 90% of the norm), the lowest of the calculated for the five year periods. On the other hand the average total of precipitation in the successive period of five years, i.e. 1995-1999, was the largest (355 mm, 112% of the norm) what affected the largest average total of precipitation, 339 mm involving the last decade of the analyzed 40 year period. In the considered years 18 times the precipitation sum in the period from April to September was lower than the average and 22 times it was higher (Figure 1). Radically different values were observed in the years: 1969 – 162 mm (51% of the average precipitation in the period of many years) and in 1987 – 477 mm (150% of the norm). The largest monthly precipitation totals occurred most frequently in July (on average 67 mm) what is characteristic of the whole of Pomerania (CZARNECKA et al. 2004), then in June (64 mm), the lowest in April (37 mm) and in September (45 mm). The standard deviation, characterizing variability of precipitation in June and in July, amounted to about 36 mm in each month and it was by about 10 to 15 mm larger than in the other analyzed months. In comparison to the neighbouring Szczecin Lowland (KALBARCZYK, KALBARCZYK 2005) the Gorzów Plain was characterized in the vegetation period by larger differentiation of precipitation conditions. No statistically significant tendency of precipitation was observed for the studied period, nor was it observed for individual months.

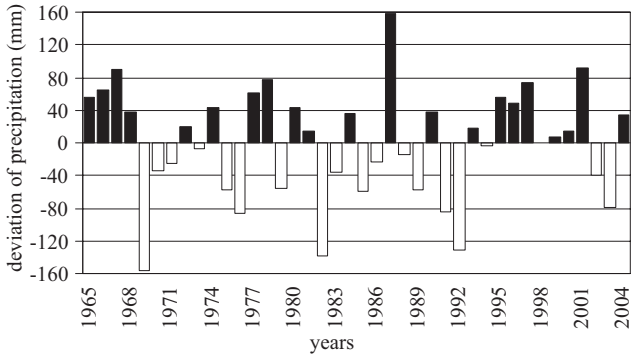


Fig. 1. Deviation of precipitation total on Gorzów Plain in the vegetative period (April-September) from a many-year average equal to 318 mm (in the years 1965-2004)

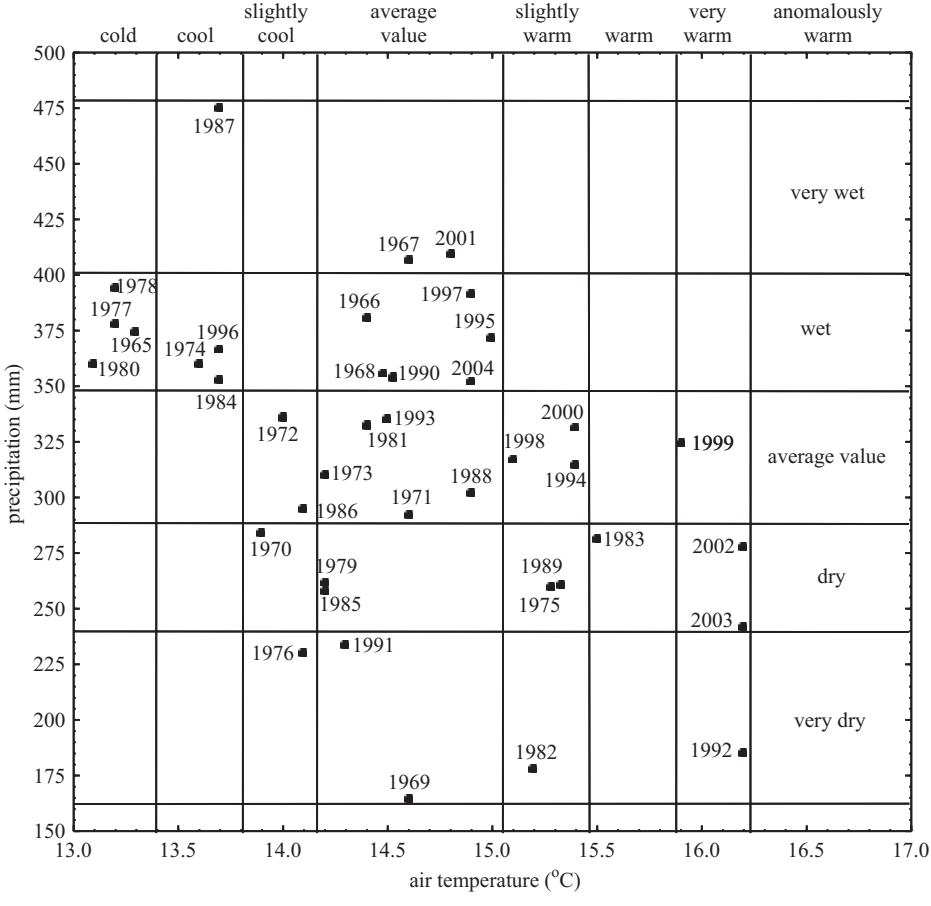


Fig. 2. Estimation of thermal and precipitation conditions on Gorzów Plain in the years 1965-2004

The analysis of the meteorological conditions in the vegetation period in individual years was carried out using a simultaneous classification of thermal conditions according to LORENC (2000) and precipitation conditions according to KACZOROWSKA (1962). In the years 1965-2004 the vegetation period with an average temperature exceeding the norm and with a simultaneous deficiency of precipitation occurred 7 times (Figure 2). The vegetation period in 1992 was very warm and at the same time it was very dry; in the years 2002 and 2003 it was very warm and dry; in 1983 – warm and dry; in 1982 – slightly warm and very dry; in 1975 and 1989 – slightly warm and dry. In the comparable period in the Szczecin Lowland simultaneously dry and warm vegetation periods occurred in the same years only in three cases: in the years 1975, 1982 and 1992 (KALBARCZYK, KALBARCZYK 2005).

On the basis of a similar analysis concerning individual months in the period from April to September it was observed that the months with a temperature exceeding the norm and with a simultaneous deficiency of precipitation occurred 35 times and it happened in three successive months in 1982 and 1992. Similarly, three successive warm and at the same time dry months occurred in the Szczecin Lowland in 1982 and 1992 (KALBARCZYK, KALBARCZYK 2005). RADZKA, KOC (2001) in their studies carried out for the town of Siedlce classified 1992 also as a dry year. Deficiency of precipitation with simultaneous surpluses of heat occurred most often in May and in July (9 times throughout 40 years), less rarely in April and in August (3 times throughout 40 years).

On the basis of the values of relative precipitation index (RPI) using KACZOROWSKA'S criteria (1962), of 40 analyzed vegetation periods 13 times precipitation conditions were observed to be below the norm, of which 8 times it was a dry period and 5 times – a very dry period, whereas no extremely dry period was observed (Figure 2). The number of months of different intensification drought in the analyzed many years' time in the vegetation period amounted on the whole to 81 and in a monthly system it varied from 11 in August to 16 in July. Thus in each month of the studied period a drought occurred on average every 2.5-3.5 years (Figure 3, Table 1). The most frequent occurrence of droughts in July is probably a local phenomenon as it can be seen in the results of the studies carried out in different regions of Poland, which are consistent, for example, with those for Szczecin and Siedlce (KALBARCZYK, KALBARCZYK 2005, RADZKA, KOC 2001), and different for the neighbourhood of Bydgoszcz or north-eastern areas of Poland (BANASZKIEWICZ et al. 2004, ŁABĘDZKI, BĄK 2004).

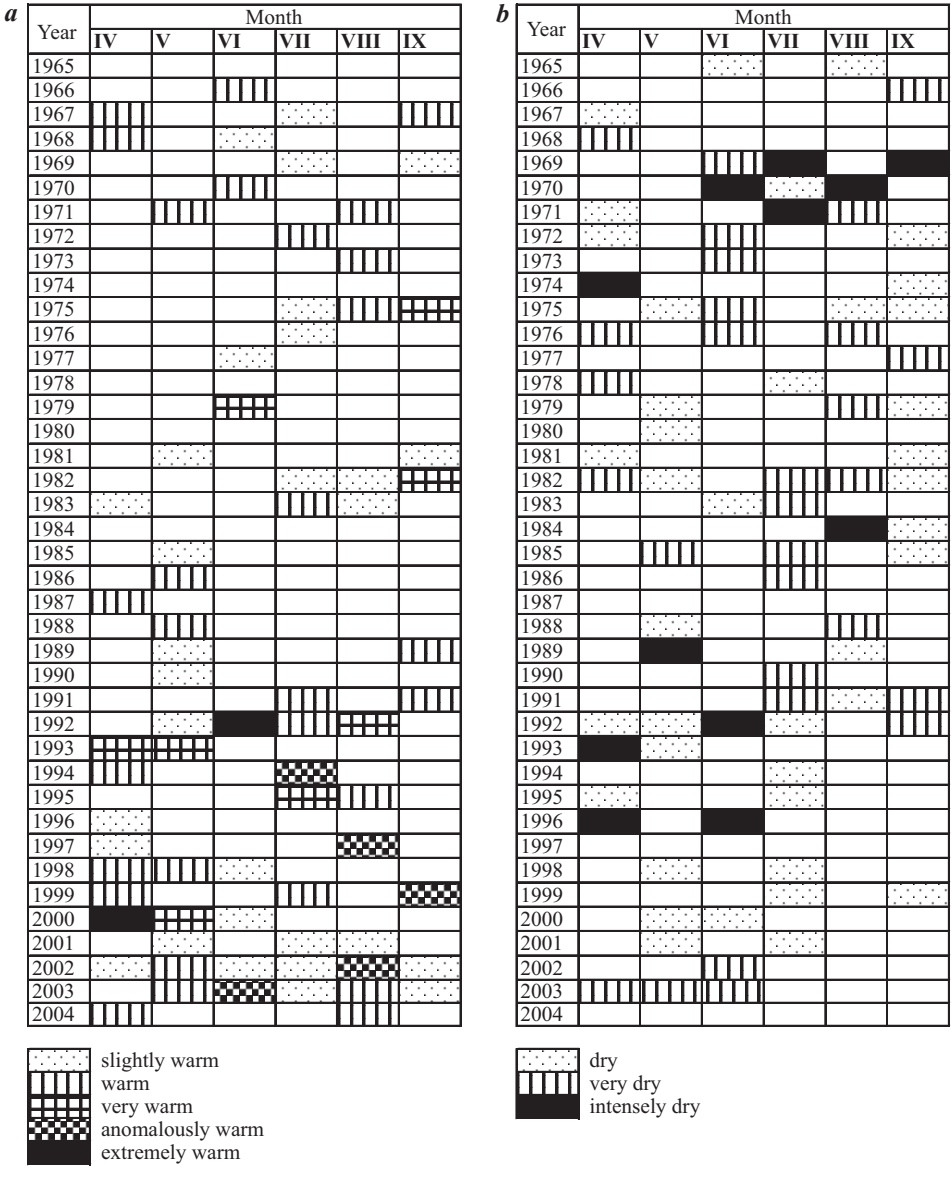


Fig. 3. Estimation of months of vegetation period on Gorzów Plain in the years 1965-2004: *a* – thermal conditions according to LORENC'S classification (2000), *b* – precipitation conditions according to KACZOROWSKA'S classification (1962)

Table 1
Frequency of atmospheric drought periods according to indices *RPI* and *K* on Gorzów Plain in the years 1965-2004

| Classification criterion | Period | Symbol | Month | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | IV | V | VI | VII | VIII | IX | Σ(IV-IX) |
| *RPI* Є<0,0; 25,0) | extremely dry | ss | 3 | 1 | 3 | 2 | 2 | 1 | 12 |
| Є<25,0; 50,0) | very dry | bs | 5 | 2 | 7 | 6 | 5 | 4 | 29 |
| Є<50,0; 75,0) | dry | s | 6 | 10 | 3 | 8 | 4 | 9 | 40 |
| | | Σ | 14 | 13 | 13 | 16 | 11 | 14 | **81** |
| *K* Є(0,0; 0,4> | extremely dry | ss | 3 | 2 | 5 | 5 | 6 | 3 | 24 |
| Є(0,4; 0,7> | very dry | bs | 2 | 5 | 7 | 6 | 5 | 7 | 32 |
| Є(0,7; 1,0> | dry | s | 6 | 9 | 3 | 6 | 14 | 12 | 50 |
| | | Σ | 11 | 16 | 15 | 17 | 25 | 22 | **106** |

Of 81 cases nearly a half was assessed as dry months (most frequently in May and in September), 36% of the cases – very dry months (most frequently in June and July). The remaining 15% of the cases were extremely dry months rarely occurring iın May and in September – only once in each of these months throughout 40 years; more often, i.e. twice in each of these months – in July and August; and the most frequently, i.e. three times – in April and in June.

The hydrothermal index *K* makes it possible to distinguish dry periods of three intensification degrees: dry, very dry and extremely dry. On the basis of the index *K* the months of different intensification of drought were determined 106 times. Nearly half of the cases (47%) were dry months, 30% – very dry months and 23% – extremely dry months (Table 1). In comparison with the classification according to *RPI* the number of the months assessed as extremely dry increased, whereas the number of the months classified as very dry, decreased.

Dry periods assessed by means of these criteria were most frequent in August and in September – they occurred more often than every two years; then every two years – from May to July; rarely in April – almost every three years and that is consistent with the differentiation of average monthly values of the index *K* of many year period for the whole of Poland (KALBARCZYK 2003), and it is partly consistent with the results obtained by RADZKA and KOC for Siedlce (2001). Analyzing individual classes of drought it can be noticed that a dry month was most often August, very dry – most often June and September, whereas extremely dry – most often August, and rarely – May.

The maximum number of dry months in the period from April to Septem-

ber in the same year amounted, according to both indices, to 5 (Figure 4). Five months, at the very least dry, during the vegetation period were observed twice – on the basis of the *RPI* index in 1982 and in 1992 and on the basis of the *K* index – also in 1982 and in 2003. According to the values of both indices no dry month occurred in 1987, nor did any in 1997 and 2004 according to *RPI*.

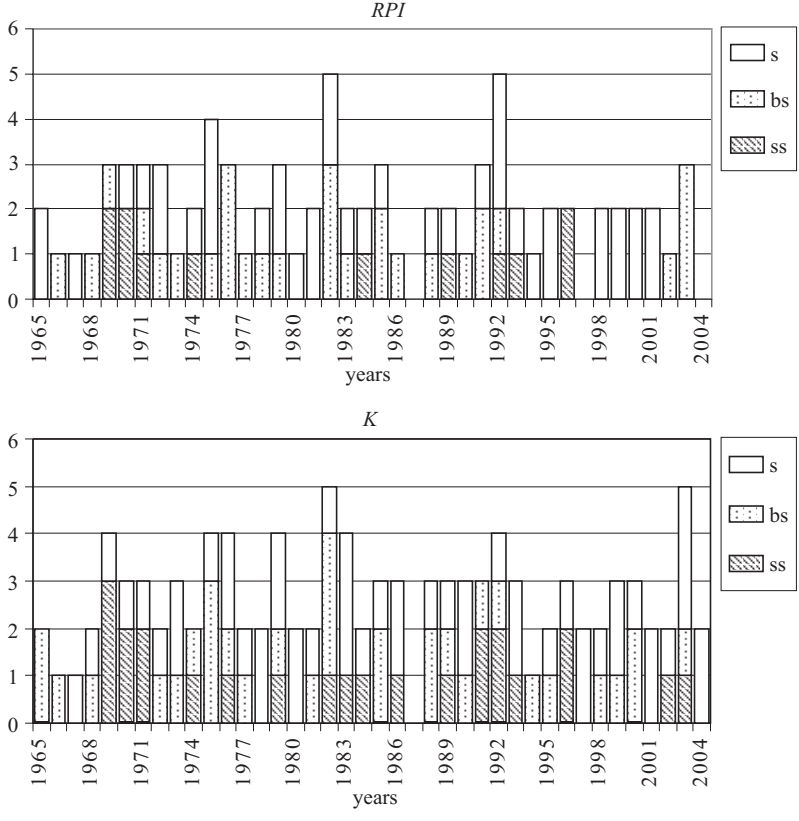


Fig. 4. Frequency of occurrence of periods: extremely dry (ss), very dry (bs), dry (s) from April to September on Gorzów Plain in the years 1965-2004 estimated according to indices *RPI* and *K*

In 1965-2004 no statistically significant tendency of the *RPI* index was observed in the years of the studies. Similarly, there are no significant time tendencies with regard to precipitation totals on the Gorzów Plain. As far as the whole of Pomerania is concerned, there were no distinct falling or rising

tendencies with regard to basic elements forming moisture conditions in the second half of the 20th century (CZARNECKA et al. 2004). However, the values of hydrothermal index show decreasing tendency with the passing of years. It is statistically significant at the level of $p < 0.1$ in May, what can indicate that there is an increasing risk of droughts in this month (Figure 5). May was characterized by the lowest value of climatic water balance, below – 40 mm (ROJEK 2004), as well as by the smallest relative air humidity (measured at 13.00) – below 55% (KOŹMIŃSKI 2004). Rainless periods lasting more than 15 days occur with frequency of about 40% (KOŹMIŃSKI, KALBARCZYK 2004).

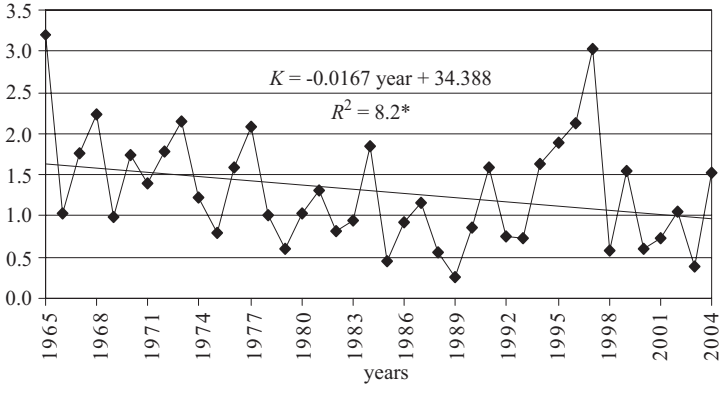


Fig. 5. Hydrothermal coefficients in May on Gorzów Plain in the years 1965-2004
* significant at $p < 0.1$

## Conclusions

1. On the Gorzów Plain atmospheric precipitation below an average of a many year period occur most often in July (an average every 2.5 years), least rarely – in August (on average every 3.5 years).

2. Nearly half of the months precipitation deficiency (determined according to Kaczorowska's classification) are dry months, 36% – very dry months, whereas 15% – extremely dry months.

3. The assessement of the hydrothermal conditions on the Gorzów Plain shows that droughts occur most frequently in August and in September (more often than every two years), least frequently – in April (on average every three years).

4. On the basis of the hydrothermal index *K* a larger number of dry periods was determined than by the index of relative precipitation *RPI*. According to the *K* index, as compared to the classification according to *RPI*, the number of

months considered as extremely dry was larger (up to 23%), whereas the number of very dry months decreased (30%).

5. During one vegetation period the occurrence of five months which were at the very least dry was observed twice – on the basis of the *RPI* index in 1982 and 1992, and on the basis of the *K* index – in 1982, but also in 2003.

6. In the years 1965-2004 no significant statistically tendency of the *RPI* index of variability. The values of hydrothermal index showed a decreasing tendency with the passing of years. It was significant statistically at the level of $p < 0.1$ in May what may indicate an increasing risk of the occurrence of dry periods in this month.

Translated by ANDRZEJ KAŁUSZYŃSKI

Accepted for print 7.11.2005

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 45-52, Y. 2006

# MINERAL COMPOSITION OF WHITE AND SAREPTA MUSTARD UNDER DIVERSIFIED CONDITIONS OF TOP DRESSING WITH NITROGEN COMPARED TO PRE-SOWING FERTILIZATION WITH SULPHUR AND MAGNESIUM

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K e y w o r d s: mustard seeds, white mustard, sarepta mustard, nitrogen, sulphur and magnesium fertilization, micro- and macroelements.

A b s t r a c t

The aim of the research was to determine the effect of diversified fertilization on the mineral composition of white and sarepta mustard seeds.

The experimental material included seeds of white and sarepta mustard obtained from experimental tillage with variable fertilization treatments: pre-sowing fertilization with sulphur and magnesium as well as different variants of top-dressing with nitrogen. The mustard seeds were determined for the contents of: macro- (Cu, Mn, Zn and Fe) and microelements (Ca, Mg,) with the atomic absorption spectrometry; Na and K – with an emission technique; P – with a colorimetric-molybdate method; and S – with a nephelometric method with $BaCO_3$.

In the mustard samples analyzed, the contents of copper and manganese were found not to depend on the diversified fertilization and accounted for 4.3-5.9 $\mu g \cdot g^{-1}$ d.m. and 20 to ca. 30 $\mu g \cdot g^{-1}$ d.m., respectively. The average content of Zn in the samples of white mustard seeds was nearly twice as high as that reported for the sarepta mustard seeds and did not depend on the fertilization applied.

The mean contents of iron ranged from ca. 70 $\mu g \cdot g^{-1}$ d.m. (sarepta mustard) to ca. 80 $\mu g \cdot g^{-1}$ d.m. (white mustard).

Compared to the seeds of sarepta mustard, those of the white mustard were characterized by a lower average content of sodium. The mean contents of potassium and magnesium in the seeds of the mustards compared were alike and ranged from 8.4 to 9.3 $mg \cdot g^{-1}$ d.m. and from 3.1 to 3.4 $mg \cdot g^{-1}$ d.m., respectively.

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Calcium and phosphorus contents of the mustard samples assayed were also alike and accounted for: Ca – 5.8 to 6.1 mg · g$^{-1}$ d.m. and P – ca. 9.5 mg · g$^{-1}$ d.m. in white mustard, and Ca – 5.6 to 6.5 mg · g$^{-1}$ d.m. and P – ca. 9.5 mg · g$^{-1}$ d.m. in sarepta mustard.

The mean contents of sulphur ranged from 10.2 to 11.6 mg · g$^{-1}$ d.m. in white mustard seeds and from 6.4 to 8.4 mg · g$^{-1}$ d.m. in sarepta mustard seeds.

**SKŁAD MINERALNY NASION GORCZYCY BIAŁEJ I SAREPSKIEJ W ZRÓŻNICOWANYCH WARUNKACH NAWOŻENIA AZOTEM, SIARKĄ I MAGNEZEM**

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S ł o w a k l u c z o w e: nasiona, gorczyca biała, gorczyca sarepska, nawożenie azotem, siarką i magnezem, mikro- i makroelementy.

A b s t r a k t

Celem podjętych badań było ustalenie wpływu zróżnicowanego nawożenia na skład mineralny nasion gorczycy białej i sarepskiej.

Materiał do badań stanowiły nasiona gorczycy białej i sarepskiej pozyskane z upraw doświadczalnych, na których stosowano zmienne nawożenie: przedsiewne siarką i magnezem oraz różne warianty nawożenia pogłównego azotem. W nasionach gorczycy oznaczono metodą spektrometrii absorpcji atomowej zawartość mikro- (Cu, Mn, Zn i Fe) i makroelementów (Ca, Mg); Na i K – techniką emisyjną; P – kolorymetryczną – molibdenianową oraz S – metodą nefelometryczną z $BaCO_3$. Wykazano, że zawartości miedzi i manganu w badanych próbach gorczycy nie zależała od zróżnicowanego nawożenia i wynosiła odpowiednio: 4,3-5,9 μg · g$^{-1}$ sm. i od 20 do ok. 30 μg · g$^{-1}$ sm. Natomiast średnia zawartość Zn w próbach nasion gorczycy białej była ok. 2-krotnie wyższa niż w nasionach gorczycy sarepskiej i nie zależała od stosowanego nawożenia.

Średnia zawartość żelaza mieściła się w granicach od ok. 70 μg · g$^{-1}$ sm. (gorczyca sarepska) do ok. 80 μg · g$^{-1}$ sm. (gorczyca biała). W nasionach gorczycy białej, w porównaniu z nasionami gorczycy sarepskiej, stwierdzono niższy średni poziom sodu. Średnia zawartości potasu i magnezu w nasionach porównywanych nasion była zbliżona i wynosiła odpowiednio: K – 8,4 – 9,3 mg · g$^{-1}$ sm. oraz Mg – 3,1 – 3,4 mg · g$^{-1}$ sm.

Zawartości wapnia i fosforu w ocenianych próbach gorczycy była zbliżona i wynosiła: gorczyca biała Ca – 5,8 – 6,1 mg · g$^{-1}$ sm., P – ok. 9,5 mg · g$^{-1}$ sm.; gorczyca sarepska Ca – 5,6 – 6,5 mg · g$^{-1}$ sm., P – ok. 9,5 mg · g$^{-1}$ sm.

Średnia zawartość siarki w nasionach gorczycy białej wahała się od 10,2 do 11,6 mg · g$^{-1}$ sm., natomiast w nasionach gorczycy sarepskiej od 6,4 do 8,4 mg · g$^{-1}$ sm.

## Introduction

Mustard is an annual crop belonging to the Brassicaceae family. It easily acclimatizes in different climatic zones, thus its cultivation has spread to almost all continents. Its most common representatives belonging to the

species *Sinapis* and *Brassica* include: white (*Sinapis alba*), black (*Brassica nigra*), and sarepta mustard (*Brassica yuncea*).

Mustard seeds have been applied not only in food processing, but also as a component of some medicines (KASZAK 1991). Used for the production of mustards, ketchups and as seasonings, they enrich the products with biologically active substances (essential oils, flavonoids, glucosinolates, etc.) as well as considerable amounts of minerals, including heavy metals.

The mineral composition of plants, its changes and ratios result from a number of factors, i.e. environment, species, development stage, cultivation and agrotechnological treatments applied (JĘDRZEJCZAK et al. 1999, KABATA-PENDIAS, PENDIAS 1999, MARKIEWICZ et al. 2002, WOJCIECHOWSKA-MAZUREK et al. 1995).

Of the components of fertilizers, a high impact on the yield and usability of seeds has been attributed to nitrogen (KOTECKI et al. 2001, KOTECKI 2002, WÓJTOWICZ, WIELEBSKI 2001), whereas fertilization with sulphur has been found indispensable for the wide availability of nitrogen and for increasing the content of alkene glucosinolates in seeds (WIELEBSKI, WÓJTOWICZ 2000, WIELEBSKI, WÓJTOWICZ 2003). As other cruciferous plants, mustard – which belongs to oil crops – requires high amounts of sulphur for proper growth and developments.

Fertilization with nitrogen is one of the key factors determining the chemical composition of mustard seeds, including their mineral composition.

The mineral composition of seeds affects, their nutritive value to a great extent, however, high contents of heavy metals are likely to disqualify them as processing raw material.

The aim of the study was to determine the effect of different variants of top-dressing with nitrogen, applied in comparison with pre-sowing fertilization with sulphur and magnesium, on the mineral composition of white and sarepta mustard seeds.

## Material and Methods

The experimental material included seeds of white and sarepta mustard obtained from plots with different variants of pre-sowing fertilization (sulphur and magnesium) and top-dressing (nitrogen). A field experiment was carried out in three series at the Production and Research Plant in Bałcyny near Ostróda. The split-plot method was employed, in three repetitions, with variable pre-sowing fertilization and top-dressing:

Pre-sowing fertilization:

(1) – sulphur, 30 kg · $ha^{-1}$;

(2) – magnesium, 6 kg · $ha^{-1}$;

top-dressing:

(a) – control (without nitrogen);

(b) – 30 kg N · $ha^{-1}$ (solid urea);

(c) – 25 kg N · $ha^{-1}$ (solid urea) + 5 kg N · $ha^{-1}$ (water solution of urea);

(d) – 60 kg N · $ha^{-1}$ (solid urea).

The level of pre-sowing fertilization with nitrogen, phosphorus and potassium was constant (60 kg N, 36 kg $P_2O_5$, and 65 kg $K_2O$). The pre-sowing fertilization was applied in the form of multicomponent fertilizers containing NPK and sulphur or magnesium. Top-dressing with solid urea was used at the beginning of plant budding, whereas foliar application was used for a 6% water solution of urea at full budding.

Each year, the experiment was established on typical grey-brown podsolic soil, medium silty, formed from light loam of a good wheat complex, with slightly acidic reaction (pH 6.1-6.2 in 1 M KCl). Soil fertility, expressed as mg of compounds per 100 g of soil, reached: 13.6-14.0 $P_2O_5$, 15.5-19.5 $K_2O$ and 9.7-10.1 Mg. Forecrop included cereals (winter wheat and triticale as well as spring barley) cultivated after a legume-cereal mixture. After the forecrop was harvested, after-harvest cultivation and autumn ploughing were carried out. Seeds of mustard were sown in the I and III decade of April, in the amount of: 120 germinating seeds of white mustard and 220 seeds of sarepta mustard per 1 $m^2$, on plots with an area of 7.5 $m^2$, in rows with 20-cm spacing. When the plants achieved technical maturity, harvest was performed in two stages.

In this experiment, the seeds of mustard were incinerated in evaporating quartz dishes at a temperature of 450°C, and the resulting white-grey ash was dissolved in 0.1 M $HNO_3$. The mineralizates obtained were determined for the contents of: Cu, Mn, Zn, Fe, Ca and Mg with atomic absorption spectrometry (WHITESIDE, MINER 1984); Na and K – with an emission technique (WHITESIDE, MINER 1984). Measurements were carried out on a Unicam 939 atomic absorption spectrometer equipped in data station ADAX, background correction and adequate cathode lamps. In the experimental seeds, the level of phosphorus was assayed with a colorimetric-molybdene method, whereas the concentration of sulphur was assayed with a nephelometric method with $BaCO_3$ (RUTKOWSKA et al. 1981).

## Results and Discussion

The results obtained (Table 1) demonstrate that in the mustard samples analysed, the content of copper ranged from 4.3 to 5.9 μg · $g^{-1}$ d.m and was not affected neither by diversified fertilization or mustard species. Likewise, no

tangible impact of fertilization and species was observed on mean levels of Mn, which ranged from ca. 20 to ca. 30 $\mu g \cdot g^{-1}$ d.m. of the mustard seeds. In contrast, the content of Zn was found to be highly affected by mustard species. The mean contents of Zn in white mustard samples ranging from 54.8 to 61.6 $\mu g \cdot g^{-1}$ d.m. were substantially higher than those reported in the seeds of sarepta mustard (28.8 to ca. 40 $\mu g \cdot g^{-1}$ d.m.), however, they did not depend on the fertilization applied.

Table 1

Contents of Cu, Zn, Mn and Fe ($\mu g \cdot g^{-1}$ d.m.) in the seeds of white mustard (WM) and sarepta mustard (SM)

| Specification | Cu | | | | Zn | | | | Mn | | | | Fe | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | fertilization | | | | | | | | | | | | | | | |
| | sulphur | | magnesium | | sulphur | | magnesium | | sulphur | | magnesium | | sulphur | | magnesium | |
| | control – without top-dressing nitrogen fertilization | | | | | | | | | | | | | | | |
| Year of study | WM | SM | WM | SM | WM | SM | WM | SM | WM | SM | WM | SM | WM | SM | WM | SM |
| 1 | 5.1 | 5.9 | 5.3 | 4.9 | 58.3 | 33.6 | 55.7 | 46.6 | 25.7 | 26.8 | 26.4 | 28.2 | 82.0 | 78.5 | 79.8 | 65.9 |
| 2 | 5.4 | 4.9 | 5.5 | 5.3 | 60.6 | 36.9 | 55.7 | 35.3 | 25.2 | 26.4 | 24.6 | 29.1 | 77.9 | 71.5 | 74.8 | 73.2 |
| 3 | 5.3 | 5.0 | 5.3 | 4.3 | 59.0 | 28.9 | 56.3 | 29.4 | 25.2 | 20.6 | 25.5 | 19.1 | 78.6 | 67.4 | 77.1 | 63.5 |
| Mean | 5.3 | 5.3 | 5.4 | 4.8 | 59.3 | 33.1 | 55.9 | 37.1 | 25.4 | 24.6 | 25.5 | 25.5 | 79.5 | 72.5 | 77.2 | 67.5 |
| SD | 0.2 | 0.6 | 0.1 | 0.5 | 1.2 | 4.0 | 0.3 | 8.7 | 0.3 | 3.5 | 0.9 | 5.5 | 2.2 | 5.6 | 2.5 | 5.1 |
| | Fertilization — 30 kg N $\cdot$ $ha^{-1}$ (solid urea) | | | | | | | | | | | | | | | |
| 1 | 5.2 | 5.0 | 5.3 | 5.0 | 60.5 | 38.4 | 60.6 | 35.1 | 25.7 | 25.9 | 25.3 | 30.6 | 79.5 | 66.3 | 79.7 | 65.9 |
| 2 | 5.5 | 5.1 | 5.5 | 5.3 | 54.8 | 39.6 | 58.3 | 37.6 | 25.3 | 30.9 | 25.8 | 26.5 | 81.7 | 73.9 | 77.7 | 69.0 |
| 3 | 5.4 | 5.0 | 5.5 | 4.1 | 58.2 | 28.9 | 59.1 | 30.5 | 26.2 | 21.6 | 25.7 | 19.9 | 80.2 | 69.3 | 77.9 | 61.0 |
| Mean | 5.4 | 5.0 | 5.4 | 4.8 | 57.8 | 35.6 | 59.3 | 34.4 | 25.7 | 26.1 | 25.6 | 25.7 | 80.5 | 69.8 | 78.4 | 65.3 |
| SD | 0.2 | 0.1 | 0.1 | 0.6 | 2.9 | 5.9 | 1.2 | 3.6 | 0.5 | 4.7 | 0.3 | 5.4 | 1.1 | 3.8 | 1.1 | 4.0 |
| | Fertilization – 25 kg N $\cdot$ $ha^{-1}$ (solid urea) + 5 kg N $\cdot$ $ha^{-1}$ (water solution of urea) | | | | | | | | | | | | | | | |
| 1 | 5.3 | 5.2 | 5.6 | 4.9 | 58.0 | 41.9 | 61.6 | 31.9 | 25.8 | 28.3 | 25.2 | 28.6 | 83.0 | 74.3 | 79.0 | 67.6 |
| 2 | 5.8 | 5.4 | 5.4 | 4.8 | 56.6 | 37.6 | 60.4 | 35.1 | 26.4 | 29.2 | 25.2 | 30.1 | 79.0 | 72.1 | 81.6 | 72.8 |
| 3 | 5.5 | 5.0 | 5.4 | 4.6 | 57.2 | 28.8 | 60.3 | 29.9 | 26.0 | 20.7 | 24.8 | 19.5 | 82.0 | 66.1 | 81.0 | 64.2 |
| Mean | 5.5 | 5.2 | 5.5 | 4.8 | 57.3 | 36.1 | 60.8 | 32.3 | 26.1 | 26.1 | 25.1 | 26.1 | 81.3 | 70.8 | 80.5 | 68.2 |
| SD | 0.3 | 0.2 | 0.1 | 0.2 | 0.7 | 6.7 | 0.7 | 2.6 | 0.3 | 4.7 | 0.2 | 5.7 | 2.1 | 4.2 | 1.4 | 4.3 |
| | Fertilization – 60 kg N $\cdot$ $ha^{-1}$ (solid urea) | | | | | | | | | | | | | | | |
| 1 | 5.4 | 4.9 | 5.2 | 5.1 | 59.9 | 39.4 | 59.1 | 33.6 | 25.9 | 27.0 | 26.1 | 30.9 | 83.8 | 72.8 | 86.6 | 68.9 |
| 2 | 5.5 | 5.0 | 5.2 | 4.9 | 58.3 | 37.9 | 59.1 | 34.0 | 26.7 | 29.0 | 26.0 | 27.0 | 85.9 | 74.8 | 83.4 | 67.1 |
| 3 | 5.4 | 4.6 | 5.3 | 4.6 | 58.6 | 34.1 | 58.3 | 29.7 | 26.4 | 22.4 | 25.8 | 20.6 | 84.4 | 66.6 | 85.1 | 65.8 |
| Mean | 5.4 | 4.8 | 5.2 | 4.9 | 58.9 | 37.1 | 58.8 | 32.4 | 26.3 | 26.1 | 26.0 | 26.2 | 84.7 | 71.4 | 85.0 | 67.3 |
| SD | 0.1 | 0.2 | 0.1 | 0.3 | 0.9 | 2.7 | 0.5 | 2.4 | 0.4 | 3.4 | 0.2 | 5.2 | 1.1 | 4.3 | 1.6 | 1.6 |

In the mustards seeds analyzed, the mean concentrations of iron fluctuated from ca. 70 $\mu g \cdot g^{-1}$ d.m. (sarepta mustard) to ca. 80 $\mu g \cdot g^{-1}$ d.m. (white mustard).

The results compiled in Table 2 indicate that the seeds of white mustard were characterized by a lower level of sodium (31.9-35.3 $\mu g \cdot g^{-1}$ d.m.), compared to the sarepta mustard seeds in which its level ranged from 37.6 to 42.3 $\mu g \cdot g^{-1}$ dm.

In the compared samples of mustard seeds, the mean contents of potassium and magnesium were alike and accounted for 8.4-9.3 $mg \cdot g^{-1}$ d.m. and 3.1--3.4 $mg \cdot g^{-1}$ d.m., respectively.

Table 2

Contents of Na, K and Mg in the seeds of white mustard (WM) and sarepta mustard (SM)

| Specification | Na ($\mu g \cdot g^{-1}$ d.m.) | | | | K ($mg \cdot g^{-1}$ d.m.) | | | | Mg ($mg \cdot g^{-1}$ d.m.) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | fertilization | | | | | | | | | | | |
| | sulphur | | magnesium | | sulphur | | magnesium | | sulphur | | magnesium | |
| | control – without top-dressing nitrogen fertilization | | | | | | | | | | | |
| Year of study | WM | SM | WM | SM | WM | SM | WM | SM | WM | SM | WM | SM |
| 1 | 33.0 | 37.1 | 31.2 | 44.0 | 8.5 | 9.1 | 8.2 | 9.2 | 3.6 | 3.5 | 3.2 | 3.6 |
| 2 | 37.1 | 39.9 | 36.1 | 39.5 | 8.7 | 8.4 | 8.6 | 9.2 | 3.2 | 2.8 | 3.2 | 3.2 |
| 3 | 35.8 | 35.7 | 34.8 | 35.9 | 8.5 | 8.9 | 8.5 | 8.6 | 3.3 | 3.1 | 3.1 | 3.2 |
| Mean | 35.3 | 37.6 | 34.0 | 39.8 | 8.6 | 8.8 | 8.4 | 9.0 | 3.4 | 3.1 | 3.2 | 3.3 |
| SD | 2.1 | 2.1 | 2.5 | 4.1 | 0.1 | 0.4 | 0.2 | 0.3 | 0.2 | 0.4 | 0.1 | 0.2 |
| | Fertilization – 30 kg $N \cdot ha^{-1}$ (solid urea) | | | | | | | | | | | |
| 1 | 35.6 | 41.5 | 33.2 | 47.2 | 8.7 | 8.7 | 8.3 | 9.5 | 3.3 | 3.6 | 3.3 | 3.6 |
| 2 | 33.7 | 37.2 | 35.6 | 41.5 | 8.7 | 8.9 | 9.0 | 8.6 | 3.3 | 3.0 | 3.4 | 3.3 |
| 3 | 34.1 | 35.8 | 34.1 | 38.2 | 8.4 | 8.6 | 8.7 | 8.9 | 3.5 | 3.2 | 3.4 | 3.2 |
| Mean | 34.5 | 38.2 | 34.3 | 42.3 | 8.6 | 8.7 | 8.7 | 9.0 | 3.4 | 3.3 | 3.4 | 3.4 |
| SD | 1.0 | 3.0 | 1.2 | 4.6 | 0.2 | 0.2 | 0.4 | 0.5 | 0.1 | 0.3 | 0.1 | 0.2 |
| | Fertilization – 25 kg $N \cdot ha^{1}$ (solid urea)+ 5 kg $N \cdot ha^{-1}$ (water solution of urea) | | | | | | | | | | | |
| 1 | 30.6 | 39.6 | 34.5 | 41.0 | 8.5 | 9.0 | 8.4 | 8.8 | 3.2 | 3.2 | 3.3 | 3.7 |
| 2 | 33.1 | 46.3 | 36.4 | 38.0 | 8.6 | 8.6 | 8.9 | 9.0 | 3.1 | 3.0 | 3.1 | 3.1 |
| 3 | 32.0 | 35.6 | 35.0 | 39.7 | 8.6 | 8.7 | 8.6 | 9.2 | 3.1 | 3.2 | 3.3 | 3.2 |
| Mean | 31.9 | 40.5 | 35.3 | 39.6 | 8.6 | 8.8 | 8.6 | 9.0 | 3.1 | 3.1 | 3.2 | 3.3 |
| SD | 1.3 | 5.4 | 1.0 | 1.5 | 0.1 | 0.2 | 0.3 | 0.2 | 0.1 | 0.1 | 0.1 | 0.3 |
| | Fertilization – 60 kg $N \cdot ha^{-1}$ (solid urea) | | | | | | | | | | | |
| 1 | 32.8 | 41.4 | 32.3 | 42.7 | 8.4 | 9.5 | 8.5 | 9.5 | 3.2 | 3.6 | 3.3 | 3.6 |
| 2 | 35.3 | 40.6 | 33.8 | 41.6 | 8.8 | 9.1 | 8.8 | 9.2 | 3.5 | 3.5 | 3.0 | 3.1 |
| 3 | 34.2 | 38.3 | 33.5 | 41.6 | 8.7 | 8.8 | 8.7 | 9.3 | 3.3 | 3.1 | 3.1 | 3.3 |
| Mean | 34.1 | 40.1 | 33.2 | 42.0 | 8.6 | 9.1 | 8.7 | 9.3 | 3.3 | 3.4 | 3.1 | 3.3 |
| SD | 1.3 | 1.6 | 0.8 | 0.6 | 0.2 | 0.4 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.3 |

As in the case of K and Mg, the mean levels of calcium and phosphorus (Table 3) were alike and ranged from 5.8 to 6.1 mg · g$^{-1}$ d.m. and ca. 9.5 mg · g$^{-1}$ d.m. in white mustard, and from 5.6 to 6.5 mg/g d.m. and ca. 9.5 mg · g$^{-1}$ d.m., respectively.

In comparing the levels of sulphur it was stated that, compared to the sarepta mustard, the seeds of white mustard were definitely richer in this element. The mean content of sulphur in the seeds of white mustard ranged from 10.2 to 11.6 mg · g$^{-1}$ d.m., whereas that in the seeds of sarepta mustard – from 6.4 to 8.4 mg · g$^{-1}$ d.m. It should be emphasized that the sulphur content

Table 3

Contents of Ca, P and S (mg · g$^{-1}$ d.m.) in the seeds of white mustard (WM) and sarepta mustard (SM)

| Specification | Ca | | | | P | | | | S | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | fertilization | | | | | | | | | | | |
| | sulphur | | magnesium | | sulphur | | magnesium | | sulphur | | magnesium | |
| | control – without top-dressing nitrogen fertilization | | | | | | | | | | | |
| Year of study | WM | SM | WM | SM | WM | SM | WM | SM | WM | SM | WM | SM |
| 1 | 5.7 | 5.4 | 5.9 | 6.0 | 9.9 | 9.1 | 9.6 | 9.8 | 11.5 | 7.8 | 10.4 | 8.2 |
| 2 | 6.0 | 6.5 | 6.2 | 6.4 | 9.3 | 9.7 | 9.6 | 9.6 | 11.2 | 9.1 | 10.2 | 8.0 |
| 3 | 5.9 | 5.4 | 6.2 | 5.4 | 9.5 | 9.3 | 9.8 | 9.3 | 11.4 | 7.3 | 10.2 | 7.0 |
| Mean | 5.9 | 5.8 | 6.1 | 5.9 | 9.6 | 9.4 | 9.7 | 9.6 | 11.4 | 8.1 | 10.3 | 7.7 |
| SD | 0.2 | 0.6 | 0.2 | 0.5 | 0.3 | 0.3 | 0.1 | 0.3 | 0.2 | 0.9 | 0.1 | 0.6 |
| | Fertilization – 30 kg N · ha$^{-1}$ (solid urea) | | | | | | | | | | | |
| 1 | 5.8 | 5.6 | 5.7 | 5.8 | 9.8 | 9.8 | 9.7 | 9.5 | 11.8 | 8.7 | 10.8 | 8.2 |
| 2 | 6.0 | 6.3 | 6.4 | 6.5 | 9.3 | 9.6 | 9.8 | 9.7 | 11.4 | 9.0 | 9.9 | 7.0 |
| 3 | 6.0 | 5.5 | 5.9 | 7.1 | 9.6 | 9.3 | 9.8 | 9.3 | 11.7 | 7.6 | 10.0 | 6.5 |
| Mean | 5.9 | 5.8 | 6.0 | 6.5 | 9.6 | 9.6 | 9.8 | 9.5 | 11.6 | 8.4 | 10.2 | 7.2 |
| SD | 0.1 | 0.4 | 0.4 | 0.7 | 0.3 | 0.3 | 0.1 | 0.2 | 0.4 | 0.7 | 0.5 | 0.9 |
| | Fertilization – 25 kg N · ha$^{-1}$ (solid urea) + 5 kg N · ha$^{-1}$ (water solution of urea) | | | | | | | | | | | |
| 1 | 5.8 | 4.9 | 5.8 | 6.0 | 9.8 | 9.4 | 9.8 | 9.5 | 10.6 | 9.3 | 11.4 | 7.2 |
| 2 | 6.0 | 6.3 | 6.1 | 6.5 | 9.2 | 9.9 | 9.7 | 9.3 | 11.1 | 8.1 | 10.1 | 6.0 |
| 3 | 5.7 | 5.7 | 6.0 | 5.5 | 9.7 | 9.3 | 9.7 | 9.5 | 10.9 | 7.5 | 10.7 | 5.9 |
| Mean | 5.8 | 5.6 | 6.0 | 6.0 | 9.6 | 9.5 | 9.7 | 9.4 | 10.9 | 8.3 | 10.7 | 6.4 |
| SD | 0.2 | 0.7 | 0.2 | 0.5 | 0.3 | 0.3 | 0.1 | 0.1 | 0.3 | 0.9 | 0.7 | 0.7 |
| | Fertilization – 60 kg N · ha$^{-1}$ (solid urea) | | | | | | | | | | | |
| 1 | 5.8 | 5.8 | 5.7 | 6.0 | 9.6 | 9.6 | 9.8 | 9.4 | 12.2 | 9.2 | 12.0 | 7.2 |
| 2 | 5.6 | 6.4 | 6.1 | 6.5 | 9.3 | 9.9 | 9.9 | 9.8 | 10.9 | 8.5 | 9.8 | 6.2 |
| 3 | 5.6 | 5.7 | 5.8 | 5.8 | 9.5 | 9.6 | 9.7 | 9.8 | 11.1 | 7.3 | 10.9 | 7.0 |
| Mean | 5.7 | 6.0 | 5.9 | 6.1 | 9.5 | 9.7 | 9.8 | 9.7 | 11.4 | 8.3 | 10.9 | 6.8 |
| SD | 0.1 | 0.4 | 0.2 | 0.4 | 0.2 | 0.2 | 0.1 | 0.2 | 0.7 | 1.0 | 1.1 | 0.5 |

of the seeds of both white and sarepta mustard fertilized with sulphur was substantially higher than that reported for mustards fertilized with magnesium (Table 3).

## Conclusions

1. Taking into account that the mustard species analyzed were cultivated under identical conditions and with the same agrotechnological treatments, the high differences observed in the concentrations of elements, especially Zn, but also Fe and S, may be acknowledged as a species-specific trait.

2. The variable top-dressing with nitrogen applied in the experiment had no effect on the accumulation of elements assayed, whereas pre-sowing fertilization with sulphur and magnesium was found to affect only a distinctively higher concentration of sulphur in the seeds of mustards fertilized with sulphur.

Translated by Joanna Jensen

Accepted for print 23.01.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 53-65, Y. 2006

# PRODUCTION OF WILLOW (*SALIX* SPP.) BIOMASS ON ARABLE LAND IN SHORT-TERM HARVESTING CYCLES

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K e y w o r d s: willow (*Salix* spp.), clones, yield of wood dry substance, harvesting frequency.

A b s t r a c t

The study attempted at identifying the productivity and morphological features of seven clones of willow, cultivated on arable land and harvested every 1, 2 and 3 years. The average yield of dry substance of the wood harvested in the experiment was 15.12 $t \cdot ha^{-1} \cdot year^{-1}$. A high yield of wood was obtained from *Salix viminalis* JORR and *Salix viminalis* 1023 clones: 17.31 and 17.20 $t \cdot ha^{-1}$ $year^{-1}$, respectively. The willow harvested in the three-year cycle gave the significantly highest yield of the dry substance of wood (18.76 $t \cdot ha^{-1}$ $year^{-1}$), which was higher by about 40% than that obtained in a one-year cycle (11.33 $t \cdot ha^{-1}$ $year^{-1}$). The average height of the trees in the one-year cycles was 264.4 cm, whereas in the 2- and 3-year cycle it was 380.0 cm and 567.2 cm, respectively. The stalk diameter increased as the harvesting cycle lengthened and was 13.3 mm, 20.0 mm and 32.5 mm for one, two- and three-year cycles, respectively.

## PRODUKCJA BIOMASY WIERZBY KRZEWIASTEJ (*Salix* spp.) NA GRUNTACH ORNYCH W KRÓTKICH ROTACJACH

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S ł o w a k l u c z o w e: wierzba krzewiasta (*Salix* spp.), klony, plon suchej masy drewna, częstotliwość zbioru.

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Abstrakt

Określono produktywność oraz cechy morfologiczne 7 klonów wierzby krzewiastej uprawianych na gruntach rolniczych i zbieranych w krótkich 1-, 2- i 3-letnich rotacjach. Plon suchej masy drewna wierzby w doświadczeniu wyniósł średnio 15,12 t · ha$^{-1}$ · rok$^{-1}$. Wysoki plon drewna dały klony *Salix viminalis* JORR oraz *Salix viminalis* 1023, odpowiednio 17,31 i 17,20 t · ha$^{-1}$ rok$^{-1}$. Istotnie najwyższy plon suchej masy drewna (18,76 t · ha$^{-1}$ rok$^{-1}$) otrzymano z wierzby pozyskiwanej w 3-letnim cyklu zbioru, i był on o ok. 40% wyższy niż z cyklu jednorocznego (11,33 t · ha$^{-1}$ rok$^{-1}$). Wysokość roślin wierzby w jednorocznych cyklach zbioru wynosiła śr. 264,4 cm, natomiast w cyklu 2- i 3-letnim odpowiednio 380,0 cm i 567,2 cm. Średnica pędu wierzby wzrastała wraz z wydłużaniem cyklu zbioru od jednorocznego do 2- i 3-letniego i wynosiła odpowiednio 13,3 mm, 20,0 mm i 32,5 mm.

## Introduction

The prospect of exhaustion of the resources of fossil fuels has sparked interest in renewable sources of energy in the European Union (UE-25).

In Poland, biomass is currently obtained mainly from wastes: from forests, the wood processing industry, urban greenery and, in small amounts, from sorted organic communal waste. In the future it could be supplemented by wood obtained from perennial energetic plants: willow – *Salix* spp., giant Chinese silver grass – *Miscanthus x giganteus*, Virginia fanpetals – *Sida hemaphrodita* Rusby (SZCZUKOWSKI, TWORKOWSKI 2003, JEŻOWSKI 2003). The greatest possibility seems to be provided by the native willow species. Their cultivation could be based, in part, on fallowed arable land ca 1.4 mln ha (GUS 2005).

Intensive research is being conducted in many countries into the possibilities of cultivating willow on arable land (LABRECQUE et al. 1997, 2003, HOFFMANN, WEIH 2005, KOPP et al. 1997, 2001, RANDERSON et al. 2000, BULLARD et al. 2002, SZCZUKOWSKI et al. 2002, 2005, SZCZUKOWSKI, TWORKOWSKI 2001). In the cited experiments, the yield of the wood dry substance ranged from a few to about 30 t · ha$^{-1}$ · year$^{-1}$ depending on the species, site, agricultural conditions and harvesting frequency.

Obtaining the lignin-cellulose willow biomass from agricultural land and its use for energy production is important from a global perspective for two reasons: it reduces the greenhouse effect and it is a source of biological carbon which is an alternative option for fossil fuels (coal, gas and crude oil). In the regional dimension it makes agriculture a producer of food as well as that of energy.

This study was aimed at determining the yield of dry substance of wood and the morphological features of seven clones of willow obtained in short, 1-, 2- and 3-year cycles.

## Methods

A field experiment was set up in the 3rd decade of April 1999 in Obory, in the Lowlands of Kwidzyn in the old valley of the Vistula, on medium-heavy alluvial soil.

The first factor of the experiment were seven clones of *Salix* spp.: *Salix viminalis* x *Salix purpurea* (1001 – the clone number in the collection), *Salix cordata* (1019), *Salix viminalis* (1023), *Salix viminalis* (1033), *Salix viminalis* var. *gigantea* (1047), *Salix viminalis* JORR, *Salix viminalis* ULV.

The second factor (b) was the frequency of plant harvesting: every year, every two and three years.

The experiment was set up in four replications. It included 84 plots, each with an area of 21.78 $m^2$ (7.26 x 3.0 m). Willow cuttings were planted with a density of 40 thousand per 1 hectare, which corresponded to the spacing of 0.75 x 0.33 m.

In the first decade of 1998, the year directly preceding the setting up of the experiment, Roundup herbicide was applied in the dose of 5 $dm^3 \cdot ha^{-1}$ in order to destroy weeds. The field was then disked and potassium fertilisers as a potassium salt were sown in the dose of 120 $kg \cdot ha^{-1}$ $K_2O$. In the III decade of October 1998 35-cm deep autumn ploughing was performed. In the first decade of April 1999 the field was levelled. No mineral fertilisers were applied. 25-cm long willow cuttings were planted in the third decade of April. This was followed by applying the Bladex 500 SC herbicide in the dose of 4 $dm3 \cdot ha^{-1}$. In addition, in order to control secondary weed infestation, manual weeding was performed twice. In the subsequent years of the experiment no cultivation was performed. In the second year, and in subsequent years, mineral fertilisers were sown on all the plots before the beginning of vegetation: N – 40 $kg \cdot ha^{-1}$, $P_2O_5$ – 40 $kg \cdot ha^{-1}$, $K_2O$ – 80 $kg \cdot ha^{-1}$ as ammonium saltpetre, superphosphate and potassium salt.

All stalks from all the plots were harvested in the 3rd decade of December 1999 in order to stimulate the growth and expansion of the stalks in subsequent years. The experiment in the first year (1999) was treated as preliminary and its results are not presented in this paper. The years 2000-2002 were treated as productive and the results for them were presented in a factor arrangement. From 2000, the plants were harvested three times in a one-year cycle (2000, 2001, 2002), once in a two-year cycle (2001) and once in a three-year one (2002). The values of biometric features of the plants obtained in the one-year cycles are presented as mean values from the years 2000-2002.

Before the harvest and after the vegetation period had ended, the number of plants on a plot was determined. To this end, the plants in the second and third rows were counted, then the number of plants in the plot was calculated

and converted to an area of 1 ha. In addition, 10 plants (2-3 from a row) were randomly chosen and their height and stalk diameters were measured (the measurement was taken 10 cm above the soil level). The number of shoots on a plant was also determined.

The harvest of willows was done with a mechanical cutter in the third decade of January. Directly after the harvest, all the plants obtained from the plots were weighed and the yield from 1 ha was calculated. Representative samples were then taken in order to determine the humidity content in wood. This was determined in a laboratory by drying at 105°C until the weight remained constant. The dry substance yield was then calculated.

The results were analysed statistically with a STATISTICA® 6,0 PL software pack. The mean values were calculated for all the measured features. The Student-Duncan test of significance was used to determine the NIR values for both factors and their combined effect at the level of significance of $p = 0.05$. A regression analysis was also performed for selected features.

## The soil at site and the meteorological condition

The experiment was conducted on alluvial soil in the old valley of the Vistula River, in the Kwidzyn Lowlands. The tests done on the soil classify it as medium heavy alluvial soil. The pH value in 1 M KCl was slightly acidic – 6.4. The $P_2O_5$ and Mg content in the soil was very high: 29.9 mg and 17.1 mg · 100 $g^{-1}$ of the soil, respectively, and that of $K_2O$ – low (10.5 mg · 100 $g^{-1}$ of the soil). The humus content was high – 5.4%. In general, in terms of the soil quality, the site must be considered favourable to the growth of willow.

The meteorological conditions in the year when the experiment was set up (1999) favoured the rooting of willow cuttings (Tables 1, 2). The air temperature and rainfall in April were higher by 1.7°C and 66.4 mm, respectively, than in that month throughout a long-term period (1961-1990). The total rainfall during the vegetation period of 1999 was very high and amounted to 543.2 mm. The rainfall in May (69.1 mm) and June (155.6 mm) was also higher than the average value for the thirty-year period, which favoured the rooting and growth of plants during its initial stages. In 2000, the first "productive" year, the average air temperature during the vegetation period was 13.1°C and was higher by 0.4°C than the average value from the thirty-year period. The total rainfall was higher by 36.8 mm than the corresponding value from the years 1961-1990. In April, May and June 2000, the average air temperature was higher and the rainfall was lower than in the long-term period. There was a humidity deficit in the soil, which limited the growth of willow, observed especially in the clones of the *Salix viminalis* species. Frequent and abundant

rains in July and August supplemented the water deficit and were followed by further secondary growth of willows; however, the yield of biomass in that year was low. In 2001, the average air temperature during the vegetation period was higher by 0.7°C than the average value in the corresponding period in the years 1961-1990. The air temperature and rainfall in July and August in that year was much higher than in the thirty-year period. The highest rainfall was recorded in July – 135.1 mm; it accounted to 28% of the total rainfall during the vegetation period of 2001. The air temperature and rainfall during the vegetation period of 2002 was higher by 1.2°C and 77.4 mm, respectively, than the corresponding values in the thirty-year period. That was a warm and humid year, which provided favourable conditions for the growth and development of willows.

Table 1
Air temperatures during the vegetation period (data from the Meteorological Station in Bałcyny)

| Year | Average monthly air temperatures (°C) | | | | | | | Average temperature for 1 IV to 31 X |
|---|---|---|---|---|---|---|---|---|
| | IV | V | VI | VII | VIII | IX | X | |
| 1999 | 8.3 | 11.1 | **16.7** | 19.1 | 16.9 | 15.3 | 7.9 | 13.6 |
| 2000 | 10.9 | 13.5 | 15.9 | 15.3 | 16.9 | 11.2 | 8.4 | 13.1 |
| 2001 | 7.3 | 12.2 | 13.8 | 19.5 | 18.4 | 12.0 | 10.5 | 13.4 |
| 2002 | 7.3 | 16.1 | 15.9 | 19.3 | 19.8 | 12.5 | 6.4 | 13.9 |
| Average 1961-1990 | 6.6 | 12.4 | 15.7 | 16.9 | 16.5 | 12.6 | 8.1 | 12.7 |

Table 2
Rainfall during the vegetation period (data from the Meteorological Station in Bałcyny)

| Year | Total monthly rainfall (mm) | | | | | | | Total for the period from for 1 IV to 31 X |
|---|---|---|---|---|---|---|---|---|
| | IV | V | VI | VII | VIII | IX | X | |
| 1999 | 101.6 | 69.1 | 155.6 | 75.5 | 53.0 | 18.4 | 70.0 | 543.2 |
| 2000 | 20.2 | 32.5 | 33.1 | 104.2 | 140.9 | 46.8 | 89.8 | 467.5 |
| 2001 | 43.5 | 31.3 | 48.8 | 135.1 | 81.8 | 99.3 | 35.1 | 474.9 |
| 2002 | 10.0 | 90.1 | 72.5 | 43.2 | 87.3 | 60.5 | 144.5 | 508.1 |
| Average 1961-1990 | 35.2 | 56.7 | 68.3 | 81.3 | 78.1 | 57.1 | 54.0 | 430.7 |

## Results

An average of 10.5% of plants were lost in relation to the initial 40 thousand cuttings planted per hectare (Figure 1). The average loss in the one-year cycle was 6.1%. The highest loss of willows in a one-year cycle was found in

*Salix viminalis* var. *gigantea* – 14.2%. The loss in the two-year cycle increased by 4% as compared to the one-year cycle, whereas further prolongation of the harvest cycle from two to three years resulted in an increase in the loss by 5.2%. The highest loss was recorded in *Salix cordata,* and the lowest – in *Salix viminalis* (1023).

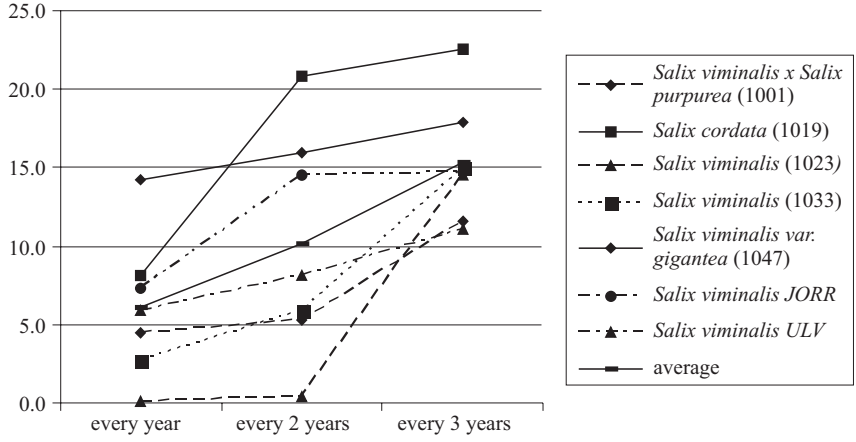


Fig. 1. The loss of willow plants in a one- two- and three-year harvesting cycles

Table 3

Number of stalks on a willow in relation to the length of the harvesting cycle

| Clone name and number in the collection (a) | Harvesting frequency (b) | | | Average |
|---|---|---|---|---|
| | every year | every 2 years | every 3 years | |
| *Salix viminalis* x *Salix purpurea* (1001) | 7.2 | 6.0 | 2.8 | 5.3 |
| *Salix cordata* (1019) | 5.4 | 6.3 | 3.9 | 5.2 |
| *Salix viminalis* (1023) | 6.6 | 4.3 | 2.4 | 4.4 |
| *Salix viminalis* (1033) | 5.8 | 4.4 | 1.9 | 4.0 |
| *Salix viminalis* var. *gigantea* (1047) | 6.7 | 5.1 | 3.2 | 5.0 |
| *Salix viminalis* JORR | 4.9 | 4.4 | 2.0 | 3.8 |
| *Salix viminalis* ULV | 6.0 | 5.4 | 2.2 | 4.5 |
| Average | 6.1 | 5.1 | 2.6 | 4.6 |
| $LSD_{0.05}$ | a-0.86 | b-0.56 | axb-ni | |

The average number of sprouts on a plant in the experiment was 4.6 (Table 3). The largest number of sprouts per plant was found in *Salix viminalis* x *Salix purpurea* – 5.3, with a similar number found in *Salix cordata* and *Salix viminalis* var. *gigantea*. The lowest number of sprouts per plant was

yielded by *Salix viminalis* JORR – 3.8. The number of sprouts in plants harvested in a one-year cycle was significantly the highest – 6.1 – and decreased with the prolongation of the harvesting cycle. The reduction in the sprout number with the prolongation of the harvesting cycle was caused by natural self-regulation. When willows were harvested in a three-year cycle, numerous dried sprouts were observed in the lower parts of plants. In consequence, the number of sprouts on a plant in a three-year cycle decreased by half as compared to the annual cycle. The clone *Salix viminalis* JORR tended to create a smaller number of sprouts on a plant as compared to the other clones in all the harvesting cycles.

The height of willows harvested every year was only 264.4 cm. The plants harvested every two years were 380.0 cm high, and those harvested every three years were 567.2 cm (Figure 2). When the plants were harvested every year, the significantly lowest plants were those of the *Salix viminalis* ULV clone (229.4 cm), whereas the highest were those of the *Salix viminalis* (1023) clone (303.5 cm). The height of plants in the three-year cycle ranged from 475.0 cm in the *Salix cordata* clone to 617.2 cm in *Salix viminalis* JORR.

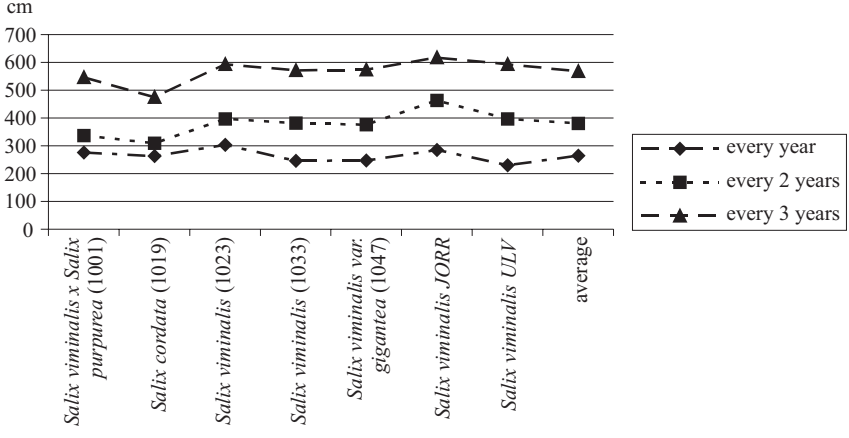


Fig. 2. The height of willows in a one-, two- and three-year harvesting cycle

Figure 3 shows the relationship between the height of plants and the number of sprouts on a plant in the harvesting cycles under study. The prolongation of the harvesting cycles resulted in increasing the plant height, but at the same time it reduced the number of sprouts per plant. A similar relationship was observed between the stalk diameter and the number of sprouts per plant.

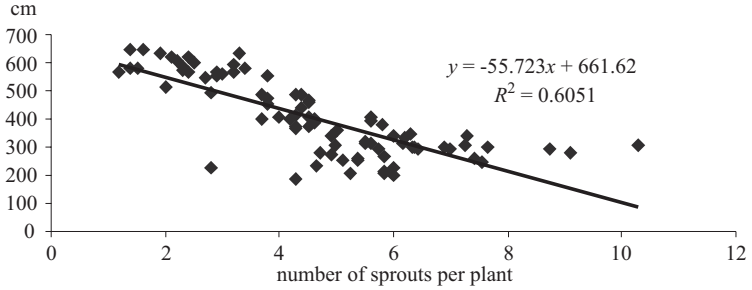


Fig. 3. The relationship between plant height and the number of sprouts per plant

The diameter of a willow stalk in the plants harvested every year was 13.3 mm and was significantly lower than in those harvested every two (20.0 mm) and three (32.5 mm) years (Figure 4). The stalk diameter in the plants harvested every year ranged from 12.5 mm in *Salix viminalis* x *Salix purpurea* to 14.7 mm in *Salix viminalis* JORR. In the plants harvested every two years, the largest diameter was found in *Salix viminalis* JORR (24.9 mm). *Salix cordata* and *Salix viminalis* x *Salix purpurea* had the stalk diameter significantly lower than the other clones.

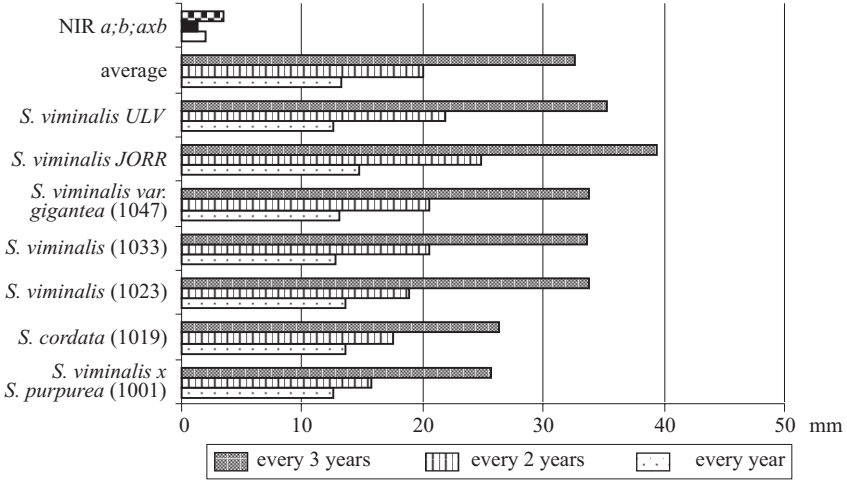


Fig. 4. The willow stalk diameter (mm) in relation to the harvesting frequency

The highest productivity in willow harvesting was achieved in the three-year harvesting cycle – 56.26 t · ha$^{-1}$ of dry substance of wood. In the same period, in the three one-year cycles, the average of 33.99 t · ha$^{-1}$ of dry

substance of wood was obtained (Figure 5). The production of dry substance of wood in a two-year cycle was 30.52 t · ha$^{-1}$ on average and was higher by 30.6% than the sum of the yields of two one-year cycles in 2000 and 2001 (Figure 5).

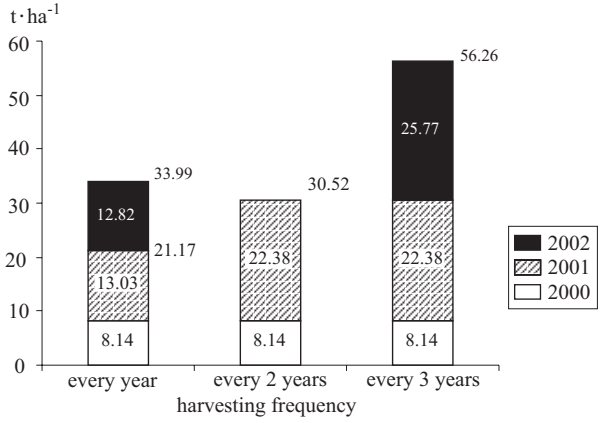


Fig. 5. Average wood dry substance yield for 7 willow clones, obtained in one-, two- and three-year harvesting cycles

Table 4

Wood dry substance yield in three harvesting cycles (t · ha$^{-1}$ · year$^{-1}$)

| Clone name and number in the collection (a) | Harvesting frequency (b) | | | Average |
|---|---|---|---|---|
| | every year | every 2 year | every 3 year | |
| *Salix viminalis* x *Salix purpurea* (1001) | 12.28 | 13.03 | 13.71 | 13.01 |
| *Salix cordata* (1019) | 10.39 | 13.60 | 12.88 | 12.29 |
| *Salix viminalis* (1023) | 13.68 | 19.09 | 18.83 | 17.20 |
| *Salix viminalis* (1033) | 11.81 | 14.06 | 22.17 | 16.01 |
| *Salix viminalis* var. *gigantea* (1047) | 10.41 | 10.28 | 22.78 | 14.49 |
| *Salix viminalis* JORR | 11.44 | 17.78 | 22.72 | 17.31 |
| *Salix viminalis* ULV | 9.30 | 18.98 | 18.26 | 15.51 |
| Average | 11.33 | 15.26 | 18.76 | 15.12 |
| $LSD_{0.05}$ | a-2.98 | b-1.95 | axb-5.16 | |

The average yield of dry substance of willow wood in the experiment was 15.12 t · ha$^{-1}$ · year$^{-1}$ (Table 4). The willows harvested every year yielded 11.33 t · ha$^{-1}$ · year$^{-1}$ on average. The yield of dry substance of one-year stalks in the years 2000, 2001, 2002 was 8.14; 13.03 and 12.82 t · ha$^{-1}$ · year$^{-1}$, respectively (Figure 5). The low yield of wood in the first "productive" year was probably caused by a deep deficit of water in soil in April, May and June. When

harvested every year, *Salix viminalis* (1023) and *Salix viminalis* x *Salix purpura* yielded 13.68 and 12.28 $t \cdot ha^{-1} \cdot year^{-1}$, respectively, which makes them useful for cultivation and one-year cycle harvesting. The willows harvested in two-year cycles yielded 15.26 $t \cdot ha^{-1} \cdot year^{-1}$ of dry substance of wood. The clones *Salix viminalis* (1023) and *Salix viminalis* ULV gave a high yield in that year.

A high yield of dry substance of wood was obtained in the three-year cycle (18.76 $t \cdot ha^{-1} \cdot year^{-1}$) and that was significantly higher than in the two-year cycle and was significantly much higher than in the one-year cycle. The clones of *Salix viminalis* var. *gigantea*, *Salix viminalis* JORR and *Salix viminalis* (1033) gave a similar, very high yield. Another group of significantly yielding clones in made up of *Salix viminalis* (1023) – 18.83 $t \cdot ha^{-1} \cdot year^{-1}$ and *Salix viminalis* ULV – 18.26 $t \cdot ha^{-1} \cdot year^{-1}$. The clones of *Salix viminalis* x *Salix purpura* (1001) and *Salix cordata* (1019) gave a low yield in the three-year cycle – 13.71 and 12.88 $t \cdot ha^{-1} \cdot year^{-1}$, respectively, and are not recommended for cultivation in this harvesting cycle.

The yield of dry substance of willow wood in the experiment depended on the plant height ($R^2 = 0.75$) and stalk diameter ($R^2 = 0.74$) – Figures 6, 7.

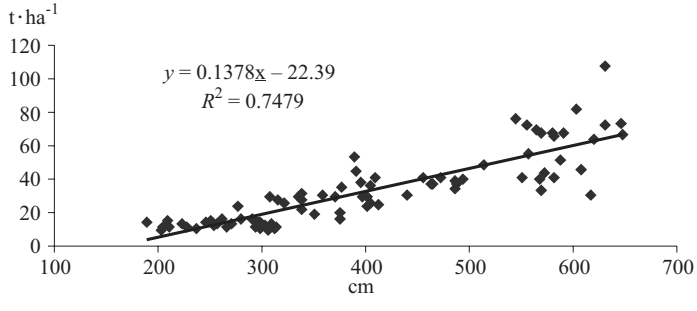


Fig. 6. The relationship between wood dry substance yield and plant height in the harvesting cycles under study

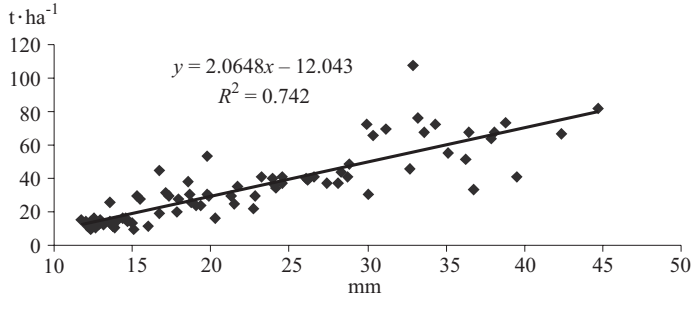


Fig. 7. The relationship between wood dry substance yield and stalk diameter in the harvesting cycles under study

## Discussion

The yield of dry substance of wood from willows cultivated on proper alluvial soil in this experiment averaged 15.12 t · ha$^{-1}$ · year$^{-1}$ and ranged from 11.33 t · ha$^{-1}$ · year$^{-1}$ when the plants were harvested every year to 18.76 t · ha$^{-1}$ · year$^{-1}$ when they were harvested every three years. The highest yield of 22,78 t · ha$^{-1}$ · year$^{-1}$ was obtained from the clone of *Salix viminalis* var. *gigantean,* harvested every three years, with a similar yield obtained in that year from *Salix viminalis* JORR and *Salix viminalis* (1033). These results are comparable with those obtained recently in Sweden, where the productivity (in terms of dry substance) of willow cultivated at sites with good soils and harvested in three-year cycles ranges from 12 to 18 t · ha$^{-1}$ · year$^{-1}$ (GIGLER et al. 1999). According to HOFFMANN and WEIH (2005), the productivity of dry substance of willow wood, depending on the location and habitat in Sweden ranges from 7 to 20 t · ha$^{-1}$ · year$^{-1}$, while in Germany the numbers are 6-14 t · ha$^{-1}$ · year$^{-1}$. In an experiment conducted in 1997 by KOPP et al. (1997), in a three-year cycle they obtained a significantly higher yield of dry substance of wood – 21.5 t · ha$^{-1}$ · year$^{-1}$ – than in one- and two-year cycles: 16.08 and 13.06 t · ha$^{-1}$ · year$^{-1}$, respectively. The highest yield (23.8 t · ha$^{-1}$ · year$^{-1}$ of dry substance) was obtained from the clone *Salix dasyclados* (SV1) in a three-year cycle with 37 thousand plants per hectare, when the plants were watered and fertilised. ADEGBIDI et al. (2001) confirmed that when watered and fertilised, the willow *Salix dasyclados* reacted to the prolongation of the harvesting cycle from one year to three years with an increase in the dry substance of wood from 12.5 to 21.7 t · ha$^{-1}$ · year$^{-1}$. LABRECQUE and TEODORESCU (2003) examined the productivity of fertilised and non-fertilised clones of *Salix viminalis* and *S. discolor* grown on sandy and clayey soil in two subsequent three-year harvesting cycles. The clone *Salix viminalis* was grown on sandy soil and fertilised, and yielded 23.5 t · ha$^{-1}$ · year$^{-1}$. However, when grown on sandy soil without fertilisation, the same plants yielded only 9.6 t · ha$^{-1}$ · year$^{-1}$. LABRECQUE, TEODORESCU (2005) cultivated 10 willow clones and two poplar clones with 18 thousand trees per hectare and harvested them every four years. The willows of *S. miyabeana* (SX64) and *S. sachalinensis* (SX61) yielded 16.90 and 15.59 t · · ha$^{-1}$ · year$^{-1}$ of dry substance of wood. The lowest yield was obtained from *S. interior x S. eriocephala* (S301) – 6.21 t · ha$^{-1}$ · year$^{-1}$. BULLARD et al. (2002) examined two clones: *Salix viminalis* var. Joruun and *S. dasyclados* in two- and three-year harvesting cycles. The highest yields for both forms were obtained in the two-year harvesting cycle.

KAJBA (1999) found that the yield of wood in field plantations of energetic plants can range from 12 to 15 t · ha$^{-1}$ · year$^{-1}$ provided the plants are properly fertilised. The best one of the clones under study – a three-species hybrid (*Salix*

*alba* x *Salix fragilis* x *Salix caprea*) harvested in a five-year cycle yielded about 26 $t \cdot ha^{-1} \cdot year^{-1}$. In Wales, attempts were made at cultivating willows on uplands with 20 thousand plants per hectare. In four-year harvesting cycles, yields of 6 to 12 $t \cdot ha^{-1} \cdot year^{-1}$ were obtained (RANDERSON et al. 2000). SZCZUKOWSKI et al. (2005) conducted their experiment on soil of good wheat complex class IIIb. When the plants were harvested every year, they obtained 13.69 $t \cdot ha^{-1} \cdot year^{-1}$ of dry substance of wood. The yield increased with the prolongation of the harvesting cycle to two, three and four years: 15.92; 17.50 and 20.04 $t \cdot ha^{-1} \cdot year^{-1}$, respectively. The clone *Salix viminalis x S. viminalis lanceolata* yielded 29.56 $t \cdot ha^{-1} \cdot rok^{-1}$ when harvested every four years, whereas *Salix triandra* yielded only 7.67 $t \cdot ha^{-1} \cdot rok^{-1}$. A similar relationship between the increase in dry substance of wood from *Salix viminalis* and the prolongation of the harvesting cycle from one to three years was observed by STOLARSKI et al. (2002).

## Conclusions

1. *Salix viminalis* (1023) and *Salix viminalis* x *Salix purpurea* (1001) can be recommended for cultivation in the region of the Lower Vistula at 40 thousand plants per hectare and a one-year harvesting cycle, *Salix viminalis* (1023) for two-year harvesting cycles and *Salix viminalis* var. *gigantea*, *Salix viminalis* JORR and *Salix viminalis* (1033) for three-year cycles.

2. The yield of dry substance of wood of willow harvested every three years amounted to 18.76 $t \cdot ha^{-1} \cdot year^{-1}$ on average; when the harvesting cycle was shortened to 2 and 1 years, it resulted in a yield decrease of 18.7% and 39.6%, respectively.

3. The productivity of willow was significantly dependent on the plant height and stalk diameter.

Translated by JOANNA JENSEN

Accepted for print 25.05.2006.

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 67:79, Y. 2006

# SPRING BARLEY OVERGROUND BIOMASS INCREASE DYNAMICS DEPENDING ON SOWING METHOD AND POSITION IN CROPS ROTATION SYSTEM

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K e y w o r d s: spring barley, Italian ryegrass intercrop, forecrop, biomass increase, soil humidity.

A b s t r a c t

The influence of intercrop (Italian ryegrass) and different forecrops (potato, spring wheat and spring barley) on spring barley overground biomass increase and topsoil humidity on medium soil were studied for a period of four years (2001-2004). The tests were carried every year during 5 development stages of barley, i.e. during seedlings formation, spreading, stems formation, heads formation and ripening.

The factors applied differentiated barley biomass during the period from spreading through the end of vegetation and the choice of forecrop showed a stronger influence than application of intercrop. The largest increases were found at the plot after potato as compared to both cereals (wheat and barley), particularly on the plot with intercrop. The sowing method (with intercrop or in pure crop field) was found insignificant as concerns biomass increases although more intensive accumulation of biomass was recorded in barley cultivated after potato during the period between stems formation and heads formation.

Studies on soil humidity showed that intercrop of Italian ryegrass in barley resulted in an increase of water reserves during barley seedlings formation and decreased moisture content during stems formation comparing to the plots without the intercrop. Introduction of the intercrop had a balancing effect on soil humidity after all forecrops while in case of "pure" barley plots (without intercrop) more favorable conditions developed on plots after potatoes.

## DYNAMIKA PRZYROSTU NADZIEMNEJ BIOMASY JĘCZMIENIA JAREGO W ZALEŻNOŚCI OD SPOSOBU SIEWU I JEGO UMIEJSCOWIENIA W ZMIANOWANIU

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S ł o w a k l u c z o w e: jęczmień jary, wsiewka życicy wielokwiatowej, przedplony, przyrost biomasy, wilgotność gleby.

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Abstrakt

W 4-leciu (2001-2004) na glebie średniej śledzono wpływ wsiewki (życicy wielokwiatowej) i zróżnicowanych przedplonów (ziemniaka, pszenicy jarej i jęczmienia jarego) na przyrosty masy nadziemnej jęczmienia jarego oraz uwilgotnienie wierzchniej warstwy gleby. Badania wykonywano corocznie w 5 fazach rozwojowych jęczmienia, tj. podczas wschodów, krzewienia, strzelania w źdźbło, kłoszenia i dojrzewania.

Wprowadzone czynniki różnicowały biomasę jęczmienia w okresie od krzewienia do końca wegetacji, przy czym silniejszy wpływ miał dobór przedplonów niż zastosowanie wsiewki. Największe przyrosty stwierdzono w stanowisku po ziemniaku, na niekorzyść obydwu zbóż (pszenicy i jęczmienia), zwłaszcza na obiekcie z wsiewką. Sposoby siewu (z wsiewką lub w łanie jednogatunkowym) okazały się bez znaczenia w dziennych przyrostach biomasy, aczkolwiek intensywniejsze jej nagromadzanie odnotowano w jęczmieniu uprawianym po ziemniaku w okresie między strzelaniem w źdźbło a kłoszeniem.

Badania wilgotności gleby wykazały, że wsiewana w jęczmień życica powodowała – w stosunku do obiektu bez wsiewki – zwiększenie zapasów wody w fazie wschodów jęczmienia, natomiast podczas strzelania w źdźbło zmniejszała jej zawartość. Wprowadzenie wsiewki działało wyrównująco na uwilgotnienie gleby po wszystkich przedplonach, natomiast w „czystych" zasiewach jęczmienia (bez wsiewki) korzystniejsze warunki ukształtowały się na stanowisku po ziemniaku.

## Introduction

Starting with 1980s the factors of economic and organizational nature have lead to major simplifications in management of arable land. One of visible manifestations of that simplification is resignation from crops rotation systems with numerous crops and cultivation of a few, most frequently cereal crops, which are frequently sown on plots after plants from the same group or even after the same crop. The above practice can lead to changes in the soil environment negatively influencing growth and development of cultivated crops making them susceptible to weeds development and infestation by pathogens and pests resulting in a decrease in yields (GAWROŃSKA 1997, WANIC et al. 2000, 2002). Cultivation of intercrops is one of traditional, long established methods aimed at mitigating those unfavorable effects that is nowadays returning to the fields (ANIL et al. 1998, JASKULSKI, KOTWICA 1999, JASKULSKI et al. 2000, KUŚ, JOŃCZYK 2000, SIUTA 1998). Generally they have a positive effect on the balance of soil organic content, prevent leaching of nitrogen, stimulate microorganic activity, limit weeds development and contribute to improving plants health status (BIS et al. 1999, DUER 1996, JACKSON et al. 1993, WANIC et al. 2005). In crops rotation systems saturated with cereals their role, however, varies as it depends on intercrop type and species sown as the intercrop, the main crop, soil conditions as well as volume and distribution of precipitations (JASKULSKI et al. 2000, KUŚ, JOŃCZYK 2000, PAWŁOWSKI, WOŹNIAK 2000). We should also consider the possibility of intercrops competing with the main crop for environmental elements, particularly water and biological components resulting in a decrease of the main crop yield (HAUG-

GAARD-NIELSEN et al. 2001). Theoretically that rivalry can start already during the early stages of vegetation and continue, with different intensity, until the end of vegetation.

This study aimed at assessment of the spring barley sowing method (with Italian ryegrass as intercrop and without intercrop) and three forecrops (potatoes, spring wheat and spring barley) influence on increase of spring barley overground part biomass and topsoil humidity.

## Material and Methods

The study was completed during the years 2001-2004 on the basis of a closed, statistical field experiment established in the autumn of 1999 at Production-Experimental Enterprise in Bałcyny. It was positioned on a medium, typical podzolic soil with balanced air and water relations, slightly acid with organic substances content of from 1.28 to 1.70%, highly abundant with available forms of potassium and phosphorus and moderate abundance of available magnesia, as concerns agricultural suitability classified to R IIIa fertility class and very good rye complex. The experiment was established on the basis of random sub-blocks in four repetitions. Spring barley (Rodion cultivar) was the assessed crop, which was cultivated yearly in the following crops rotation pairs:

potato – spring barley; spring wheat – spring barley; spring barley-spring barley.

The main investigated factors were:

1) barley sowing methods: pure cultivation and cultivation with Italian ryegrass intercrop;

2) forecrops: potato, spring wheat, spring barley.

Italian ryegrass was sown (at 24 kg · ha$^{-1}$) together with spring barley (at density of 350 seedlings per 1 m$^2$) at times optimal for that cereal (2001 – 17.04.; 2002 – 04.04.; 2003 – 17.04. and 2004 – 06.04). After barley harvest the grass was left on the field and next it was turned during pre-winter tillage. The dry mass of intercrop introduced to the soil for the experimental period averaged 2.87 t · ha$^{-1}$ (from 1.91 t in 2003 to 3.55 in 2004). The applied doses of mineral fertilizers (NPK) were identical in barley with and without intercrop, however, they were differentiated depending on the forecrop; after potato cultivated on manure at 25 t · ha$^{-1}$ – 220 kg · ha$^{-1}$ (N – 60; $P_2O_5$ – 80; $K_2O$ – 80), and after spring wheat and spring barley – 240 (80, 80 and 80 kg respectively).

The scope of yearly detailed studies covered:

1. Determination of dry mass of overground parts of spring barley during 5 phenophases: seedlings formation, spreading, stems formation, heads

Table 1

Precipitations and air temperatures during spring barley vegetation season

| Month | Decade | Precipitations (mm), years | | | | | Temperature (°C), years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2001 | 2002 | 2003 | 2004 | average for 1961--1995 | 2001 | 2002 | 2003 | 2004 | average for 1961--1995 |
| April | I | 7.0 | 0.0 | 12.9 | 34.0 | *x* | 9.1 | 2.4 | 1.1 | 5.4 | *x* |
| | II | 18.7 | 5.6 | 6.6 | 3.5 | | 2.9 | 9.8 | 6.6 | 8.3 | |
| | III | 17.8 | 4.4 | 4.1 | 14.0 | | 9.9 | 9.8 | 10.1 | 6.7 | |
| | sum or average | 43.5 | 10.0 | 23.6 | 51.5 | 35.4 | 7.3 | 7.3 | 5.9 | 6.8 | 7.0 |
| May | I | 4.5 | 1.5 | 0.0 | 65.1 | *x* | 13.1 | 18.2 | 14.2 | 14.6 | *x* |
| | II | 11.6 | 47.0 | 36.9 | 15.0 | | 12.9 | 13.7 | 7.0 | 13.3 | |
| | III | 15.2 | 41.6 | 41.7 | 7.0 | | 10.6 | 16.4 | 15.6 | 9.8 | |
| | sum or average | 31.3 | 90.1 | 78.6 | 87.1 | 57.6 | 12.2 | 16.1 | 12.3 | 12.6 | 12.5 |
| June | I | 15.7 | 10.2 | 0.6 | 21.4 | *x* | 11.9 | 15.3 | 18.4 | 15.9 | *x* |
| | II | 5.9 | 44.3 | 30.6 | 47.2 | | 13.6 | 17.0 | 12.6 | 15.2 | |
| | III | 27.2 | 18.0 | 29.5 | 22.0 | | 15.9 | 15.3 | 15.6 | 14.6 | |
| | sum or average | 48.8 | 72.5 | 60.7 | 90.6 | 69.5 | 13.8 | 15.9 | 15.5 | 15.2 | 15.8 |
| July | I | 42.4 | 1.5 | 66.5 | 19.3 | *x* | 19.4 | 18.8 | 16.5 | 15.9 | *x* |
| | II | 67.4 | 54.0 | 16.7 | 11.2 | | 19.3 | 20.2 | 15.6 | 15.5 | |
| | III | 25.3 | 6.7 | 35.0 | 18.3 | | 19.6 | 19.1 | 21.4 | 17.5 | |
| | sum or average | 135.1 | 43.2 | 118.2 | 48.8 | 81.6 | 19.4 | 19.3 | 17.8 | 16.3 | 17.2 |
| August | I | 26.0 | 74.3 | 1.5 | 16.5 | *x* | 17.7 | 20.7 | 19.0 | 19.0 | *x* |
| | II | 16.9 | 13.0 | 12.9 | 41.1 | | 20.5 | 19.6 | 18.5 | 20.6 | |
| | III | 38.9 | 0.0 | 20.5 | 31.7 | | 17.1 | 19.2 | 15.4 | 19.3 | |
| | sum or average | 81.8 | 87.3 | 34.9 | 89.3 | 75.2 | 18.4 | 19.8 | 17.6 | 19.6 | 16.8 |
| Sum or average from April through August | | 340.5 | 322.1 | 316.0 | 367.3 | 319.3 | 14.2 | 15.7 | 13.8 | 14.1 | 13.9 |

formation and ripening. The determination was done on 25. representative plants from each plot. At the laboratory the plants were dried to air dry mass and next weighted; that data was also used to calculate the day increases of the studied biomass.

2. After the barley reached harvest ripeness, the yield of grain from each plot was determined ($t \cdot ha^{-1}$).

3. Topsoil humidity measurements (0-20 and 20-30 cm) were taken at 5 selected points in each plot during the 5 earlier identified barley development stages. The measurements were taken using an Easy Test TDR Multimeter (by the Institute of Agrophysics of the Polish Academy of Sciences in Lublin).

4. The results obtained were processed by means of variance analysis estimating the average values using the Tukey;s test and correlation coefficients between the studied variables were calculated.

The weather conditions during the four years covered by the experiment differed significantly (Table 1). As concerns precipitations during the period from April to August, according to the criteria set by PRZEDPEŁSKA (after MARKS 2000) years 2001, 2002, and 2003 were considered moderately wet (340.5; 322.1 and 316.0 mm of rain respectively) while the season of 2004 was considered wet (367.3 mm). The season of 2002 was warm as for the region of northeastern Poland (15.7°C), while the other seasons were moderately warm (2001 – 14.2°C; 2003 – 13.8; 2004 – 14.1°C). According to the study by RUDNICKI (1995), the season of 2004 was relatively optimal for barley as concerns precipitations and temperatures while the other seasons were not favorable for its vegetation: in 2001 droughts in May and June (45.7 and 70.2% of long-term standard) and abundant rainfall in July (exceeding the long-term average by almost 70%) coupled with high air temperature (19.4°C), in 2002 – almost no rain in April (10.0 mm only) and too high temperatures in May (16.1°C), in 2003 – little rain in April (23.6 mm) and July with occasional small rain (118.1 mm).

Occurrence dates for developmental stages of barley during the examined 4-year period are presented in Table 2.

Table 2

Development stages of spring barley

| Development stage | Year | | | |
|---|---|---|---|---|
| | 2001 | 2002 | 2003 | 2004 |
| Seedlings formation | 02.05. – 11.05. | 22.04. – 01.05. | 30.04. – 09.05. | 20.04. – 03.05. |
| Spreading | 12.05. – 22.05. | 02.05. – 15.05. | 10.05. – 21.05. | 04.05. – 26.05. |
| Stems formation | 23.05. – 13.06. | 16.05. – 02.06. | 22.05. – 09.06. | 27.05. – 13.06. |
| Heads formation | 14.06. – 06.07. | 03.06. – 07.07. | 10.06. – 05.07. | 14.06. – 11.07. |
| Ripening | 07.07. – 30.07. | 25.06. – 26.07. | 06.07. – 05.08. | 12.07. – 04.08. |

## Results

During barley seedlings formation the intercrop and tested forecrops had no significant influence on the biomass of plants mass (Table 3). At further stages of growth and development it differentiated more as a consequence of the plot selection and less as a consequence of the intercrop presence. It should be pointed out that during none of the determination times an influence of sowing method on the studied feature was observed on plots after potatoes. The negative consequential influence of wheat (as forecrop) on barley appeared

at spreading stage and it continued with varying intensity until the end of vegetation; during spreading the studied mass was lower by 15.5% on the plot with pure crop and lower by 20.5% on the plot with the intercrop. Those gaps were closed during the stems formation period (with a minor favorable trend for sowing with the intercrop) and during the stage of heads formation they appeared again showing lower biomass than on plots after potato reaching 13.3% in pure crop and 14.1% with intercrop. At the end of vegetation the difference reached 19.0 and 21.4% respectively. Barley reacted less clearly in case of cultivation after barley. During spreading and stems formation stages a significant decrease of biomass by 4.5% was observed; the differences were slightly larger in pure crop (without intercrop) and during heads formation and ripening its significant reduction occurred on the plot with the intercrop. During the heads formation that decrease, as compared to the plot after potato, was: in pure crop – 15.2%, with intercrop – 17.7%, while during ripening it was 14.1 and 22.0% respectively.

Table 3

Dry mass of 1 barley plant (g); average values for years 2001-2004

| Development stage | Cultivation without intercrop | | | | Cultivation with intercrop | | | |
|---|---|---|---|---|---|---|---|---|
| | forecrop | | | | | | | |
| | potato | spring wheat | spring barley | average | potato | spring wheat | spring barley | average |
| Seedlings formation | 0.07 | 0.05 | 0.06 | 0.06 | 0.06 | 0.06 | 0.07 | 0.06 |
| Spreading | 0.46 | 0.39 | 0.42 | 0.42 | 0.42 | 0.31 | 0.39 | 0.39 |
| Stems formation | 1.83 | 1.80 | 1.79 | 1.81 | 1.91 | 1.87 | 1.91 | 1.90 |
| Heads formation | 5.05 | 4.38 | 4.28 | 4.57 | 5.03 | 4.32 | 4.14 | 4.50 |
| Ripening | 5.16 | 4.18 | 4.43 | 4.59 | 5.10 | 4.01 | 3.98 | 4.36 |

$NIR_{0.05}$ (g): seedlings formation – differences insignificant; spreading: sowing method – differences insignificant, concurrence (sowing method x forecrop) – 0.07; stems formation: sowing method – 0.08, concurrence (sowing method x forecrop) – 0.06; heads formation: sowing method – differences insignificant, concurrence (sowing method x lots) – 0.59; ripening: sowing method – 0.19, concurrence (sowing method x lots) – 0.67.

Table 4 presents the daily increases of barley dry mass. The discussed process achieved the highest rate during the period from heads formation to ripening, During the intermediate phases of seedlings development-spreading and spreading-stems formation no differences in barley biomass increases; only at the plot with intercrop certain trends indicating slowing down of the process on fields after wheat and after barley during the first period and acceleration of that rate on the plot after barley were observed. During the dynamic growth of plants at the time of stems formation-heads formation and

with a slightly lower intensity of dry mass accumulation at the plot with the intercrop a barley growth stimulating influence of the plot after potato, as opposed to plot after barley where the increases were smaller, was observed.

The described rate of dry mass accumulation shows high correlation with the grain yields (Table 5). In case of both compared sowing methods they were as similar levels (no significant differences) but the forecrops had a significant influence. Potato had the most favorable effect. As compared to it the consecutive cultivation of barley after cereals resulted in a significant yield

Table 4

Day increases of dry mass of the overground parts of spring barley (g); average values for years 2001-2004

| Periods between phases | Cultivation without intercrop | | | | Cultivation with intercrop | | | |
|---|---|---|---|---|---|---|---|---|
| | forecrop | | | | | | | |
| | potato | spring wheat | spring barley | average | potato | spring wheat | spring barley | average |
| Seedlings formation – spreading | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 | 0.02 |
| Spreading – stems formation | 0.08 | 0.09 | 0.08 | 0.08 | 0.09 | 0.10 | 0.09 | 0.09 |
| Stems formation – heads formation | 0.13 | 0.12 | 0.10 | 0.12 | 0.13 | 0.10 | 0.09 | 0.11 |
| Heads formation – ripening | 0.004 | 0.007 | 0.005 | 0.004 | 0.002 | -0.010 | -0.005 | -0.004 |

$NIR_{0.05}$ (g/day) seedlings formation – spreading: sowing methods – differences insignificant, concurrence (sowing methods x forecrop) – differences insignificant; spreading – stems formation: sowing methods – differences insignificant, concurrence (sowing methods x forecrop) – differences insignificant; stems formation – heads formation: sowing methods – differences insignificant, concurrence (sowing methods x forecrop) – 0.02; heads formation – ripening: sowing methods – differences insignificant, concurrence (sowing methods x forecrop) – differences insignificant.

Table 5

Yield of spring barley grain; average values for years 2001-2004

| Cultivation without intercrop | | | | Cultivation with intercrop | | | |
|---|---|---|---|---|---|---|---|
| Forecrop | | | | | | | |
| potato | spring wheat | spring barley | average | potato | spring wheat | spring barley | average |
| Grain yield ($t \cdot ha^{-1}$) | | | | | | | |
| 6.20 | 5.03 | 5.30 | 5.51 | 6.15 | 4.94 | 5.25 | 5.45 |
| Variability of yield between years (%) | | | | | | | |
| 12.98 | 7.75 | 7.30 | 9.34 | 15.61 | 8.28 | 6.24 | 10.04 |

$NIR_{(0.05)}$ for grain yield ($t \cdot ha^{-1}$): sowing methods: differences insignificant; forecrop – 0.23; concurrence (sowing methods x forecrop) – 0.33.

Table 6

Soil humidity of plots (%) under spring barley; average values for years 2001-2004

| Determination times (phenophases) | Soil layer (cm) | Cultivation without intercrop | | | | Cultivation with intercrop | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | forecrop | | | | | | | |
| | | potato | spring wheat | spring barley | average | potato | spring wheat | spring barley | average |
| Seedlings formation | 0-20 | 9.9 | 10.6 | 10.7 | 10.4 | 13.7 | 12.5 | 13.7 | 13.3 |
| | 20-30 | 11.7 | 11.6 | 12.0 | 11.8 | 14.9 | 13.1 | 13.6 | 13.9 |
| Spreading | 0-20 | 19.6 | 18.3 | 18.4 | 18.8 | 19.9 | 19.2 | 18.5 | 19.2 |
| | 20-30 | 19.2 | 18.5 | 19.1 | 18.9 | 20.6 | 18.7 | 19.2 | 19.5 |
| Stems formation | 0-20 | 15.8 | 13.4 | 13.5 | 14.2 | 11.8 | 10.2 | 11.9 | 11.3 |
| | 20-30 | 13.5 | 12.5 | 12.8 | 12.9 | 11.6 | 10.3 | 11.7 | 11.2 |
| Heads formation | 0-20 | 11.7 | 11.6 | 12.0 | 11.8 | 12.6 | 12.7 | 12.6 | 12.6 |
| | 20-30 | 12.0 | 12.2 | 12.5 | 12.2 | 12.5 | 12.8 | 13.0 | 12.8 |
| Ripening | 0-20 | 18.6 | 19.7 | 18.3 | 18.8 | 20.0 | 20.1 | 18.6 | 19.5 |
| | 20-30 | 15.9 | 16.7 | 15.8 | 16.1 | 16.8 | 17.2 | 16.0 | 16.7 |
| Average for determination times | 0-20 | 15.1 | 14.7 | 14.6 | 14.8 | 15.6 | 14.9 | 15.0 | 15.2 |
| | 20-30 | 14.5 | 14.3 | 14.4 | 14.3 | 15.3 | 14.4 | 14.7 | 14.8 |

$NIR_{0,05}$ (%): seedlings formation: sowing methods – soil layer 0-20 cm – 2.20, 20-30 cm – 1.9; concurrence (sowing method x forecrops) – 0-20 cm – 0.9, 20-30 cm – 1.3; spreading: sowing methods – 0-20 cm and 20-30 cm – differences insignificant; concurrence (sowing methods x forecrop) – 0-20 cm – 1.10, 20-30 cm – 0.9; stems formation: sowing methods – 0-20 cm – 2.30, 20-30 cm – 1.60; concurrence (sowing methods x forecrop) 0-20 cm – 2.90, 20-30 cm – differences insignificant; heads formation: sowing methods – 0-20 cm and 20-30 cm – differences insignificant, concurrence (sowing methods x forecrop) – 0-20 and 20-30 cm – differences insignificant; ripening: sowing methods – 0-20 and 20-30 cm – differences insignificant, concurrence (sowing methods x forecrop) – 0-20 cm – 1.20, 20-30 cm – 0.60.

decreases. That decrease reached higher values on the plot after spring wheat. It should be pointed out that the negative consequential influence of wheat on barley was more pronounced on the plot with ryegrass. In case of cultivation without the intercrop the yield decrease as compared to the plot after potato was 18.9%, and with intercrop – 19.7%. Additionally, the numbers in the discussed table indicate that introduction of the intercrop resulted in larger year-to-year differences in yields. Indifferent of the sowing method, the plot after potato increased the year-to-year difference (particularly on plot with intercrop) and after cereals decreased it; as a consequence the yield after cereals was lower but with less year-to-year difference. The calculated general averages presented in table 6 inform that during the entire barley vegetation period the method of sowing and the forecrops had little influence on differences in soil humidity. Only the trend of slightly higher water resources on plots with the intercrop, particularly on the plot after potato, was observed. Analysis

of the situation during individual phenophases of barley indicates that experiment variables had different influence on that characteristic. Starting with the seedlings development stage it was found that ploughed intercrop secured significantly better soil humidity on all assessed plots. Its positive influence was clearly visible in 0-20 cm layer in which, as well known, the basic mass of roots is positioned. In the experiment it was established that, as compared to the plots of barley without intercrop, the highest increase in soil humidity was found in the plot after potato. This should undoubtedly be linked to more effective soil moisture retention by ploughed organic intercrop mass and application of manure under that forecrop; the lowest water reserves were recorded after spring wheat and at the deeper level (20-30 cm) also after barley. During the spreading phase, as compared to the preceding period, the soil moisture level increased (as a result off abundant rainfalls) with simultaneous elimination of differences between plots observed earlier. As a consequence no significant influence of the intercrop on soil humidity but just a minor trend to improve the situation in the deeper layer, particularly on the plot after potato, was observed. Indifferent of the sowing method, cultivation of barley after cereals lead to a decrease in water reserves in the 0-20 cm layer and the intercrop moderated that process slightly at the plot after spring wheat.

During the period of intensive barley growth, that is stalks formation, soil humidity decreased by about 7% as compared to the spreading phase. Under the described conditions the intercrop became an important competitor of the cereal for water. Exhaustion of water reserve applied more to the 0-20 cm layer, particularly at the plots after potato (decrease by 4%) and spring wheat (3%). The favorable influence of potato as forecrop observed during the earlier phases was still maintained but only where barley was cultivated as pure crop because at the plot with the intercrop the moisture status after all forecrops equalized (differences insignificant).

During the period of heads formation the reserves of water decreased slightly as compared to the preceding phase and the experimental variables did not have any significant influence on differentiation. On the plots with the intercrop, as compared to pure barley cultivation, the trend of slightly better soil humidity at 0-20 cm level was observed. It was recorded on plots after wheat and barley where it was maintained until the end of vegetation. On the other hand, during ripening of barley growing with the intercrop on the plot after potato the reserves of water were significantly larger in the entire layer tested during the study.

Analysis of correlations showed that in the plot with the intercrop the shapeliness of plants at different crop rotation plots (larger biomass) at barley stalks formation stage showed a significant positive relation between soil humidity in both tested layers during the seedlings formation stage ($r = 0.89$

and 0.90), during spreading – in the deeper layer ($r$ = 0.59), and negative with water content in 0-20 cm layer (-0.58). During the heads formation stage the intercrop limited significantly the yield of dry mass or barley, despite better soil humidity conditions ($r$ = -0.57 and -0.64). During the same period and at the end of vegetation the biomass was also negatively correlated with humidity status of both layers during spreading (-0.86 and -0.63; -0,75 and -0.67), and in the ripening phase also with the water content during heads formation (-0.68; -0.65). Finally, the yield of grain showed no relation with soil humidity during barley vegetation and the differences observed between the assessed plots were correlated with poor vegetative mass increase during the stages of spreading ($r$ = 0.77), heads formation (0.92) and ripening (0.96).

## Discussion

Following the rhythm of spring barley biomass increase showed that it changed depending on the introduced experimental variables starting with the spreading phase. It was strongly differentiated by forecrops, less by presence or absence of intercrop. The best conditions for barley growth were recorded at the plot after potato and without modifying effects of the intercrop. Negative consequential influence of spring wheat as a forecrop of barley continued until the end of vegetation and, what is important, it intensified in presence of the intercrop. Barley showed a slightly less pronounced reaction to cultivation after itself; the intercrop limited its biomass only during heads formation and ripening. JASKULSKI (2004) and WOŹNIAK (2000) draw attention to the negative role of sowing Italian ryegrass as intercrop in barley on the yields of the following crops – winter wheat and Westland ryegrass introduced to monoculture of winter triticale. JASKULSKI (2004), KUŚ, JOŃCZYK (2000), PŁAZA, CEGLAREK (2004) as well as SIUTA (1998) demonstrate that the influence of intercrop on yields of cereals depends on habitat conditions, mainly the volume and distribution of precipitations. During the years with optimum rainfall the intercrop has a positive or no influence on the yield of barley while in dry seasons it decreases the yields. This experiment did not confirm that fully. It was carried out during three years with moderate precipitations and one wet year but during each of those years on plots after cereal forecrops the intercrop reduced barley biomass additionally leading to greater variability from year to year. That last finding stands in opposition to the publication by IGNACZAK (1993), according to which introduction of intercrops to cultivated cereals influences a higher stability of yields.

According to some authors, the positive effects of enriching agricultural biocenoses, i.e. increasing the biodiversity of them by introducing intercrops

into rotation systems saturated with cereals and into cereal monocultures become visible after an extended time mainly as a consequence of organic matter accumulation in soil (BURACZYŃSKA, CEGLAREK 2002, PŁAZA, CEGLAREK 2004). This paper presents analysis of the experiment where the intercrop in barley was applied for 5 years. It is possible that its positive effects will be visible at a later time.

The presented studies have shown that the intercrop of ryegrass significantly increased the water content in soil during spring barley seedlings formation. During the later vegetation stages its role decreased and during the stems formation phase it became a competitor of barley for water (soil on the plot with intercrop was dryer). The positive influence of the intercrop was visible most clearly on the plot after potato cultivated on manure. It is hard to confront the results obtained with those available in literature as the majority of studies in that field are based on measurements taken during a selected phase of cereal vegetation (most frequently at its final stage). They show, e.g. that the intercrop has a varied influence on soil humidity, which depends on environmental factors, cereal type, intercrop type and plants sown together with the cereal (DUER 1996, JASKULSKI, KOTWICA 1999, PŁAZA, CEGLAREK 2004). Among abiotic factors a significant role is that of the soil and the water (KUŚ, JOŃCZYK 2000). During years with low precipitations the intercrop causes excessive drying of the soil and during seasons abundant with precipitations it has no influence or marginal influence on its status (DUER 1996, PARYLAK 1996). That is confirmed by JASKULSKI, KOTWICA (1999), who showed, on the basis of studies carried out after harvest of barley with ryegrass as intercrop, that the intercrop caused decrease of water content in the soil. It partly matched the results of the here-analyzed experiment, which, as mentioned earlier, was carried out during years with moderate precipitations or wet years. The intercrop in this case decreased the water content significantly during the dynamic barley biomass increase (stems formation) only. Among the compared forecrops potato had a positive influence on soil humidity level while cultivation of barley after spring wheat or spring barley resulted in decreasing the water reserves. The intercrop evidently decreased the differences caused by the forecrops. The above results are in contradiction with those presented by PARYLAK et al. (2002), which lead to conclusion that short cultivation of winter triticale in monoculture increases soil humidity.

## Conclusions

1. Factors of the experiment (intercrop and three forecrops) did not differentiate spring barley biomass during the seedlings formation. Starting

with the spreading phase the plot after potato (with or without intercrop) had a positive influence on its accumulation. The negative consequential influence of spring wheat as a forecrop on barley started at the spreading stage and it continued with different intensity until the end of vegetation increasing under the influence of intercrop (excluding the stems formation phase). A weaker reaction was recorded in spring barley cultivated after spring barley during spreading and stems formation but during heads formation and ripening the intensity of that influence increased, particularly on the plot with intercrop.

2. Cultivation of barley with or without intercrop did not differentiate day biomass increases. A significantly higher rate of biomass accumulation was recorded on plot after potato during the period between stem formation and heads formation.

3. Introduction of the intercrop (or not) had no influence on barley grain yield. That cereal offered the highest yield cultivated after potato and he lowest cultivated after spring wheat, particularly with intercrop.

4. On all three rotation plots tested (after potato, spring wheat and spring barley) introduction of Italian ryegrass as intercrop with barley secured better humidity of the soil during seedlings formation as compared to pure crop cultivation. During the stems formation phase the intercrop acted as a competitor for water. During the remaining phases of barley growth and development no significant influence of sowing method on soil humidity was observed.

5. The plot after potato, particularly with pure barley cultivation had a positive influence on soil humidity during the entire vegetation period. The intercrop moderated the differences caused by forecrops.

Translated by Jerzy Gozdek

Accepted for print 11.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 81-90, Y. 2006

# EFFECTS OF GROWTH REGULATORS ON THE GROWTH AND YIELD OF SPRING WHEAT UNDER CONDITIONS OF DIFFERENTIATED POTASSIUM FERTILIZATION

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K e y w o r d s: spring wheat, growth regulators, potassium fertilization, grain weight, vegetative organs weight.

A b s t r a c t

A pot experiment was established on light soil with medium abundance of available macroelements to determine the effects of growth regulators on the growth and yield of spring wheat under conditions of increasing potassium fertilization rates. The application of gibberellin enabled to significantly elongate ears and increase plant height. The influence of kinetin and auxin was less pronounced and varied. All growth promoters, especially kinetin, reduced grain weight per plant. Gibberellin increased, while kinetin and auxin decreased the biomass of above-ground vegetative organs in wheat.

The highest grain weight per plant was achieved when potassium was applied at a rate of 1 g K per pot. The optimum fertilization efficiency was recorded when potassium levels increased from 0.5 to 1.0 g K per pot.

## WPŁYW REGULATORÓW WZROSTU NA WZROST I PLONOWANIE PSZENICY JAREJ W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA POTASEM

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Abstrakt

W doświadczeniu wazonowym, na glebie lekkiej o średniej zasobności w przyswajalne makroskładniki, badano wpływ regulatorów wzrostu na wzrost i plonowanie pszenicy jarej w warunkach wzrastającego poziomu nawożenia potasem. Giberelina zdecydowanie wpłynęła na wydłużenie kłosów oraz zwiększenie wysokości roślin. Działanie kinetyny, gibereliny i auksyny było słabsze i niejednoznaczne. Wszystkie badane regulatory wzrostu, zwłaszcza kinetyna, wpływały na zmniejszenie masy ziarna z rośliny. Giberelina zwiększała nadziemną masę wegetatywną pszenicy, natomiast auksyna i, przede wszystkim, kinetyna ją redukowały.

Największą masę ziarna z rośliny uzyskano po zastosowaniu nawożenia na poziomie 1 g K na 1 wazon. Wzrost poziomu nawożenia od 0,5 – 1,0 g K na wazon gwarantował najwyższą efektywność nawożenia.

## Introduction

The root system of spring wheat develops in a deeper soil layer than winter wheat. At first the root system develops at a slow rate, which makes plants more sensitive to periodic droughts and determines their response to fertilization.

Potassium, supplied to the roots in the form of $K^+$, is transported with the transpiration stream to above-ground parts. Among all nutrients, nitrogen and potassium are absorbed in the largest amounts, but potassium uptake takes more time. The effect-forming of potassium fertilization depends on both crop species and soil abundance of this nutrient. A decrease of potassium fertilization is followed by a decrease in yield. This concerns cereals (MERBACH et al. 1999) to a lower degree than sensitive plants (potatoes, sugar beets, rape, peas). In addition, potassium supply enables to enhance the yield-forming effect of nitrogen (SZCZEPANIAK 2004).

A key factor of metabolic control in plant organisms are biologically active compounds. The control over plant growth and development by means of bioregulators allows to increase plant biomass. These substances may support fertilization, thus contributing to an increase of yield and an improvement of its quality (HARMS, NOWAK 1990).

The aim of the study was to determine the effects of growth regulators on spring wheat yield under conditions of increasing potassium fertilization rates.

## Materials and Methods

A pot experiment was performed in the years 1999-2002. Mitscherlich pots were filled with 6.5 kg of light loamy sand with pH 6.4 in 1 mol $KCl \cdot dm^{-3}$ and medium abundance of available phosphorus, potassium and magnesium. Mineral fertilizer application:

N – 1.5 g per pot ($NH_4NO_3$),
P – 0.5 g per pot ($KH_2PO_4$),
K – 0-3.0 g per pot ($KH_2PO_4$ supplemented KCl and $K_2SO_4$ rate 1:1),
Mg – 0.25 g per pot ($MgSO_4 \cdot 7H_2O$).

Total rates of phosphorus and magnesium were applied to the soil pre--sowing. Nitrogen and potassium were applied at three rates: $^1/_2$ pre-sowing, $^1/_4$ at the beginning of tillering and $^1/_4$ at the stage of stem formation. 20 spring wheat plants, cv. Jasna, were grown in each pot. The experiment was conducted in four replications.

The effects of growth regulators were compared under conditions of increasing potassium fertilization rates. In order to do the pots were divided into groups.

| Experiment variant | Sprayning time | |
|---|---|---|
| | Initial tillering | Initial florescence |
| I – control | water | water |
| II – kinetin | BAP (6-benzylaminopurine) | FAP (furfurylaminopurine) |
| III – gibberellin | $GA_3$ (gibberellic acid) | $GA_3$ (gibberellic acid) |
| IV – auxin | IAA (indole-3-acetic acid) | NAA($\alpha$-naphthaaleneacetic) |

In each of the groups wheat plants were sprayed with 0.5 $dm^3$ of a solution of each growth regulator, at a concentration of 50 $mg \cdot dm^{-3}$.

Wheat plants were collected at the stage of full ripeness, and the length of whole plants and ears was measured. Then the plants divided into the following organs: grain, glumes with rachis, stem, leaf $1^{st}$ (flag), leaf $2^{nd}$ and the remaining leaves. The results of measurements and weights of particular organs were subjected to an analysis of variance for two-factorial experiments in a completely randomized design.

## Results and Discussion

Growth regulators had a stronger effect on the length of spring wheat ears and stems than increasing potassium fertilization rates (Table 1). The application of gibberellin enabled to significantly elongate ears and whole plants, by 9.5% and 38.7% respectively, in comparison with the control treatment. Kinetin shortened wheat ears by 5.1% and had no effect on plant height. Plant elongation caused by auxin was slight, but statistically significant.

The control plants provided the longest ears. The lowest potassium rate resulted in their significant shortening. A further increase of potassium fertilization had no considerable influence on this parameter. Plant height

increase was recorded to a level of 1.5 – 2.0 g K per pot, and was strongly interacted with gibberellin. The highest potassium rates significantly inhibited plant elongation.

Table 1

Lenght of spike and wheat height (cm)

| Plant growth regulators | Dose K in g per pot | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | |
| Spike | | | | | | | | |
| Control | 9.70 | 9.12 | 8.77 | 8.70 | 8.44 | 8.60 | 8.62 | 8.85 |
| Kinetin | 9.07 | 8.31 | 8.05 | 8.38 | 7.98 | 8.25 | 8.78 | 8.40 |
| Gibberellin | 10.40 | 9.62 | 9.73 | 9.75 | 9.50 | 9.37 | 9.48 | 9.69 |
| Auxine | 9.76 | 8.85 | 9.08 | 8.86 | 8.90 | 8.32 | 8.73 | 8.93 |
| Means | 9.73 | 8.97 | 8.91 | 8.92 | 8.71 | 8.63 | 8.90 | – |
| $LSD_{0.01}$ | I – 0.32 | | | II – 0.42 | | I x II – n.s. | | |
| Wheat height | | | | | | | | |
| Control | 74.43 | 80.32 | 81.12 | 81.11 | 80.21 | 73.10 | 75.13 | 77.92 |
| Kinetin | 76.92 | 68.86 | 78.88 | 82.91 | 81.73 | 77.70 | 77.18 | 77.74 |
| Gibberellin | 106.88 | 105.30 | 106.56 | 112.02 | 111.08 | 107.75 | 107.07 | 108.09 |
| Auxine | 82.65 | 82.30 | 81.10 | 81.66 | 84.43 | 76.60 | 73.70 | 80.35 |
| Means | 85.22 | 84.19 | 86.92 | 89.42 | 89.36 | 83.79 | 83.27 | – |
| $LSD_{0.01}$ | I – 2.03 | | | II – 2.69 | | I x II – 5.38 | | |

* I – regulators
II – dose K
I x II – interaction

Other authors also observed a stimulating effect of gibberellin and an inhibitory effect of kinetin on the length of spring wheat ears and stems (WIERZBOWSKA et al. 2001). Experiments with triticale showed that only NAA significantly shortened ears, and that stems elongated under the influence of gibberellin and IBA (CZAPLA et al. 2000).

The effects of growth regulators and potassium fertilization on yield structure elements are presented in Table 2. The proportion of yield in the total biomass produced by a plant is referred to as the harvest index (HI). Only auxin contributed to an increase in the value of this index. The application of kinetin and gibberellin significantly reduced the value of this parameter (by 6.9% and 13% respectively, as compared with the control treatment). An increase of grain percentage in total biomass was observed to a level of 2.0 g K per pot, and reached the highest value for the interaction between this fertilizer rate and auxin. Growth promoters had no effect on unproductive and productive tillering, but these parameters increased proportionally to potassium rates.

Table 2

Yield components

| Plant growth regulators | Dose K in g per pot | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | |
| Hervest index | | | | | | | | |
| Control | 31.39 | 32.24 | 33.43 | 32.29 | 33.79 | 34.07 | 34.62 | 33.22 |
| Kinetin | 29.24 | 29.69 | 32.39 | 31.69 | 32.03 | 31.56 | 29.82 | 30.92 |
| Gibberellin | 26.11 | 28.40 | 30.46 | 29.45 | 32.57 | 28.48 | 26.83 | 28.90 |
| Auxine | 30.99 | 31.23 | 35.14 | 34.42 | 37.55 | 31.14 | 33.23 | 33.38 |
| Means | 29.43 | 30.39 | 32.86 | 32.14 | 33.98 | 31.31 | 31.12 | – |
| $LSD_{0.01}$ | I – 1.95 | | | II – 1.88 | | I x II – 2.77 | | |
| Total tillering | | | | | | | | |
| Control | 1.35 | 1.35 | 1.22 | 1.22 | 1.42 | 1.62 | 1.30 | 1.35 |
| Kinetin | 1.32 | 1.30 | 1.31 | 1.20 | 1.28 | 1.06 | 1.43 | 1.27 |
| Gibberellin | 1.30 | 1.45 | 1.19 | 1.45 | 1.19 | 1.25 | 1.59 | 1.35 |
| Auxine | 1.20 | 1.24 | 1.27 | 1.39 | 1.35 | 1.35 | 1.60 | 1.34 |
| Means | 1.29 | 1.34 | 1.25 | 1.32 | 1.31 | 1.33 | 1.48 | – |
| $LSD_{0.01}$ | I – n.s. | | | II – 0.13 | | I x II – n.s. | | |
| Productive tillering | | | | | | | | |
| Control | 1.29 | 1.29 | 1.18 | 1.18 | 1.36 | 1.46 | 1.25 | 1.29 |
| Kinetin | 1.29 | 1.25 | 1.26 | 1.16 | 1.20 | 1.05 | 1.40 | 1.23 |
| Gibberellin | 1.24 | 1.43 | 1.16 | 1.31 | 1.21 | 1.16 | 1.51 | 1.29 |
| Auxine | 1.14 | 1.06 | 1.26 | 1.29 | 1.35 | 1.33 | 1.53 | 1.28 |
| Means | 1.24 | 1.26 | 1.21 | 1.23 | 1.18 | 1.25 | 1.42 | – |
| $LSD_{0.01}$ | I – n.s. | | | II – 0.16 | | I x II – n.s. | | |

* legends as Table 1

Studies on the effects of growth regulators on yield structure elements provided varied results. According to DIAZ-MIGUEL (1989), kinetin applied to the soil caused an early stimulation of tillering, followed by a decrease of the number of fertile shoots. Gibberellin delayed tillering, which resulted in a high number of sterile shoots. AUFHAMMER and FEDEROLF (1992) demonstrated that kinetin had no effect on the number of ear-bearing shoots. Kinetin applied during flowering and grain setting was conducive to late tillering, which resulted in the production of green, unfertile shoots during harvest. On the other hand, WIERZBOWSKA et al. (2001) found that gibberellin increased, while kinetin decreased the number of both ears and stems.

Growth regulators generally had a negative effect on wheat grain weight, but this was confirmed by a statistical analysis only in the case of kinetin, which contributed to a 20.6% yield decrease (Table 3). The highest grain weight was obtained when potassium was applied in the amount of 1g K per pot, which permitted a yield increase by 18.4%, in relation to the control

Table 3

Weight of wheat organs in g per plant

| Plant growth regulators | Dose K in g per pot | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | |
| Grain | | | | | | | | |
| Control | 0.97 | 0.96 | 1.11 | 0.96 | 0.99 | 0.99 | 1.17 | 1.02 |
| Kinetin | 0.81 | 0.76 | 0.98 | 0.90 | 0.82 | 0.77 | 0.68 | 0.81 |
| Gibberellin | 0.82 | 0.92 | 0.99 | 0.96 | 1.08 | 0.97 | 0.88 | 0.95 |
| Auxine | 0.88 | 0.79 | 1.04 | 0.95 | 1.04 | 0.85 | 1.03 | 0.94 |
| Means | 0.87 | 0.86 | 1.03 | 0.94 | 0.98 | 0.90 | 0.94 | – |
| $LSD_{0.01}$ | I – 0.09 | | | II – 0.06 | | I x II – 0.12 | | |
| Glume | | | | | | | | |
| Control | 0.41 | 0.48 | 0.50 | 0.42 | 0.38 | 0.40 | 0.46 | 0.43 |
| Kinetin | 0.38 | 0.39 | 0.41 | 0.42 | 0.32 | 0.30 | 0.28 | 0.36 |
| Gibberellin | 0.42 | 0.41 | 0.44 | 0.43 | 0.37 | 0.45 | 0.43 | 0.42 |
| Auxine | 0.36 | 0.39 | 0.39 | 0.38 | 0.35 | 0.40 | 0.39 | 0.38 |
| Means | 0.39 | 0.42 | 0.44 | 0.41 | 0.35 | 0.39 | 0.39 | – |
| $LSD_{0.01}$ | I – 0.04 | | | II – 0.05 | | I x II – 0.09 | | |
| Stem | | | | | | | | |
| Control | 0.63 | 0.60 | 0.68 | 0.65 | 0.62 | 0.66 | 0.67 | 0.65 |
| Kinetin | 0.54 | 0.52 | 0.55 | 0.60 | 0.58 | 0.56 | 0.37 | 0.53 |
| Gibberellin | 0.85 | 0.90 | 0.89 | 0.92 | 0.87 | 0.90 | 1.00 | 0.90 |
| Auxine | 0.60 | 0.62 | 0.67 | 0.61 | 0.60 | 0.62 | 0.65 | 0.62 |
| Means | 0.65 | 0.66 | 0.70 | 0.69 | 0.67 | 0.68 | 0.67 | – |
| $LSD_{0.01}$ | I – 0.03 | | | II – n.s. - n.i. | | I x II – 0.06 | | |
| Flag leaf | | | | | | | | |
| Control | 0.36 | 0.34 | 0.35 | 0.30 | 0.31 | 0.31 | 0.37 | 0.34 |
| Kinetin | 0.29 | 0.26 | 0.31 | 0.29 | 0.25 | 0.25 | 0.33 | 0.28 |
| Gibberellin | 0.30 | 0.34 | 0.29 | 0.29 | 0.25 | 0.30 | 0.34 | 0.30 |
| Auxine | 0.34 | 0.35 | 0.30 | 0.28 | 0.27 | 0.34 | 0.34 | 0.32 |
| Means | 0.33 | 0.32 | 0.31 | 0.29 | 0.27 | 0.30 | 0.35 | – |
| $LSD_{0.01}$ | I – 0.02 | | | II – 0.02 | | I x II – 0.04 | | |
| Second leaf | | | | | | | | |
| Control | 0.20 | 0.17 | 0.19 | 0.16 | 0.18 | 0.23 | 0.22 | 0.19 |
| Kinetin | 0.19 | 0.18 | 0.19 | 0.20 | 0.18 | 0.16 | 0.17 | 0.18 |
| Gibberellin | 0.21 | 0.23 | 0.18 | 0.18 | 0.16 | 0.19 | 0.21 | 0.20 |
| Auxine | 0.18 | 0.15 | 0.14 | 0.17 | 0.15 | 0.16 | 0.21 | 0.17 |
| Means | 0.20 | 0.17 | 0.17 | 0.18 | 0.17 | 0.18 | 0.20 | – |
| $LSD_{0.01}$ | I – 0.02 | | | II – 0.02 | | I x II – 0.05 | | |
| Remaining leaves | | | | | | | | |
| Control | 0.56 | 0.52 | 0.47 | 0.49 | 0.42 | 0.45 | 0.49 | 0.49 |
| Kinetin | 0.54 | 0.44 | 0.46 | 0.48 | 0.42 | 0.47 | 0.42 | 0.46 |
| Gibberellin | 0.48 | 0.45 | 0.42 | 0.37 | 0.36 | 0.36 | 0.48 | 0.42 |
| Auxine | 0.45 | 0.46 | 0.43 | 0.41 | 0.40 | 0.45 | 0.45 | 0.44 |
| Means | 0.51 | 0.47 | 0.45 | 0.44 | 0.40 | 0.43 | 0.46 | – |
| $LSD_{0.01}$ | I – 0.03 | | | II – 0.04 | | I x II – 0.09 | | |

* legends as Table 1

treatment. A further increase of fertilization levels was followed by a slight decrease in grain weight, but its value was still above that recorded in the control plants. The worst results were achieved for the interaction between kinetin and the highest potassium rate. Similar tendencies were observed also in glume weight.

Growth regulators, affecting plant elongation, differentiated stem weight to the highest degree. Stem weight was substantially increased by gibberellin (by 38.6%), and decreased by kinetin (19.3%), compared with the control. Just like in the case of grain, wheat plants fertilized with 1.0 g K per pot provided the highest stem weight. Both growth regulators and increasing potassium rates usually reduced the biomass, especially of the oldest leaves.

Previous studies conducted by WIERZBOWSKA et al. (2001) and WIERZBOWSKA and SIENKIEWICZ (2004) confirmed a negative effect of growth regulators on grain weight. In the experiment performed by WOJCIESKA (1992) the yield of oat treated with auxin was comparable with that of control plants. AUFHAMMER and FEDEROLF (1992) observed no significant effect of kinetin on wheat grain yield. However, the results obtained by other authors (NOWAK, WIERZBOWSKA 1991, WIERZBOWSKA, NOWAK 1998, CZAPLA et al. 2000) indicated a positive influence of growth regulators, especially auxin and giberellin applied immediately before ear formation, on grain yield (WŁODKOWSKI 1990). Studies on the effect of potassium fertilization on potassium-deficient soils showed that the

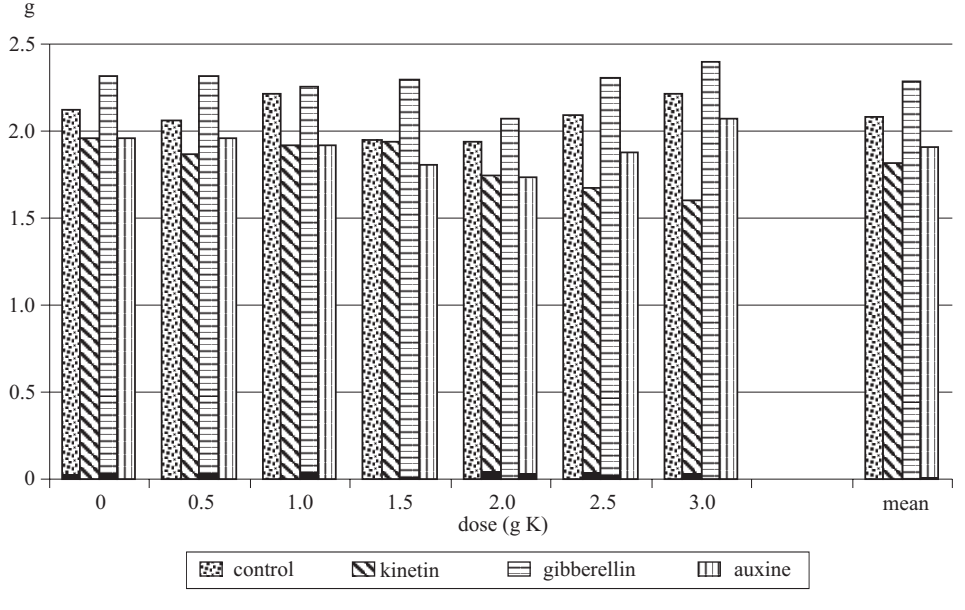


Fig. 1. Aboveground vegetative mass of spring wheat

highest tricticale grain yield (STĘPIEŃ et al. 1997) and the highest spring wheat grain yield (MERCIK et al. 1976) could be achieved already at the lowest fertilizer rate, and that a further increase of fertilization levels caused yield recession. On the other hand, straw yield increase was directly proportional to potassium rates. Moreover, JOHANSON et al. (1990) found a close correlation between grain yield and flag leaf biomass, and the harvest index.

The mass of wheat vegetative organs was affected by growth regulators and potassium fertilization to a slight degree only (Figure 1). Only gibberellin displayed a tendency towards an increase of vegetative part biomass, mostly due to stem increment stimulation. Kinetin and auxin reduced the mass of vegetative organs. Similar effects of growth promoters on the production of above-ground vegetative parts were also observed by WIERZBOWSKA et al. (2001) in experiments with spring wheat, and by CZAPLA et al. (2000) in studies on triticale. However, other authors reported that the development of vegetative parts was stimulated by kinetin and auxin, as well as mineral fertilization (NOWAK, WIERZBOWSKA 1991, WIERZBOWSKA, NOWAK 1998).

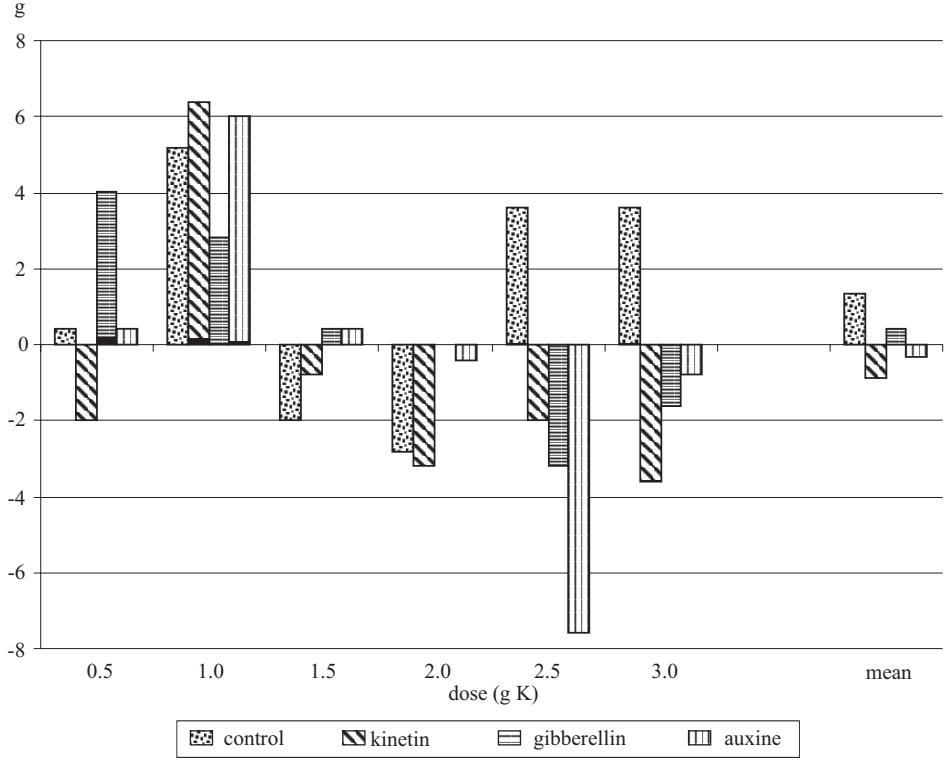


Fig. 2. Agricultural efficiency of potassium fertilization of spring wheat

The optimum fertilization efficiency was recorded at potassium levels of 0.5 to 1.0 g K per pot (Figure 2). This indicates that under experimental conditions potassium rates above 1 g per pot did not produce a yield-forming effect. Higher potassium rates could disturb cation equilibrium in the soil due to an antagonistic effect exerted primarily on $Mg^{2+}$. Depending on the growth regulator, the fertilization efficiency of potassium applied at the above rate varied widely, i.e. kinetin – 6.4, auxin – 6.0, control – 5.2, gibberellin – only 2.8 g grain per g of potassium. The lowest fertilization efficiency, recorded in the treatment with gibberellin, was related to the strong effect of this growth regulator on plant elongation, as well as on vegetative part biomass. Within the entire range of potassium rates, the mean fertilization efficiency was positive only in the control treatment and in the treatment with gibberellin (1.3 and 0.4 g per g of potassium).

## Conclusions

1. The application of gibberellin enabled to significantly elongate ears (by 9.5%) and stems (by 38.7%). The influence of the other growth regulators was less pronounced and varied.

2. All growth promoters, especially kinetin, reduced grain weight per plant.

3. Giberellin increased, while kinetin and auxin decreased the biomass of above-ground vegetative organs in wheat.

4. The highest grain weight per plant was achieved when potassium was applied at a rate of 1 g K per pot.

Translated by Aleksandra Poprawska

Accepted for print 21.02.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 91-100, Y. 2006

# NITROGEN AND PHOSPHORUS MANAGEMENT OF SPRING WHEAT PLANTS FOLLOWING THE APPLICATION OF GROWTH REGULATORS AND INCREASING POTASSIUM FERTILIZATION RATES

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K e y w o r d s: spring wheat, growth regulators, potassium fertilization, nitrogen and phosphorus content, nitrogen and phosphorus accumulation.

A b s t r a c t

A pot experiment was performed to determine the effects of kinetin, gibberellin and auxin on the concentration, accumulation and distribution of nitrogen and phosphorus in spring wheat grown under conditions of increasing potassium fertilization rates. Auxin increased the nitrogen content of wheat grain and glumes, whereas kinetin and gibberellin decreased nitrogen concentration in wheat grain and vegetative organs. All phytohormones increased the phosphorus content of wheat grain, glumes and flag leaf, and in the case of kinetin and gibberellin the concentration of this element increased also in wheat stems. The highest nitrogen content of wheat grain was achieved as a result of the application of the highest potassium rates (2.0 and 2.5 g K per pot). Phosphorus concentration in wheat grain increased proportionally to potassium levels. Phytohormones reduced nitrogen accumulation and increased phosphorus concentration in wheat grain. All growth regulators caused a decrease in nitrogen accumulation in whole wheat plants, while phosphorus accumulation increased following the application of gibberellin. Gibberellin and auxin increased nitrogen accumulation in grain by 4.23%, and all phytohormones increased phosphorus accumulation in grain.

## GOSPODARKA AZOTEM I FOSFOREM W ROŚLINACH PSZENICY JAREJ W WARUNKACH STOSOWANIA REGULATORÓW WZROSTU I WZRASTAJĄCEGO POZIOMU NAWOŻENIA POTASEM

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S ł o w a k l u c z o w e: pszenica jara, regulatory wzrostu, nawożenie potasem, zawartość azotu i fosforu, akumulacja azotu i fosforu.

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Abstrakt

W doświadczeniu wazonowym badano wpływ kinetyny, gibereliny i auksyny na koncentrację, akumulację i dystrybucję azotu i fosforu w pszenicy jarej, uprawianej w warunkach wzrastającego nawożenia potasem. Auksyna zwiększyła zawartość azotu w ziarnie i plewach pszenicy, natomiast kinetyna i giberelina zmniejszyły koncentrację tego składnika w ziarnie i organach wegetatywnych. Wszystkie fitohormony zwiększyły zawartość fosforu w ziarnie, plewach i liściu flagowym, a kinetyna i giberelina również w źdźble pszenicy. Najwyższą zawartość azotu w ziarnie uzyskano w wyniku zastosowania najwyższych dawek potasu (2,0 i 2,5 g K na 1 wazon). Koncentracja fosforu w ziarnie pszenicy rosła analogicznie do dawek potasu. Fitohormony zmniejszyły akumulację azotu, a zwiększyły fosforu w ziarnie pszenicy. W całych roślinach pszenicy akumulacja azotu zmniejszyła się w wyniku oprysku wszystkimi regulatorami wzrostu, a akumulacja fosforu wzrosła po zastosowaniu giberelliny. Giberelina i auksyna zwiększyły o 4,23% udział ziarna w gromadzeniu azotu, a wszystkie fitohormony w gromadzeniu fosforu.

## Introduction

Potassium supply enables to enhance the yield-forming effect of nitrogen (FOTYMA 2005, 1999, SZCZEPANIAK 2004), whereas potassium deficiency slows down the process of nitrogen uptake, thus reducing the rate of assimilation area growth. The uptake and transport of $NO_3^-$ ions are also related to potassium supply (MARSCHNER et al. 1996, MARSCHNER 1986).

Phosphorus is indispensable for normal growth and development of plants, animals and humans. Phosphorus requirement increases during plant development, to reach the maximum at the flowering stage through full ripeness. Phosphorus uptake increases during flowering, but often decreases at further developmental stages (WOJCIESKA et al. 1989, WIERZBOWSKA, NOWAK 1999, LÀSZTITY 1988).

The aim of the study was to determine the effects of growth regulators on nitrogen and phosphorus management in spring wheat plants under conditions of increasing potassium fertilization rates.

## Materials and Methods

A pot experiment was performed in a greenhouse of the University of Warmia and Mazury in Olsztyn, in the years 1999-2002. Mitscherlich pots (four replications) were filled with 6.5 kg of light loamy sand with pH 6.4 in 1 mol $KCl \cdot dm^{-3}$ and medium abundance in available compounds of P, K and Mg. Potassium was applied in the form of three chemical compounds, i.e. $KH_2PO_4$, KCl and $K_2SO_4$, at the following rates: 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g per pot. The other nutrients were supplied at rates considered optimum for normal growth and development of plants, i.e. N – 1.5 g per pot in the form

of $NH_4NO_3$, P – 0.5 g per pot in the form of $KH_2PO_4$, Mg – 0.25 g per pot in the form of $MgSO_4 \cdot 7H_2O$.

Total rates of phosphorus and magnesium were applied to the soil pre-sowing. Nitrogen and potassium were applied at three rates: 1/2 pre-sowing, 1/4 at the beginning of tillering and 1/4 at the stage of stem formation. 20 spring wheat plants, cv. Jasna, were grown in each pot.

The second experimental factor was the application of growth regulators (kinetin, gibberallin and auxin). They were applied twice over the growing season (at the beginning of tillering and at the beginning of flowering). Wheat plants were sprayed with 0.5 $dm^3$ of a solution of each growth regulator, at a concentration of 50 $mg \cdot dm^{-3}$. In the control treatment wheat plants were sprayed with an identical amount of distilled water.

Wheat plants were collected at the stage of processing maturity, and divided into the following organs: grain, glumes with rachis, stem, flag leaf, leaf 2$^{nd}$ and the remaining leaves. Dried and ground plant material was subjected to wet mineralization in concentrated sulfur acid with $H_2O_2$. Nitrogen was determined colorimetrically by the hypochlorite method, and phosphorus was determined by the vanadium-molybdenum method.

## Results and Discussion

Growth regulators, especially cytokinins, may indirectly induce remobilization of nitrogen compounds in the vegetative organs of plants, and accelerate their transport to developing seeds. This causes an increase of protein concentration, followed by an increase of the nitrogen content in seeds and a decrease in the amount of this element in straw (CZAPLA et al. 2000, WIERZBOWSKA, NOWAK 1999, WIERZBOWSKA et al. 2002).

In this study nitrogen concentration in spring wheat grain and vegetative organs was modified by both growth regulators and potassium fertilization (Table 1). Gibberellin and kinetic decreased, while auxin increased the nitrogen content of wheat grain. In glumes nitrogen concentration was slightly reduced by gibberellin and elevated by the remaining growth promoters, as compared with the control plants. In the remaining vegetative organs, especially in the flag leaf and the other leaves, growth regulators, particularly gibberellin, decreased nitrogen content (by 31.88% and 15.92% respectively), in comparison with the control treatment.

Low and medium potassium rates reduced, while high potassium rates elevated the nitrogen content in wheat grain, compared with the unfertilized treatment. In most cases potassium fertilization contributed to a decrease of nitrogen concentration in vegetative organs, particularly in stems and the

Table 1

Nitrogen content in organs of spring wheat ($g \cdot kg^{-1}$ d.w.)

| Plant growth regulators | Dose K in g per pot | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | |
| Grain | | | | | | | | |
| Control | 23.90 | 26.00 | 26.00 | 26.60 | 23.90 | 30.40 | 28.20 | 26.43 |
| Kinetin | 28.70 | 23.90 | 26.00 | 2600 | 27.30 | 23.90 | 26.00 | 25.97 |
| Gibberellin | 26.00 | 25.90 | 22.30 | 23.70 | 25.20 | 28.00 | 26.00 | 25.30 |
| Auxine | 26.70 | 24.50 | 29.40 | 23.00 | 26.70 | 28.90 | 32.90 | 27.44 |
| Means | 26.33 | 25.08 | 25.93 | 24.83 | 25.78 | 27.80 | 28.28 | – |
| Glume | | | | | | | | |
| Control | 11.10 | 10.20 | 8.30 | 13.90 | 6.50 | 13.00 | 9.20 | 10.31 |
| Kinetin | 13.00 | 11.10 | 12.00 | 9.20 | 11.10 | 8.30 | 11.00 | 10.81 |
| Gibberellin | 8.30 | 12.00 | 8.30 | 11.00 | 9.20 | 11.00 | 9.20 | 9.86 |
| Auxine | 11.00 | 9.20 | 19.20 | 7.40 | 12.00 | 11.00 | 14.00 | 11.97 |
| Means | 10.85 | 10.63 | 11.95 | 10.38 | 9.70 | 10.83 | 10.85 | – |
| Stem | | | | | | | | |
| Control | 12.30 | 8.40 | 9.80 | 12.30 | 11.40 | 9.80 | 10.50 | 10.64 |
| Kinetin | 15.70 | 11.40 | 12.30 | 9.10 | 12.60 | 12.30 | 10.50 | 11.99 |
| Gibberellin | 9.80 | 9.80 | 9.10 | 9.80 | 10.50 | 10.50 | 7.70 | 9.60 |
| Auxine | 8.40 | 8.60 | 9.80 | 9.10 | 9.10 | 12.30 | 11.20 | 9.79 |
| Means | 11.55 | 9.55 | 10.25 | 10.08 | 10.09 | 11.23 | 9.98 | – |
| Flag leaf | | | | | | | | |
| Control | 32.00 | 34.50 | 28.00 | 22.70 | 27.20 | 29.40 | 35.20 | 29.86 |
| Kinetin | 21.90 | 19.80 | 27.20 | 29.40 | 19.20 | 19.10 | 27.20 | 23.40 |
| Gibberellin | 18.80 | 21.00 | 19.80 | 24.50 | 16.50 | 18.30 | 23.50 | 20.34 |
| Auxine | 28.70 | 20.10 | 20.10 | 19.80 | 28.10 | 16.60 | 21.60 | 21.03 |
| Means | 25.35 | 21.90 | 23.78 | 24.10 | 22.75 | 20.85 | 26.88 | – |
| Second leaf | | | | | | | | |
| Control | 19.20 | 14.80 | 19.70 | 17.30 | 16.60 | 18.10 | 24.40 | 18.59 |
| Kinetin | 16.60 | 18.30 | 20.40 | 22.80 | 19.30 | 21.00 | 16.50 | 19.27 |
| Gibberellin | 21.30 | 16.60 | 20.10 | 20.40 | 15.00 | 15.30 | 21.90 | 18.66 |
| Auxine | 26.80 | 26.00 | 18.00 | 17.50 | 19.70 | 14.20 | 14.00 | 19.46 |
| Means | 20.98 | 18.93 | 19.55 | 19.50 | 17.65 | 17.15 | 19.20 | – |
| Remaining leaves | | | | | | | | |
| Control | 22.50 | 30.80 | 25.00 | 23.30 | 22.50 | 30.80 | 25.80 | 25.81 |
| Kinetin | 21.70 | 19.20 | 30.80 | 24.20 | 20.00 | 23.30 | 30.00 | 24.17 |
| Gibberellin | 22.50 | 23.10 | 25.00 | 25.50 | 19.20 | 13.80 | 25.80 | 21.70 |
| Auxine | 28.30 | 22.50 | 21.30 | 20.00 | 26.70 | 20.00 | 20.40 | 22.74 |
| Means | 23.75 | 23.90 | 25.53 | 22.50 | 22.10 | 21.98 | 25.50 | – |

oldest leaves. However, STĘPIEŃ et al. (2005) did not observe any effects of potassium fertilization on the amount of nitrogen in barley grain and straw. The studies on the effects of growth regulators on phosphorus concentration in cereal grain, conducted so far, provided inconsistent results (WIERZBOWSKA,

NOWAK 1999, 2002, BENEDYCKA et al. 2001, WIERZBOWSKA, SIENKIEWICZ 2004). In the present experiment growth regulators increased the phosphorus content in wheat grain (from 14.18% in the case of auxin to 29.26% in that of kinetin), glumes, straw and flag leaf (Table 2), and decreased the amount of this element in leaf 2nd and the other leaves, as compared with the control plants.

Table 2

Phosphorus content in organs of spring wheat ($g \cdot kg^{-1}$ d.w.)

| Plant growth regulators | Dose K in g per pot | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | |
| Grain | | | | | | | | |
| Control | 3.90 | 4.10 | 4.20 | 4.10 | 4.10 | 5.00 | 5.00 | 4.34 |
| Kinetin | 5.60 | 5.70 | 5.50 | 5.90 | 5.20 | 5.70 | 5.70 | 5.61 |
| Gibberellin | 5.20 | 5.50 | 5.30 | 5.00 | 5.40 | 5.40 | 5.20 | 5.29 |
| Auxine | 5.20 | 5.50 | 5.20 | 5.20 | 5.10 | 4.40 | 5.00 | 5.09 |
| Means | 4.98 | 5.20 | 5.05 | 5.05 | 4.95 | 5.10 | 5.23 | – |
| Glume | | | | | | | | |
| Control | 2.10 | 4.10 | 2.50 | 2.90 | 2.10 | 3.10 | 2.40 | 2.89 |
| Kinetin | 2.50 | 3.40 | 2.80 | 3.70 | 3.00 | 3.10 | 3.10 | 3.09 |
| Gibberellin | 3.70 | 3.10 | 3.00 | 3.10 | 3.40 | 4.40 | 3.40 | 3.44 |
| Auxine | 3.00 | 3.10 | 3.40 | 2.70 | 2.90 | 3.00 | 2.50 | 2.94 |
| Means | 2.83 | 3.43 | 2.93 | 3.35 | 2.85 | 3.40 | 2.85 | – |
| Stem | | | | | | | | |
| Control | 0.70 | 0.40 | 0.50 | 0.50 | 0.40 | 0.30 | 0.30 | 0.44 |
| Kinetin | 0.30 | 0.60 | 0.60 | 0.40 | 0.50 | 0.70 | 0.70 | 0.51 |
| Gibberellin | 0.30 | 0.60 | 0.60 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Auxine | 0.50 | 0.30 | 0.30 | 0.50 | 0.40 | 0.50 | 0.50 | 0.43 |
| Means | 0.45 | 0.48 | 0.50 | 0.48 | 0.45 | 0.50 | 0.50 | – |
| Flag leaf | | | | | | | | |
| Control | 1.60 | 2.10 | 1.40 | 1.50 | 1.90 | 2.20 | 1.40 | 1.73 |
| Kinetin | 2.00 | 2.00 | 1.70 | 1.70 | 2.10 | 2.20 | 2.10 | 1.97 |
| Gibberellin | 2.60 | 2.10 | 1.60 | 1.60 | 1.60 | 1.60 | 1.60 | 1.81 |
| Auxine | 2.00 | 1.90 | 1.90 | 2.00 | 2.00 | 2.00 | 1.80 | 1.94 |
| Means | 2.05 | 2.03 | 1.65 | 1.70 | 1.90 | 2.00 | 1.73 | – |
| Second leaf | | | | | | | | |
| Control | 1.50 | 2.10 | 1.90 | 1.30 | 1.40 | 1.30 | 1.00 | 1.50 |
| Kinetin | 1.40 | 2.10 | 1.30 | 1.40 | 1.40 | 1.40 | 1.30 | 1.47 |
| Gibberellin | 1.10 | 1.50 | 1.50 | 1.50 | 1.30 | 1.60 | 1.50 | 1.46 |
| Auxine | 1.30 | 1.20 | 1.20 | 1.30 | 1.30 | 1.30 | 0.90 | 1.21 |
| Means | 1.33 | 1.73 | 1.48 | 1.38 | 1.35 | 1.45 | 1.18 | – |
| Remaining leaves | | | | | | | | |
| Control | 2.70 | 3.00 | 2.50 | 2.40 | 2.20 | 2.60 | 2.20 | 2.51 |
| Kinetin | 2.50 | 2.70 | 2.30 | 2.60 | 2.20 | 2.50 | 2.90 | 2.53 |
| Gibberellin | 2.20 | 2.20 | 2.00 | 1.90 | 2.10 | 2.40 | 1.90 | 2.10 |
| Auxine | 2.40 | 2.10 | 2.20 | 1.90 | 1.80 | 2.60 | 2.20 | 2.17 |
| Means | 2.45 | 2.50 | 2.25 | 2.20 | 2.08 | 2.53 | 2.30 | – |

Increasing potassium fertilization rates positively affected the phosphorus content in wheat grain. The effect of potassium levels on phosphorus concentration in vegetative organs was not unequivocal.

The highest nitrogen accumulation was recorded in control wheat plants, which resulted primarily from higher grain weight, in comparison with wheat plants sprayed with growth promoters (Table 3). The lowest nitrogen accumulation (by 19.13% lower than in the control treatment) was observed following kinetin application. Nitrogen accumulation in particular organs and whole wheat plants was modified by potassium fertilization to a slight degree only, and fertilizer rates produced differentiated effects.

Table 3

Nitrogen accumulation in spring wheat plants (mg N per plant)

| Plant growth regulators / Dose K (g per pot) | Grain | Glume | Stem | Flag leaf | Second leaf | Remaining leaves | Total |
|---|---|---|---|---|---|---|---|
| Control | 27.51 | 4.48 | 6.86 | 10.06 | 3.63 | 12.30 | 64.83 |
| Kinetin | 21.03 | 3.88 | 6.39 | 6.72 | 3.50 | 10.91 | 52.43 |
| Gibberellin | 23.40 | 4.16 | 8.65 | 5.96 | 3.64 | 10.00 | 55.81 |
| Auxine | 26.28 | 4.56 | 6.12 | 6.60 | 3.20 | 9.52 | 56.28 |
| 0.0 | 22.81 | 4.23 | 7.40 | 8.32 | 4.07 | 12.40 | 59.23 |
| 0.5 | 22.32 | 4.43 | 6.28 | 7.08 | 3.38 | 10.85 | 54.35 |
| 1.0 | 26.36 | 5.05 | 7.02 | 7.50 | 3.44 | 11.72 | 61.09 |
| 1.5 | 23.38 | 4.31 | 7.01 | 7.00 | 3.49 | 9.60 | 54.79 |
| 2.0 | 24.75 | 3.41 | 7.24 | 5.86 | 2.95 | 9.00 | 53.22 |
| 2.5 | 25.39 | 4.26 | 7.61 | 6.26 | 3.18 | 9.52 | 56.21 |
| 3.0 | 26.86 | 4.18 | 6.48 | 9.33 | 3.93 | 11.69 | 62.47 |

Gibberellin increased phosphorus accumulation in wheat, by 7.00% in comparison with the control plants, mainly due to the highest concentration of this element in grain (+13.18%) and stems (+60.71%) – Table 4. The other regulators, especially auxin, increased phosphorus accumulation in grain, but slightly decreased in whole plants.

Potassium fertilization had a positive effect on phosphorus accumulation both in wheat grain and whole plants, because potassium supply stimulated phosphate anion uptake.

Phytohormones had different effects on nitrogen accumulation in particular wheat organs. Kinetin and auxin may stimulate remobilization of nitrogen compounds in vegetative organs, and nitrogen transport to grain (WIERZBOWSKA, NOWAK 1999, CZAPLA et al. 2000). In other studies growth regulators, particularly gibberellin, reduced nitrogen accumulation in grain. The amount of nitrogen accumulated in glumes and stems decreased as well, and consideably increased in leaves (WIERZBOWSKA et al. 2002).

Table 4

Phosphorus accumulation in spring wheat plants (mg N per plant)

| Plant growth regulators / Dose K (g per pot) | Grain | Glume | Stem | Flag leaf | Second leaf | Remaining leaves | Total |
|---|---|---|---|---|---|---|---|
| Control | 4.39 | 1.27 | 0.28 | 0.57 | 0.29 | 1.20 | 8.00 |
| Kinetin | 4.64 | 1.11 | 0.27 | 0.56 | 0.27 | 1.14 | 7.98 |
| Gibberellin | 4.85 | 1.45 | 0.45 | 0.55 | 0.28 | 0.97 | 8.56 |
| Auxine | 4.73 | 1.12 | 0.27 | 0.62 | 0.20 | 0.91 | 7.84 |
| 0.0 | 4.29 | 1.11 | 0.29 | 0.65 | 0.26 | 1.29 | 7.89 |
| 0.5 | 4.49 | 1.44 | 0.32 | 0.65 | 0.32 | 1.13 | 8.35 |
| 1.0 | 4.45 | 1.26 | 0.35 | 0.51 | 0.26 | 1.03 | 7.87 |
| 1.5 | 5.08 | 1.39 | 0.33 | 0.49 | 0.24 | 0.94 | 8.48 |
| 2.0 | 4.66 | 1.01 | 0.27 | 0.51 | 0.23 | 0.86 | 7.54 |
| 2.5 | 4.90 | 1.34 | 0.34 | 0.60 | 0.27 | 1.08 | 8.53 |
| 3.0 | 4.71 | 1.10 | 0.32 | 0.59 | 0.24 | 1.06 | 8.02 |

Gibberellin and auxin intensified nitrogen translocation from the oldest leaves and flag leaf to grain and glumes, which enabled to increase the accumulation of this element in grain by 4.23% (Figure 1). Kinetin inhibited nitrogen remobilization in the vegetative organs of wheat plants, and its translocation to grain. Potassium fertilization positively affected nitrogen transport in wheat plants; applied at a rate of 2.0 g K per pot, potassium contributed to an approx. 8% increase of nitrogen accumulation in grain.

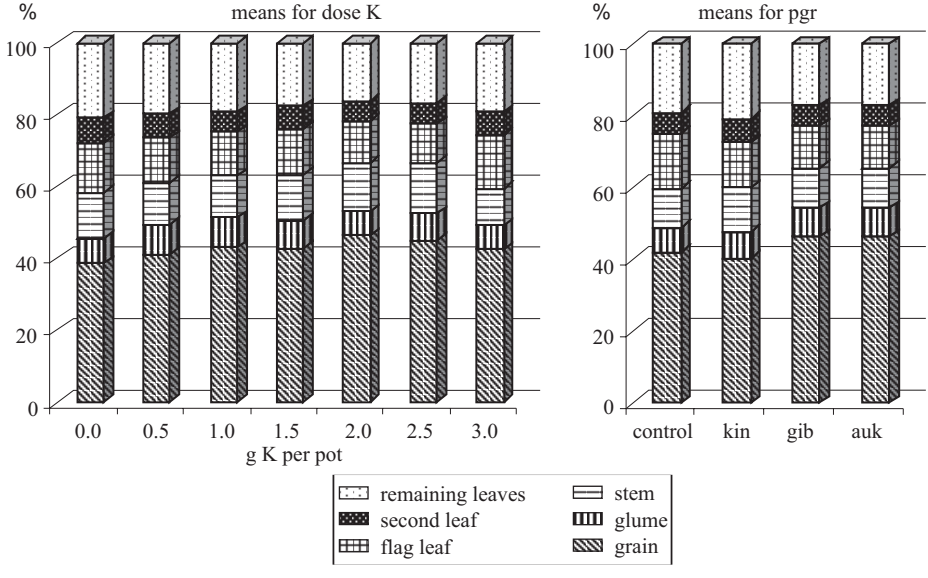


Fig. 1. Nitrogen distribution in spring wheat plants (control, kin – kinetin, gib – gibberellin, auk – auxine)

Growth regulators enhanced also phosphorus remobilization in the oldest leaves, and promoted the translocation of this element to grain. This enabled to increase phosphorus accumulation in the generative organs of wheat plants by 1.80% to 5.75% (Figure 2). BENEDYCKA et al. (2001) also reported a beneficial effect of auxin (IBA) on phosphorus accumulation in grain.

An increase of potassium fertilization, in a range of 0.0 to 2.0 g K per pot, resulted in an increase in the contribution of grain to phosphorus retention in wheat, from 54.35% to 61.73%.

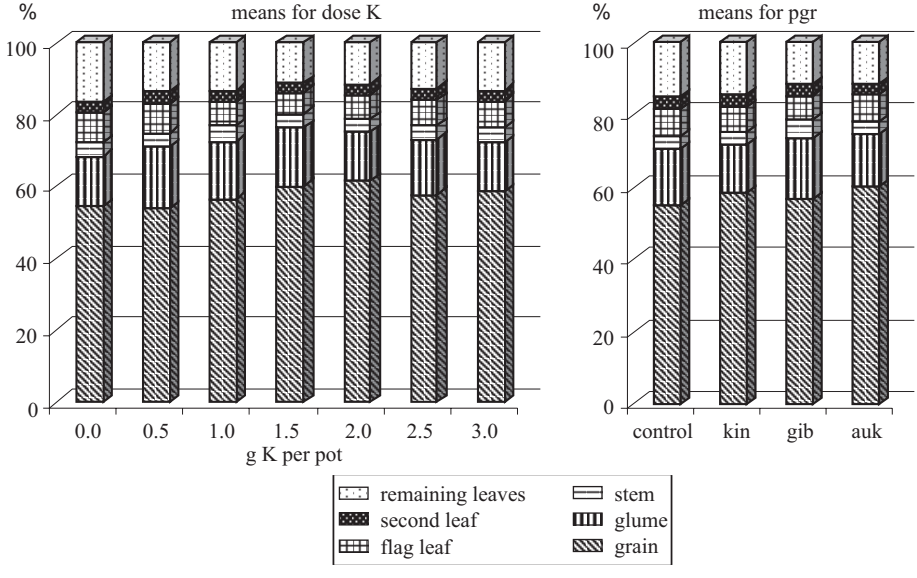


Fig. 2. Phosphorus distribution in spring wheat plants (legends as Fig. 1)

The results of the study indicate that phytohormones may significantly affect nitrogen and phosphorus management in spring wheat. These compounds influence the basic physiological functions of plants, thus stimulating the development of generative and vegetative organs, and modifying the content of nitrogen and phosphorus. Especially gibberellin and auxin produced good effects. It should be also stressed that potassium supply regulates water economy in plants and determines proper functioning of cell membranes, which in turn contributes to nitrogen and phosphorus translocation to grain.

## Conclusions

1. Auxin increased the nitrogen content in wheat grain and glumes, whereas kinetin and gibberellin decreased nitrogen concentration in wheat grain and vegetative organs.

2. All phytohormones increased the phosphorus content in wheat grain, glumes and flag leaf, and in the case of kinetin and gibberellin the concentration of this element increased also in wheat stems.

3. Low and medium potassium rates decreased, and high potassium rates increased the nitrogen content in wheat. Phosphorus concentration in wheat grain increased proportionally to potassium rates.

4. Phytohormones, especially kinetin, reduced nitrogen accumulation in whole wheat plants and grain. Phosphorus concentration in wheat grain increased as a result of the application of all growth regulators, while the phosphorus content in whole plants increased in the case of gibberellin only.

5. Gibberellin and auxin increased nitrogen accumulation in grain by 4.23%, and all phytohormones increased phosphorus accumulation in grain. The highest content of nitrogen and phosphorus in wheat grain was achieved when potassium was applied at a rate of 2.0 g K per pot.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 21.02.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 101-110, Y. 2006

# NITRIFICATION PROCESS IN SOIL CONTAMINATED WITH COBALT

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Key words: nitrifying activity, cobalt, soil.

Abstract

The effect of soil contamination with cobalt at 200 and 400 mg Co · $kg^{-1}$ d.m. of soil was analyzed in a laboratory experiment. The soil used for the trials was light loam of 7.0 pH.

The results suggested that the influence of cobalt on nitrification process depended on the dose of this element applied. The lower rate of 200 mg Co · $kg^{-1}$ d.m. of soil had little impact of nitrification, while the higher dose of 400 mg Co · $kg^{-1}$ d.m. of soil considerably inhibited the process. The nitrifying activity of soil under the influence of the higher cobalt dose was 2.3-fold depressed at maximum. This effect, however, was short-lived and disappeared on day 60 of the experiment. Cobalt present in excessive amounts in soil contributed to the lowering of the concentration of mineral nitrogen ($N\text{-}NH_4^+$ + $N\text{-}NO_3^-$).

## PROCES NITRYFIKACJI W GLEBIE ZANIECZYSZCZONEJ KOBALTEM

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Słowa kluczowe: aktywność nitryfikacyjna, kobalt, gleba.

Abstrakt

W doświadczeniu laboratoryjnym badano wpływ zanieczyszczenia gleby kobaltem w dawce 200 i 400 mg Co · $kg^{-1}$ s.m gleby na przebieg procesu nitryfikacji. Do badań użyto materiału glebowego o składzie granulometrycznym gliny lekkiej i pH 7,0.

Stwierdzono, że oddziaływanie kobaltu na proces nitryfikacji zależało od wielkości zastosowanej dawki tego pierwiastka. Dawka 200 mg Co · $kg^{-1}$ s. m. gleby wywierała niewielki wpływ na przebieg tego procesu, natomiast dawka 400 mg Co · $kg^{-1}$ istotnie go hamowała. Aktywność nitryfikacyjna gleby pod wpływem wyższej dawki kobaltu była zmniejszona nawet 2,3-krotnie. Działanie to było jednak krótkotrwałe i zanikało w 60. dniu trwania doświadczenia. Kobalt występujący w nadmiarze w glebie przyczynił się do obniżenia zawartości azotu mineralnego ($N\text{-}NH_4^+$ + $N\text{-}NO_3^-$).

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## Introduction

The concentration of cobalt in soil varies from 0.1 to 100 mg mg $Co \cdot kg^{-1}$ (KABATA-PENDIAS, PENDIAS 1999). In Poland soils typically contain from 3 to 8 mg $Co \cdot kg^{-1}$ d.m. of soil. Cobalt can easily undergo sorption by organic matter. The admissible level of this element in the surface horizon of soils in Poland (0-30 cm) should not exceed 20 $mg \cdot kg^{-1}$. In soil solutions, cobalt appears in concentrations between 0.3 and 87 μg $dm^{-3}$ (KABATA-PENDIAS, PENDIAS 1999). The admissible amount of this element in the surface horizon of soils in Poland (0-30 cm) should not exceed 20 $mg \cdot kg^{-1}$ (Ordinance of the Minister for the Environment 2002).

The literature dealing with the issues of the impact of heavy metals in microbiological and biochemical activity of soil is quite abundant (GILLER et al. 1998). What seems to lack is sufficient information of a possible negative influence of cobalt on the primary processes occurring in soil, including nitrification, which rather faithfully reflects the quality of soil (BARABASZ et al. 2002, BRIERLEY, WOOD 2001, DE BOER, KOWALCHUK 2001, KUCHARSKI 1985, KUCHARSKI 2000). The more intensive the nitrification process, the better the soil's quality and fertility. The factors that modify the soil's properties exert strong influence of the course of nitrification. This means and the process of nitrification is a good indicator of soil pollution with all xenobiotics, including heavy metals (DENI, PENNICKX 1999, KARA et al. 2004, PRZYBULEWSKA et al. 2003, WYSZKOWSKA 2002). In some cases nitrification may lead to nitrogen losses, which is why the process is artificially impeded by selective nitrification inhibitors (KUCHARSKI 1985, AULAKH et al. 2001, CARRASCO et al. 2004, SHEN et al. 2003).

For these reasons, the nitrification process has been chosen in the present study for analysis of a possible negative effect of cobalt on soil metabolism. Thus, the aim of the study has been to determine the effect of cobalt soil contamination on the course of the process of nitrification.

## Material and Methods

The experiment carried out under laboratory conditions. Soil samples collected from the arable humus horizon were used for the trials. In its natural state, this was proper brown soil formed from light loam (61% sand, 12% silt, and 27% fine particles), of the $pH_{KCl}$ equal 7.0. The soil contained 7.7 g $C_{org} \cdot kg^{-1}$. Its hydrolytic acidity was 13.5 $mmol \cdot kg^{-1}$, and sum of alkaline exchangeable cations was 113.0 $mmol \cdot kg^{-1}$.

The tested variables were:
– cobalt contamination in mg $Co \cdot kg^{-1}$ d.m. of soil: 0, 200 and 400;
– N rate in mg $N \cdot kg^{-1}$ d.m. of soil: 0 and 250;
– time of soil incubation in days: 0, 20, 40 and 60.

The trials were carried out on 100 $cm^3$ beakers. Prior to the establishment of the experiment, the soil was passed through a sieve with a mesh diameter of 1 mm. After that samples weighing 50 g d. m. were placed in beakers. Aqueous solutions of $CoCl_2 \cdot 6H_2O$ (factor 1) and $(NH_4)_2SO_4$ (factor 2) were added to appropriate objects. The content in each beaker was carefully mixed and the soil moisture was brought up (with distilled water) to the level of 60% of water capillary capacity. The soils maintained in this state of moisture content were kept in a incubator at 25°C (factor 3). The experiment was designed with 6 replications. In total 144 beakers with soil were used. When the time set for the experiment (factor 3) elapsed, some of the beakers were removed and the soil was used for determination of the concentration of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ and soil reaction.

## Extraction of mineral nitrogen

The whole mass of the incubated soil in a beaker was transferred quantitatively to a 300 $cm^3$ flask using 250 $cm^3$ of 1% aqueous solution of $K_2SO_4$. The suspension in the flask was shaken from 0.5 h in a laboratory mixer ELPIN$_{SC}$$^+$358A. Then the flask content was filtered and the filtrate was analyzed to determine its reaction and the concentration of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$.

## Determination of $N\text{-}NH_4^+$ concentration

Depending on the expected amount of ammonium nitrogen, 50 $cm^3$ measuring flasks were filled with 1 to 4 $cm^3$ of clear soil filtrate. Afterwards, 30 $cm^3$ distilled $H_2O$ was added, followed with 2 $cm^3$ 25% aqueous solution of sodium-potassium tartrate ($C_4H_4KNaO_6 \cdot 4H_2O$). The contents of each flasks were mixed by rotation, and then 2 $cm^3$ Nessler's reagent was added. The flask was subsequently filled up with distilled water to the capacity of 50 $cm^3$, and after 10 minutes extinction against the control samples was performed on a PerkinElmer Lambda 25 spectrophotometer (410 nm). Based on the extinction of the analyzed sample and model curve, the content of $N\text{-}NH_4^+$ in 1 kg d.m. of oil was calculated. Aqueous solution of $(NH_4)_2SO_4$ containing 10 mg N in 1 $cm^3$ was used to establish the model curve.

### Determination of $N\text{-}NO_3^-$ concentration

In order to determine the concentration of ammonium nitrogen, 100 $cm^3$ beakers were filled with 0.5 $cm^3$ of aqueous solution of 0.1 M NaOH. Then from 1 to 4 $cm^3$ (depending on the expected amount of $N\text{-}NO_3^-$) of clear soil filtrate were filled, and the content of the beakers was completely evaporated (until dry state) in a water bath. Having evaporated the filtrate, 2 $cm^3$ of phenoldisulphonic acid was added to each beaker. The precipitate left after the evaporation was dissolved in phenoldisulphonic acid. Then 0.5 $cm^3$ distilled water was added, the contents were mixed and 20 $cm^3$ of alkalising mixture was added (150 g NaOH + 23 g disodium versenate [$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$] in 1 $dm^3$ distilled water). When the beakers had been cooled, their content was transferred quantitatively using distilled water to 50 $cm^3$ measuring flasks. The flasks were filled up to full capacity with water and, after mixing carefully the whole content, extinction against a control sample was carried out on a PerkinElmer Lambda 25 spectrophotometer (410 nm). Taking into consideration the extinction of the analyzed sample and the model curve, the content of $N\text{-}NO_3^-$, which contained 10 mg N in 1 $cm^3$, was calculated.

The results enabled us to draw conclusions on the course of nitrification. In order to obtain more detailed knowledge of this process, nitrification activity of the soil was assayed with Kandler's method (KANDELER 1996). The results were elaborate statistically with Duncan;s multiple gap test, and three-factor analysis of variance. All statistical calculations were completed using the software package Statistica (Statsoft 2003).

## Results and Discussion

The nitrifying activity of the soil tested was nearly 5% (Figure 1). The contamination with 200 mg Co $kg^{-1}$ d.m. of soil depressed the nitrification process by just 4.56%, while the double high dose of cobalt (400 mg $Co \cdot kg^{-1}$ d.m. of soil) caused a 2.3-fold decline in the nitrification process. This was also reflected in the amount of $N\text{-}NO_3^-$, especially in the soil fertilized by sulphate of ammonia (Figure 2). The soil treated with 400 $Co \cdot kg^{-1}$ contained 22.7% less nitrate nitrogen than the control soil, which was not polluted by adding cobalt. In the soil which received 200 mg $Co \cdot kg^{-1}$, this difference was set at just 5.0%.

The inhibition of nitrification by 400 mg $Co \cdot kg^{-1}$ was not persistent (Figure 3). This rate depressed the concentration of $N\text{-}NO_3^-$ by 50% during the first 20 days. With time, the effect became weaker to disappear completely on day 60.

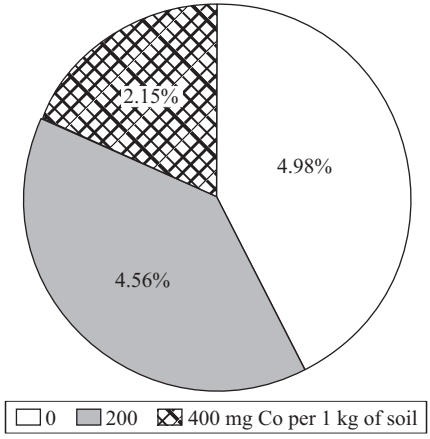


Fig. 1. Effect of cobalt on the nitrification activity of soil express as percentage of nitrificated N $d^{-1}$

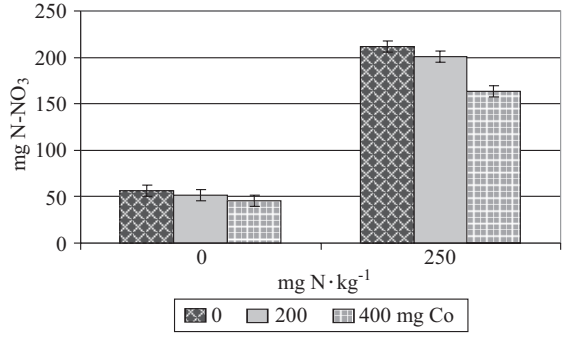


Fig. 2. The content of $N\text{-}NO_3$ (in 1 kg d.m. of soil) depending on the rate of cobalt and rate of nitrogen

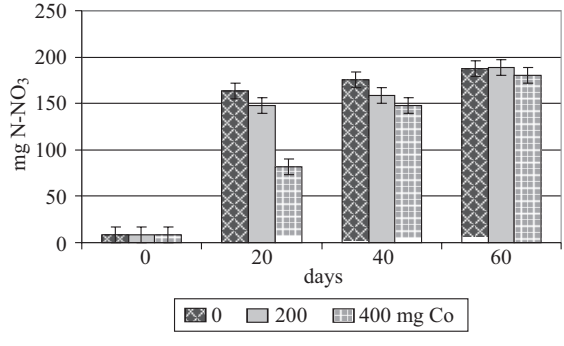


Fig. 3. The content of $N\text{-}NO_3$ (in 1 kg d.m. of soil) depending on the rate of cobalt and time of soil incubation

The unfavourable effect of the higher cobalt contamination rate on the process of nitrification is also made evident by the continually elevated (as compared to the control soil) level of $N\text{-}NH_4^+$ in the soil polluted with cobalt (Figure 4). The concentration of $N\text{-}NH_4^+$ was negatively correlated (Table 1) with the content of $N\text{-}NO_3^-$. The correlation coefficients varied from -0.95 to -0.98. With time, the effect of cobalt on the quantity of $N\text{-}NH_4^+$ became weaker, which is reflected by its depressed effect on the process of nitrification (Figure 5).

The total content of $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ in soil (Figures 6, 7) shows that the cobalt contamination periodically restricted the amount of nitrogen available to plants. This effect, nonetheless, was not permanent and vanished completely before the termination of the experiment (day 60). This could result from the sorption of cobalt by organic matter, which this metal undergoes

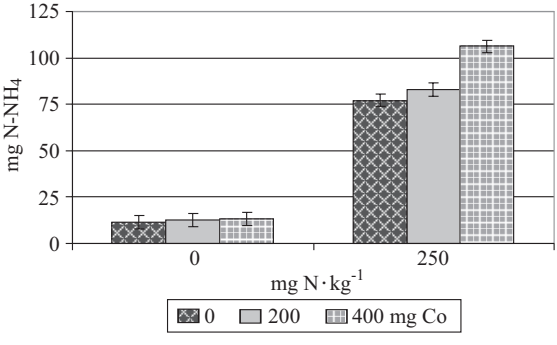


Fig. 4. The content of $N\text{-}NH_4$ (in 1 kg d.m. of soil) depending on the rate of cobalt and rate of nitrogen

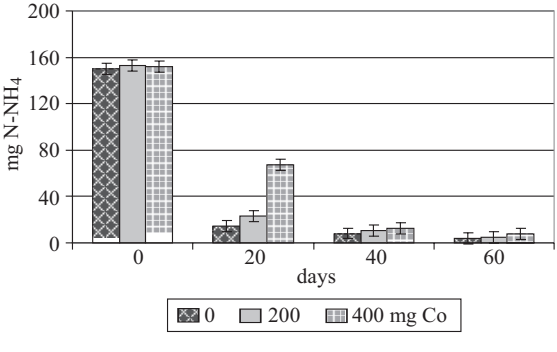


Fig. 5. The content of $N\text{-}NH_4$ (in 1 kg d.m. of soil) depending on the rate of cobalt and time of soil incubation

Table 1

Correlation coefficients between the rate of cobalt and forms of mineral nitrogen in soil

| Variable | Co rate | $N\text{-}NO_3^-$ content |
|---|---|---|
| Without N | | |
| $N\text{-}NO_3^-$ | -0.16 | |
| $N\text{-}NH_4^+$ | 0.08 | -0.98* |
| $N\text{-}NO_3^- + N\text{-}NH_4^+$ | -0.20 | 0.99* |
| With N | | |
| $N\text{-}NO_3^-$ | -0.16 | |
| $N\text{-}NH_4^+$ | 0.11 | -0.95* |
| $N\text{-}NO_3^- + N\text{-}NH_4^+$ | -0.21 | 0.30* |

* correlation coefficients significant for $p < 0.01$, $n = 72$

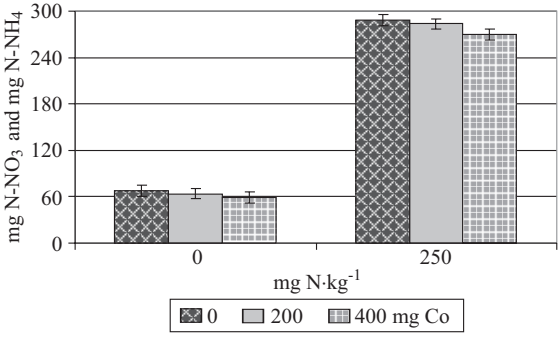


Fig. 6. The total content of $N\text{-}NO_3$ and $N\text{-}NH_4$ (in 1 kg d.m. of soil) depending on the rate of cobalt and rate of nitrogen

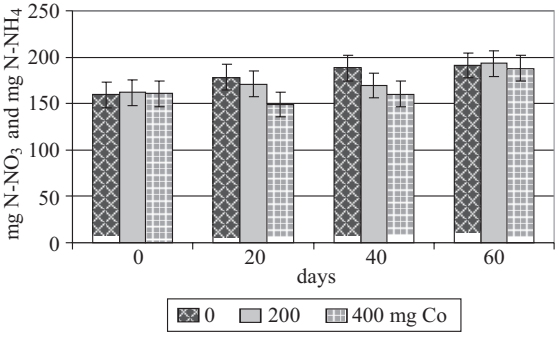


Fig. 7. The total content of $N\text{-}NO_3$ and $N\text{-}NH_4$ (in 1 kg d.m. of soil) depending on the rate of cobalt and time of soil incubation

quite readily (KABATA-PENDIAS, PENDIAS 1999). Other kinds of contamination, e.g. with fuel oil (WYSZKOWSKA, KUCHARSKI 2004), or chromium (WYSZKOWSKA 2002) cause long-lasting changes in the nitrification process and immobilization of mineral nitrogen. Hexavalent chromium (WYSZKOWSKA 2002), even when applied at a low dose (10 mg $kg^{-1}$), depressed this process by up to 80%. Such strong influence was not determined in the case of cobalt, although the lower dose tested was 20-fold higher than the level of chromium.

The adverse, albeit brief, impact of cobalt contamination of soil on the process of nitrification may possibly derive from the direct negative effect of this element on nitrifying bacteria, as was in the case of chromium (WYSZKOWSKA 2002). In part, it could have resulted from the unfavourable change in th soil reaction caused by $CoCl_2 \cdot 6H_2O$ (Table 2). In addition, these undesirable effects may have been aggravated by the soil acidification associated with the nitrate nitrogen being produced. According to SAUVE et al. (1999), it is difficult to determine the mechanism of depressing the nitrification potential of soils contaminated by metals. This is due to the fact that both organic matter and soil;s pH produce direct and indirect effects on speciations of metals. There is little doubt, however, that heavy metals present in excessive amounts in soil decrease the total biomass of bacteria (GILLER et al. 1998), and inhibit the enzymatic activity of soil (HINOJOSA et al. 2004). These two phenomena may play a crucial role in the modification of the nitrification process in soils polluted with metals, with the degree of contamination being an important factor. In a study reported by YIN et al. (2003), zinc depressed the activity of nitrifying soil enzymes only when applied at rates higher than 200 mg per kg of soil, which means that the influence of this metal was similar to that of cobalt, which could significantly inhibit nitrification if used at 400 mg per kg of soil, but was far less effective when applied at a lower dose (200 mg).

Table 2

pH soil contaminated with cobalt

| Time of soil incubation in days | N rate in mg · $kg^{-1}$ of soil | | | | | |
|---|---|---|---|---|---|---|
| | 0 | | | 250 | | |
| | Co rate in mg · $kg^{-1}$ of soil | | | | | |
| | 0 | 200 | 400 | 0 | 200 | 400 |
| 0 | 6.95 | 6.63 | 6.45 | 6.86 | 6.63 | 6.45 |
| 20 | 6.69 | 6.69 | 6.66 | 6.36 | 6.36 | 6.34 |
| 40 | 6.66 | 6.63 | 6.60 | 6.34 | 6.36 | 6.27 |
| 60 | 6.63 | 6.60 | 6.45 | 6.31 | 6.25 | 6.13 |
| $LSD_{p=0.01}$ | a – 0.02; b – 0.02; c – 0.02; a x b – 0.03; a x c – 0.04; b x c – 0.03; a x b x c – 0.05 | | | | | |

LSD for: a – Co rate, b – N rate, c – time of soil incubation

## Conclusions

1. The influence of cobalt on the nitrification process in soil depended in the rate of this element applied: 200 mg Co · $kg^{-1}$ d.m. of soil had little effect on the course of the nitrifying process, whereas 400 mg Co · $kg^{-1}$ inhibited significantly the activity of nitrifying bacteria. The nitrifying activity of soil under the influence of the higher cobalt rate was 2.3-fold lower. This effect, however, was short-lived and disappeared completely on day 60 of the experiment.

2. The excessive amounts of cobalt in soil contributed to the depression of the concentration of mineral nitrogen ($N\text{-}NH_4^+$ + $N\text{-}NO_3^-$).

Translated by JOLANTA IDŹKOWSKA

Accepted for print 11.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 111-120, Y. 2006

# NITRIFICATION PROCESS IN NICKEL-CONTAMINATED SOIL*

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Key words: nickel, soil contamination, nitrification.

Abstract

A laboratory experiment was established to study the effect of soil contamination with nickel at a dose from 100 to 400 mg Ni · $kg^{-1}$ of soil on the process of nitrification. The soil samples used for the trials consisted of typical brown soil formed from heavy loamy sand and light silty clay. Nickel was introduced in the form of $NiCl_2 \cdot 6H_2O$ and $NiSO_4 \cdot 7H_2O$. The concentration of $N\text{-}NO_3$ and $N\text{-}NH_4$ was determined on days 14, 28, 42 and 56 of incubation. During the whole experiment, a constant moisture level was maintain, equal 60% of the capillary water capacity.

The results of the experiment showed that contamination of samples of typical brown soil formed from heavy loamy sand and typical brown soil formed from light clay with nickel at a dose of 100 to 400 mg Ni · $kg^{-1}$ of soil had an inhibitory effect of the process of nitrification. Nickel chloride was a stronger inhibitor that nickel sulphate. The negative effect of nickel on the course of nitrification was much weaker in a more compact soil (light soil) than in a lighter soil (heavy loamy sand). Determinations of nitrifying activity in soil can be a good indicator of the degree of soil pollution with nickel.

## PROCES NITRYFIKACJI W GLEBIE ZANIECZYSZCZONEJ NIKLEM

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Słowa kluczowe: nikiel, zanieczyszczenie gleby, nitryfikacja.

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* Suppored by the Ministry of Education and Science of the Poland, Project No. 2 P06S 014 28

Abstrakt

W doświadczeniu laboratoryjnym badano wpływ zanieczyszczenia gleby niklem w ilości od 100 do 400 mg Ni · kg$^{-1}$ gleby na przebieg procesu nitryfikacji. Do badań wykorzystano próbki gleby brunatnej właściwej, wytworzonej z piasku gliniastego mocnego i gliny lekkiej pylastej. Nikiel wprowadzono w postaci $NiCl_2 \cdot 6H_2O$ oraz $NiSO_4 \cdot 7H_2O$. Zawartość $N\text{-}NO_3$ i $N\text{-}NH_4$ oznaczano w 14., 28., 42. i 56. dniu inkubacji. Przez cały okres trwania doświadczenia utrzymywano stałą wilgotność gleby na poziomie 60% kapilarnej pojemności wodnej.

W wyniku badań stwierdzono, że zanieczyszczenie próbek gleby brunatnej właściwej wytworzonej z piasku gliniastego mocnego i brunatnej właściwej wytworzonej z gliny lekkiej niklem w ilości od 100 do 400 mg Ni · kg$^{-1}$ wpłynęło hamująco na proces nitryfikacji. Silniejszym inhibitorem nitryfikacji był chlorek niklu niż siarczan niklu. Negatywny wpływ niklu na proces nitryfikacji był znacznie słabszy w glebie zwięźlejszej (glinie lekkiej) niż lżejszej (piasku gliniastym mocnym). Oznaczenie aktywności nitryfikacyjnej może być dobrym wskaźnikiem stanu jej zanieczyszczenia niklem.

## Introduction

Heavy metals introduced to natural environment and accumulated in soil affect soil biological activity (ANTIL et al. 2001, CIEĆKO et al. 2004, ROPEK, PARA 2003, OMAR, ISMAIL 1999). Mircoorganisms are the most essential component of the environment in providing nutrients available to plants. For agriculture, the most important are nitrogen-transforming microorganisms (BRIERLEY, WOOD 2001, BARABASZ et al. 2002). Out of all types of nitrogen transformation processes, researchers most often determine N mineralisation activity and nitrification (MACCARTY 1999, BURGER, JACKSON 2003). In many soils nitrification is a key process in nitrogen conversion, as it transforms $NH_4^+$ exchangeable cations into $NO_2^-$ and $NO_3^-$ mobile anions, which undergo subsequent transformation (denitrification to $NO_2$ and $N_2O$) or else are easily washed away from soil (DINCER, KARGI 2000, KUCHARSKI 2000, SIMEK 2000). The following are the principal factors limiting the intensity of oxygenation of non-reduced mineral nitrogen forms by nitrifying bacteria: amount of oxygenated substrate, pH of the environment, its oxygenation, Corg : N ratio, contamination of soil with various xenobiotics, including heavy metals, temperature and moisture (DENI, PENNINCKX 1999, PRZYBULEWSKA et al. 2003, WYSZKOWSKA, KUCHARSKI 2004). The type of soil is another important factor influencing the process of nitrification, and soils of high sorptive capacity can reduce the toxic effect of xenobiotics, which means that nitrification in such soils can be more intensive (PRZYBULEWSKA et al. 2003, MYŚKÓW et al. 1996, WYSZKOWSKA, KUCHARSKI 2001). On the other hand, in soils which are excessively compact, the air and water conditions may not favour multiplication of nitrifying bacteria. Therefore, it cannot be excluded that two factors which inhibit nitrification, such as poor oxygenation and contamination with xenobiotics, may overlap.

These considerations have encouraged us to undertake a study with an aim of determining the influence of nickel contamination in soils of different granulometric composition on the rate of nitrification. Another objective of the trials was to find out whether determination of nitrifying activity in soils can serve as a good indicator of the degree of soil contamination with this metal.

## Material and Methods

A laboratory experiment (in 6 replications) was conducted on soil material sampled from the arable humus horizon. Two types of soil Eutric Camisols according to WRB (1998) were examined. A more detailed description of the soils is presented in Table 1. The experiment was performed in flasks of the capacity of 100 $cm^3$. In each flask 50 g of air dry soil, passed through a sieve of a mesh size of 2 mm, was placed. The soil samples were contaminated with different rates of nickel (factor 1) in the form of two chemical compounds (factor 2) and enriched with nitrogen in the form of ammonium sulphate (factor 3). The soil samples were carefully mixed and then their moisture content was brought up to 60% of capillary water capacity. Afterwards, they were incubated for 14-56 days (factor 4) at a constant temperature of 25°C. Thus, the variables in the experiment embraced:

– dose of nickel contamination in mg Ni $kg^{-1}$ of soil: 0, 100, 200, 300, 400;
– type of a nickel compound: $NiCl_2 \cdot 6H_2O$ and $NiSO_4 \cdot 7H_2O$;
– two types of soil Eutric Camisols (characteristics of soils are in Table 1);
– rate of ammonium sulphate converted into N in mg $kg^{-1}$ of soil: 0 and 250;
– time of soil incubation in days: 14, 28, 42 and 56 days.

Table 1

Characteristics of Eutric Cambisols soils

| Type of soil | Granulometric composition (mm) | | | $pH_{KCl}$ | $C_{organic}$ ($g \cdot kg^{-1}$) | CEC (mmol (+) $kg^{-1}$) | BS (%) |
|---|---|---|---|---|---|---|---|
| | 1-0.1 | 0.1-0.02 | < 0.02 | | | | |
| hls | 66 | 17 | 17 | 6.9 | 7.5 | 100.5 | 88.9 |
| lsc | 42 | 32 | 26 | 7.0 | 11.2 | 167.8 | 94.7 |

hls – heavy loamy sand, lsc – light silty clay
CEC – cation exchange capacity, BS – base saturation

The actual process of the nitrification process was deduced from the content of the mineral nitrogen forms ($N\text{-}NH_4$ and $N\text{-}NO_3$) after 14, 28, 42 and 56 days of incubation. The experiment was curried out and content of mineral nitrogen was determined according to SCHINNER at al. (1996).

The results were subjected to statistical analysis using Duncan's multiple range test and a four-factor analysis of variance. All statistical computations were aided by the software package Statistica (Statsoft, Inc. 2004).

## Results and Discussion

Soil contamination with nickel had an indisputably negative influence on the course of nitrification (Table 2). This adverse influence of nickel, however, depended on the degree of soil contamination, duration of the experiment and type of soil. The nitrification of ammonium nitrogen was particularly strongly affected by the rates of 300 and 400 mg $Ni \cdot kg^{-1}$, which inhibited this process by up to 70-80% during the first 28 days. Smaller rates of the metal (100 and 300 mg $Ni \cdot kg^{-1}$) also depressed the nitrifying activity of soil, but the inhibition was much weaker. Correlation coefficients between the amount of $N\text{-}NO_3$ produced in the nitrification process and the content of nickel in soil were highly negative and equalled -0.93 in heavy loamy sand not fertilised with nitrogen and -0.78 when the sand was nitrogen treated. In light silty clay the correlation coefficients were -0.71 without nitrogen fertilisation and -0.76 when nitrogen was added to soil (Table 3). The inhibitory effect of nickel was much stronger in heavy loamy sand than in light silty clay, and this was particularly evident in the case of soil without additional nitrogen introduced, which may have resulted from the fact that heavier soil is characterised by much better physicochemical properties (including sorptive capacity). Such relationships are confirmed in the studies completed by WYSZKOWSKA, KUCHARSKI (2001), PRZYBULEWSKA et al. (2003).

Another significant factor which determined the course of nitrification process, next to the degree of soil contamination with nickel, time of incubation and type of soil, was the type of compound in which the metal was introduced to soil (Table 2). Nickel chloride turned out to be a stronger inhibitor than nickel sulphate, both in the soil with and without ammonium sulphate. The highest rate of $NiCl_2 \cdot 6H_2O$ (400 mg $Ni \cdot kg^{-1}$), irrespective of the type of soil, inhibited the nitrification of ammonium nitrogen by 49% on average, whereas the same dose of nickel introduced as $NiSO_4 \cdot H_2O$ was responsible for a 38% decline in nitrification.

The process of nitrification observed in this study was very slow (Table 4, Figure 1). The slow rate of nitrification is confirmed by amounts of nitrified nitrogen. The highest rate of nitrogen transformation in unpolluted soil samples occurred in the first 14 days of the experiment, when in heavy loamy sand 51% of nitrogen was nitrified, and in light silty clay the percentage of nitrified nitrogen was 69%. The contamination of soil with nickel, either as

Table 2

Retardation of the nitrification process in soil by nickel (%)

| Ni dose (mg $kg^{-1}$ of soil) | Soil type | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | heavy loamy sand (hls) | | | | light silty clay (lsc) | | | |
| | day of analysis (no. of days) | | | | | | | |
| | 14 | 28 | 42 | 56 | 14 | 28 | 42 | 56 |
| $NiCl_2 \cdot 6H_2O$ | | | | | | | | |
| Without ammonium sulphate | | | | | | | | |
| 100 | 18.28 | 10.56 | 14.58 | 18.57 | 10.57 | 8.89 | 2.90 | 5.13 |
| 200 | 24.73 | 15.56 | 30.21 | 39.05 | 25.20 | 11.85 | 10.14 | 8.33 |
| 300 | 39.78 | 23.89 | 43.23 | 39.52 | 34.80 | 25.19 | 26.09 | 10.90 |
| 400 | 53.23 | 42.78 | 43.23 | 45.71 | 39.02 | 30.74 | 31.16 | 11.54 |
| *r* | 0.99 | 0.95 | 0.94 | 0.89 | 0.97 | 0.97 | 0.98 | 0.88 |
| With ammonium sulphate | | | | | | | | |
| 100 | 20.83 | 13.27 | 1.82 | 4.80 | 14.76 | 12.62 | 17.57 | 11.11 |
| 200 | 52.24 | 13.58 | 12.12 | 11.30 | 40.48 | 25.95 | 26.80 | 13.33 |
| 300 | 77.40 | 49.23 | 26.97 | 31.64 | 57.62 | 43.33 | 30.41 | 14.22 |
| 400 | 84.29 | 72.38 | 61.82 | 60.73 | 68.10 | 50.48 | 47.30 | 40.44 |
| *r* | 0.97 | 0.95 | 0.96 | 0.97 | 0.98 | 0.99 | 0.96 | 0.83 |
| $NiSO_4 \cdot 7H_2O$ | | | | | | | | |
| Without ammonium sulphate | | | | | | | | |
| 100 | 3.33 | 1.67 | 7.81 | 10.10 | 3.25 | 5.19 | 5.07 | 15.71 |
| 200 | 15.56 | 11.67 | 16.67 | 17.68 | 9.76 | 15.56 | 18.84 | 18.59 |
| 300 | 35.00 | 34.44 | 25.00 | 26.77 | 21.14 | 19.26 | 20.29 | 27.88 |
| 400 | 41.67 | 40.00 | 36.98 | 38.38 | 23.98 | 34.07 | 27.90 | 32.69 |
| *r* | 0.98 | 0.97 | 0.99 | 0.99 | 0.97 | 0.97 | 0.94 | 0.98 |
| With ammonium sulphate | | | | | | | | |
| 100 | 20.83 | 1.85 | 2.12 | 5.37 | 16.67 | 8.57 | 11.04 | 10.67 |
| 200 | 50.96 | 10.19 | 9.70 | 14.12 | 33.57 | 8.81 | 13.51 | 14.22 |
| 300 | 70.03 | 32.10 | 13.64 | 23.73 | 43.33 | 24.52 | 14.19 | 15.11 |
| 400 | 77.24 | 58.33 | 41.82 | 25.99 | 52.62 | 33.81 | 19.37 | 20.00 |
| *r* | 0.96 | 0.98 | 0.92 | 0.97 | 0.99 | 0.95 | 0.94 | 0.96 |
| $LSD_{0.01}$* | $a$ – 0.32; $b$ – 0.23; $c$ – 0.23; $d$ – 0.23; $e$ – 0.32; $a \cdot b$ – 0.46; $a \cdot c$ – 0.46; $b \cdot c$ – 0.32; $a \cdot d$ – 0.46; $b \cdot d$ – 0.32; $c \cdot d$ – 0.32; $a \cdot e$ – 0.64; $b \cdot e$ – 0.46; $c \cdot e$ – 0.46; $d \cdot e$ – 0.46; $a \cdot b \cdot c$ – 0.64; $a \cdot b \cdot d$ – 0.64; $a \cdot c \cdot d$ – 0.64; $b \cdot c \cdot d$ – 0.46; $a \cdot b \cdot e$ – 0.91; $a \cdot c \cdot e$ – 0.91; $b \cdot c \cdot e$ – 0.64; $a \cdot d \cdot e$ – 0.91; $b \cdot d \cdot e$ – 0.64; $c \cdot d \cdot e$ – 0.64; $a \cdot b \cdot c \cdot d$ – 0.91; $a \cdot b \cdot c \cdot e$ – 1.29; $a \cdot b \cdot d \cdot e$ – 1.219; $a \cdot c \cdot d \cdot e$ – 1.29; $b \cdot c \cdot d \cdot e$ – 0.91; $a \cdot b \cdot c \cdot d \cdot e$ – 1.82 | | | | | | | |

* LSD (least statistical difference) for: $a$ – nickel dose, $b$ – nickel compound, $c$ – type of soil, $d$ – source of nitrogen, $e$ – day of analysis;
$r$ – correlation coeffictiens significant difference for $p < 0.01$; $n = 12$

Table 3

Soil pH during the nitrification process

| Ni dose (mg $kg^{-1}$ of soil) | Soil type | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | heavy loamy sand (hls) | | | | light silty clay (lsc) | | | |
| | day of analysis (no. of days) | | | | | | | |
| | 14 | 28 | 42 | 56 | 14 | 28 | 42 | 56 |
| $NiCl_2 \cdot 6H_2O$ | | | | | | | | |
| Without ammonium sulphate | | | | | | | | |
| 0 | 7.13 | 7.33 | 7.23 | 7.23 | 7.23 | 7.33 | 7.33 | 7.33 |
| 100 | 7.13 | 7.23 | 6.63 | 6.63 | 7.23 | 7.40 | 6.83 | 6.93 |
| 200 | 7.13 | 7.13 | 6.63 | 6.53 | 7.23 | 7.33 | 6.80 | 6.83 |
| 300 | 7.13 | 7.13 | 6.53 | 6.53 | 7.23 | 7.13 | 6.73 | 6.73 |
| 400 | 7.03 | 7.03 | 6.53 | 6.53 | 7.13 | 7.13 | 6.63 | 6.63 |
| *r* | -0.71 | -0.97 | -0.80 | -0.78 | -0.71 | -0.84 | -0.87 | -0.94 |
| With ammonium sulphate | | | | | | | | |
| 0 | 7.03 | 7.03 | 7.03 | 7.03 | 7.23 | 7.23 | 7.13 | 7.13 |
| 100 | 7.03 | 7.03 | 5.63 | 5.53 | 6.93 | 6.83 | 6.13 | 6.03 |
| 200 | 6.83 | 6.83 | 5.33 | 5.23 | 6.73 | 6.73 | 5.93 | 5.93 |
| 300 | 6.43 | 6.73 | 5.23 | 5.03 | 6.63 | 6.63 | 5.73 | 5.83 |
| 400 | 6.13 | 6.73 | 5.03 | 5.03 | 6.43 | 6.53 | 5.73 | 5.83 |
| *r* | -0.95 | -0.94 | -0.87 | -0.85 | -0.99 | -0.94 | -0.87 | -0.80 |
| $NiSO_4 \cdot 7H_2O$ | | | | | | | | |
| Without ammonium sulphate | | | | | | | | |
| 0 | 7.13 | 7.33 | 7.23 | 7.23 | 7.23 | 7.33 | 7.33 | 7.33 |
| 100 | 7.23 | 7.33 | 6.63 | 6.53 | 7.23 | 7.13 | 6.93 | 6.83 |
| 200 | 7.13 | 7.33 | 6.53 | 6.53 | 7.23 | 7.13 | 6.93 | 6.73 |
| 300 | 7.13 | 7.23 | 6.53 | 6.53 | 7.23 | 7.13 | 6.83 | 6.73 |
| 400 | 7.03 | 7.13 | 6.53 | 6.53 | 7.13 | 7.13 | 6.73 | 6.63 |
| *r* | -0.67 | -0.88 | -0.80 | -0.78 | -0.71 | -0.71 | -0.90 | -0.85 |
| With ammonium sulphate | | | | | | | | |
| 0 | 7.03 | 7.03 | 7.03 | 7.03 | 7.23 | 7.23 | 7.13 | 7.13 |
| 100 | 7.03 | 6.73 | 5.33 | 5.13 | 7.03 | 6.73 | 6.03 | 5.83 |
| 200 | 6.73 | 6.53 | 5.23 | 5.13 | 6.73 | 6.23 | 5.83 | 5.83 |
| 300 | 6.33 | 6.53 | 5.03 | 4.93 | 6.63 | 6.23 | 5.83 | 5.83 |
| 400 | 6.13 | 6.33 | 5.03 | 4.83 | 6.53 | 6.23 | 5.83 | 5.73 |
| *r* | -0.97 | -0.94 | -0.87 | -0.85 | -0.98 | -0.88 | -0.78 | -0.75 |
| $LSD_{0.01}$* | $a$ – 0.01; $b$ – 0.01; $c$ – 0.01; $d$ – 0.01; $e$ – 0.01; $a \cdot b$ – 0.01; $a \cdot c$ – 0.01; $b \cdot c$ – 0.01; $a \cdot d$ – 0.01; $b \cdot d$ – 0.01; $c \cdot d$ – 0.01; $a \cdot e$ – 0.01; $b \cdot e$ – 0.01; $c \cdot e$ – 0.01; $d \cdot e$ – 0.01; $a \cdot b \cdot c$ – 0.01; $a \cdot b \cdot d$ – 0.01; $a \cdot c \cdot d$ – 0.01; $b \cdot c \cdot d$ – 0.01; $a \cdot b \cdot e$ – 0.02; $a \cdot c \cdot e$ – 0.02; $b \cdot c \cdot e$ – 0.01; $a \cdot d \cdot e$ – 0.02; $b \cdot d \cdot e$ – 0.01; $c \cdot d \cdot e$ – 0.01; $a \cdot b \cdot c \cdot d$ – 0.02; $a \cdot b \cdot c \cdot e$ – 0.02; $a \cdot b \cdot d \cdot e$ – 0.02; $a \cdot c \cdot d \cdot e$ – 0.02; $b \cdot c \cdot d \cdot e$ – 0.02; $a \cdot b \cdot c \cdot d \cdot e$ – 0.03 | | | | | | | |

* explanations under Table 2

chloride or as sulphate, significantly depressed the amount of nitrified nitrogen in both types of soil. As a result, only 1.3% of the nitrogen introduced to heavy loamy sand as $NiCl_2 \cdot 6H_2O$ was nitrified in the objects which received 400 mg $Ni \cdot kg^{-1}$. When nitrogen was added to the same type of soil and in the same dose but as $NiSO_4 \cdot 7H_2O$, the percentage of the nitrified nitrogen was 4.3%. In light clay these values were: 13.8% for nickel chloride and 24.6% for nickel sulphate. The negative effect of nickel on the course of nitrification (during 56 days) finds further confirmation in the highly significant negative correlation coefficients between the degree of soil contamination with nickel chloride or nickel sulphate and the amount of nitrified nitrogen, which ranged from -0.75 to -0.99. This long-term inhibitory influence of nickel on the process of nitrification results most probably from the fact that nitrifying bacteria are predominantly chemolitotrophic microorganisms with high environmental requirements (WYSZKOWSKA 2002). The negative effect of nickel, especially

Table 4

Effect of nickel on the amount of nitrified nitrogen in soil containing ammonium sulphate (%)

| Ni dose $mg \cdot kg^{-1}$ of soil | Soil type | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | heavy loamy sand (hls) | | | | light silty clay (lsc) | | | |
| | day of analysis (no. of days) | | | | | | | |
| | 14 | 28 | 42 | 56 | 14 | 28 | 42 | 56 |
| $NiCl_2 \cdot 6H_2O$ | | | | | | | | |
| 0 | 51.45 | 55.07 | 54.83 | 58.33 | 68.95 | 67.43 | 72.10 | 68.60 |
| 100 | 39.90 | 46.78 | 56.47 | 58.68 | 57.87 | 56.93 | 54.13 | 58.80 |
| 200 | 18.43 | 47.60 | 52.03 | 58.33 | 36.87 | 44.80 | 46.90 | 57.63 |
| 300 | 3.38 | 22.40 | 43.52 | 41.65 | 22.82 | 31.97 | 48.30 | 57.63 |
| 400 | 1.28 | 8.87 | 16.68 | 19.13 | 13.77 | 26.72 | 32.43 | 30.33 |
| *r* | -0.97 | -0.94 | -0.86 | -0.87 | -0.99 | -0.99 | -0.94 | -0.86 |
| $NiCl_4 \cdot 7H_2O$ | | | | | | | | |
| 0 | 51.45 | 55.07 | 54.83 | 58.33 | 68.95 | 67.43 | 72.10 | 68.60 |
| 100 | 37.33 | 53.55 | 54.72 | 57.40 | 53.90 | 59.73 | 61.60 | 63.12 |
| 200 | 17.97 | 49.35 | 50.87 | 51.92 | 39.20 | 62.77 | 63.47 | 60.43 |
| 300 | 8.17 | 37.57 | 49.70 | 46.08 | 32.90 | 48.53 | 63.23 | 62.88 |
| 400 | 4.32 | 18.90 | 30.68 | 46.90 | 24.62 | 44.10 | 60.32 | 59.50 |
| *r* | -0.99 | -0.92 | -0.94 | -0.95 | -0.98 | -0.93 | -0.75 | -0.82 |
| $LSD_{0.01}$* | $a$ – 0.18; $b$ – 0.29; $c$ – 0.18; $d$ – 0.26; $a \cdot b$ – 0.40; $a \cdot c$ – 0.26; $a \cdot d$ – 0.36; $b \cdot c$ – 0.40; $b \cdot d$ – 0.57; $c \cdot d$ – 0.36; $a \cdot b \cdot c$ – 0.57; $a \cdot b \cdot d$ – 0.81; $a \cdot c \cdot d$ – 0.51; $b \cdot c \cdot d$ – 0.81; $a \cdot b \cdot c \cdot d$ – 1.14 | | | | | | | |

* LSD (least statistical difference) for: $a$ – nickel dose, $b$ – nickel compound, $c$ – type of soil, $e$ – day of analysis;

$r$ – correlation coeffictiens significant difference for $p < 0.01$; $n = 12$

when applied in smaller doses (100 and 200 mg Ni · $kg^{-1}$ of soil) was becoming less pronounced with time. Under the conditions of the experiment reported in this paper, irrespective of the degree of soil contamination with nickel or the compound in which nickel was introduced to soil, nitrification appeared to be more intensive in heavier than in lighter soil. This was probably due to the differences in the physicochemical properties of the soils used for the trials (Table 1) as well as to the change in their pH (Table 3). The correlation coefficients between the rate of nickel and pH were highly significant and varied between -0.49 and -0.56 (Table 5). Acid reaction activates mobilisation of heavy metals and affects their mobility (DENI, PENNINCKX 1999). The unfavourable effect of changes in soil reaction on the process of nitrification has also been verified by the studies completed by WYSZKOWSKA, KUCHARSKI (2004), PRZYBULEWSKA et al. (2003).

Table 5
Correlation coeffictiens between nickel dose and content of nitrogen mineral forms and pH in soil

| Variable | $N\text{-}NO_3$ | $N\text{-}NH_4$ | $N\text{-}NO_3 + N\text{-}NH_4$ | pH |
|---|---|---|---|---|
| Heavy loamy sand (hls) | | | | |
| Without ammonium sulphate | | | | |
| Ni | -0.93** | 0.56** | -0.93** | -0.50** |
| $N\text{-}NO_3$ | | -0.68** | 0.97** | 0.40** |
| $N\text{-}NH_4$ | | | -0.49** | -0.14* |
| $N\text{-}NO_3 + N\text{-}NH_4$ | | | | 0.44** |
| With ammonium sulphate | | | | |
| Ni | -0.78** | 0.67** | -0.37** | -0.51** |
| $N\text{-}NO_3$ | | -0.94** | 0.26** | 0.15* |
| $N\text{-}NH_4$ | | | 0.09 | -0.12 |
| $N\text{-}NO_3 + N\text{-}NH_4$ | | | | 0.09 |
| Light silty clay (lsc) | | | | |
| Without ammonium sulphate | | | | |
| Ni | -0.71** | 0.51** | -0.71** | -0.49** |
| $N\text{-}NO_3$ | | -0.80** | 0.99** | 0.08 |
| $N\text{-}NH_4$ | | | -0.73** | 0.10 |
| $N\text{-}NO_3 + N\text{-}NH_4$ | | | | 0.11 |
| With ammonium sulphate | | | | |
| Ni | -0.76** | 0.59** | -0.32** | -0.56** |
| $N\text{-}NO_3$ | | -0.88** | 0.22** | 0.24** |
| $N\text{-}NH_4$ | | | 0.27** | -0.05 |
| $N\text{-}NO_3 + N\text{-}NH_4$ | | | | 0.39** |

$r$ – correlation coeffictiens significant difference for: ** $p < 0.01$; * $p < 0.05$; n = 240

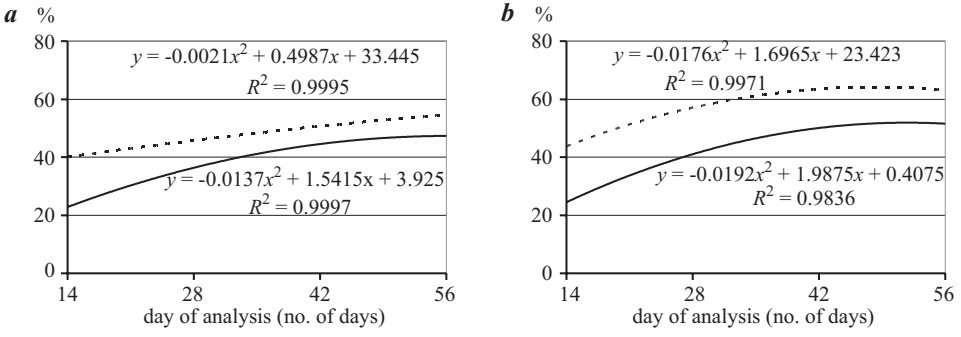


Fig. 1. Dynamics of the amounts of nitrogen nitrified in soil contaminated with nickel as: *a* – $NiCl_2 \cdot 6H_2O$, *b* – $NiSO_4 \cdot 7H_2O$

The negative impact of nickel on nitrification, demonstrated in the present study, finds further evidence in the results reported by PATRA et al. (1992), ANTIL et al. (2001). Less unambiguous are the opinions on how the duration of such negative influence exerted by heavy metals affects nitrification, as this is dependent on the kind and amount of contaminants, type of soil and its properties. While in the author's own research, nickel introduced in the amount of 300 – 400 mg $\cdot$ $kg^{-1}$ significantly inhibited the process of nitrification for 56 days, YEATES et al. (1994) showed that excessive amounts of chromium, copper and arsenic slowed down nitrification for only 14 days. In contrast, another study conducted by WYSZKOWSKA (2002) revealed that that Cr(VI) added in as small a dose as 10 mg depressed transformation of ammonium nitrogen for 84 days.

## Conclusions

1. Contamination of samples of typical brown soil formed from heavy loamy sand or typical brown soil formed from light clay with nickel in a dose from 100 to 400 Ni $\cdot$ $kg^{-1}$ inhibited nitrification.

2. Nickel chloride was a stronger inhibitor than nickel sulphate.

3. The adverse effect of nickel on nitrification was much weaker in a more compact soil (light clay) than in a lighter soil (heavy loamy sand).

4. Determination of nitrifying activity can be a good indicator of soil contamination with nickel.

Translated by JOLANTA IDŹKOWSKA

Accepted for print 27.03.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 121-133, Y. 2006

# PRODUCTIVITY OF SPRING BARLEY GROWN WITH RED CLOVER AS UNDERSOWN CROP

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Key words: spring barley, red clover, cover crop, undersowing, productivity.

Abstract

The paper presents the productivity of spring barley grown with undersown red clover. The study was based on the results of a three-year series (2001-2003) of two-factorial experiments established in a split-plot design with di- and tetraploid forms of red clover and various sowing rates (4, 8, 12, 16 kg · ha$^{-1}$). It was found that spring barley yield depended on the environmental conditions prevailing in particular years of the study, and varied in a wide range from 2.7 to 4.5 t · ha$^{-1}$. The forms of red clover had no impact on plant growth or grain yield, whereas increasing sowing rates over 8 kg · ha$^{-1}$ might result in yield decrease. The interdependences between spring barley traits were similar. A significant simple correlation and direct path effects between grain weight per ear and grain yield, calculated for red clover treatments (forms and sowing rates), suggest that grain weight per ear can be used as a predictor of spring barley yield when sown with spring barley. Stubble-clover yield amounted to 7.25 t · ha$^{-1}$ and was determined by rainfall. Plant habit of red clover was closely related to sowing rate. An increase in sowing rates was followed by a significant decrease in the number of branches and the dry matter content of the aboveground parts of plants.

## PRODUKCYJNOŚĆ JĘCZMIENIA JAREGO JAKO ROŚLINY OCHRONNEJ W UPRAWIE Z KONICZYNĄ CZERWONĄ

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Abstrakt

Przedstawiono ocenę produktywności jęczmienia jarego jako rośliny ochronnej w uprawie z koniczyną czerwoną. Podstawą oceny były wyniki z opisów biometrycznych oraz plony ziarna jęczmienia z doświadczenia polowego założonego w układzie split-plot z di- oraz tetraploidalnymi formami koniczyny czerwonej oraz różną ilością wysiewu (4, 8, 12, 16 kg · ha$^{-1}$), z trzech lata badań 2001-2003.

Stwierdzono, że poziom plonu ziarna jęczmienia jarego zależy od układu warunków glebowo-klimatycznych w latach badań, i może się zawierać w szerokich granicach od 2.7 do 4.5 t · ha$^{-1}$. Formy koniczyny czerwonej nie mają istotnego wpływu na zróżnicowanie pokroju roślin jęczmienia i jego plon, natomiast zwiększenie ilości wysiewu koniczyny powyżej 8 kg · ha$^{-1}$ może powodować jego zniżkę. Miary zależności między cechami jęczmienia były zbliżone, przy czym silne istotne pozytywne korelacje proste oraz wartości współczynników ścieżki bezpośredniej obliczone dla badanych form koniczyny czerwonej i ilości wysiewu dowodzą, że jedynie masa ziarn z kłosa może być wykorzystana jako predykat plonu jęczmienia. Plon ściernianki wynosił 7.25 t · ha$^{-1}$ i uwarunkowany był korzystnym przebiegiem opadów. Pokrój roślin koniczyny czerwonej był ściśle związany z ilością wysiewu. Wraz ze wzrostem tego czynnika zmniejszała się istotnie liczba rozgałęzień i sucha masa części nadziemnych roślin.

## Introduction

Red clover sowing into a cover crop is very common (FORDOŃSKI 1977, HOGH-JENSEN, SCHJOERRING 1997, SPANCER, TODD 2003, ZAJĄC et al. 1999). According to KELLY et al. (1996), sowing into a cover crop is more economical as it enables, among other, to reduce nitrogen fertilization rates. Red clover is considered the best cover crop (VRZAL et al. 1999, VYN et al. 1999). VYN et al. (1999) demonstrated that the mean nitrogen content of the biomass of maize grown with red clover as a cover crop was by 40.4 kg · ha$^{-1}$ higher at the flowering stage, compared with pure sowing. The suitability of red clover as an undersown crop results primarily from a slow growth rate after emergence, which indicates its low competitiveness with respect to a cover crop in this development phase. This has been also confirmed by PROTAS et al. (1982), who observed high weed infestation within a few weeks following red clover emergence and a relatively lower seed yield in pure sowing, compared with sowing into a cover crop.

The effects of a cover crop on the growth and development of undersown clover are complex. Allelopathy is observed at the germination stage, followed by competition for nutrients, space, light or water (ZAJĄC, WITKOWICZ 1999). In addition, cover crops regulate weed infestation in plantations, whereas undersown crops reduce nutrient leaching and prevent erosion (KANKANEN et al. 2001). Ideal undersown crops develop slowly and minimally compete with cover crops until the main harvest (ABDIN et al. 1997). However, yield in the year of sowing and in the first year of full utilization of legumes grown without cover crop cannot make up for yield losses in cereals, considered traditional cover crops (EDWARDS 1998, SPANCER, TODD 2003).

The objectives of the present study were: (1) to evaluate grain yield components and productivity of spring barley grown with red clover as

undersown crop, and (2) to determine the significant traits of undersown crop as affected by various sowing rates of red clover.

## Material and Methods

A series of exact field experiments with red clover sown into a cover crop (spring barley) was performed in the years 2001-2003, in a split-plot design, in four replications, at the Experimental Station in Bałcyny (53°40'N, 19°50'E), Poland. Plot area was 16 $m^2$. The experiment was located in a former winter wheat field, on pseudopodsolic silty soil of class IIIb, formed from light loam underlain by heavy loam. The first experimental factor were di- and tetraploid forms of red clover: Krynia (2*n*), Parada (2*n*), Bona (4*n*) and Jubilatka (4*n*), and the second experimental factor was the sowing rate of red clover seeds: 4, 8, 12 and 16 kg · $ha^{-1}$. Dressed seeds of red clover were sown at the spacing of 20 cm, at a depth of 1-1.5 cm. Red clover was the undersown crop while the main crop was spring barley cv. Orthega grown for grain, sown at a rate of 100 kg · $ha^{-1}$ immediately before red clover sowing. Presowing mineral fertilization was applied at the following rates: N – 30 kg · $ha^{-1}$, 70 kg · $ha^{-1}$ $P_2O_5$ and 100 kg · $ha^{-1}$ $K_2O$. In addition, at the stage of stem formation, the plants were supplied with nitrogen at a rate of 30 kg · $ha^{-1}$. The emergence of red clover and spring barley, and yield level and structure were determined in the years of sowing. Spring barley was harvested at the full maturity stage, in the first week or in the middle of August. Stubble-clover yield and growth characteristics traits of red clover plants were determined in 2002 only. The measurements were done in autumn. The dry matter content of the aboveground parts and roots (at a depth of 15 cm) of red clover was estimated by the oven-drying method. Root crown thickness and chlorophyll concentration were also measured.

Results were analyzed statistically by an analysis of variance. The significance of differences was verified by the Tukey T test, determining HSD (*Honest Significant Difference*) at a significance level $\alpha = 0.05$. Relationships between the variables, yield components, were determined using simple correlation coefficients and path coefficients. Analysis of variance, correlation and regression was performed using Statistica software, and path coefficients were calculated using own statistical software (IDŹKOWSKA et al. 1996).

### Climatic condition during the seasons

Air temperature over the growing season was higher in 2002 than in 2001 and 2003 (Table 1). The heaviest rainfall was recorded in summer 2002. During the growing season spring barley entered successive development phases at the same time, regardless of the density of red clover plants.

Table 1
Mean daily air temperature and precipitation over the experimental period 2001-2003 vs. mean multiannual temperature and precipitation. Meteorological Station in Bałcyny

| | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Year | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII |
| Mean daily air temperature (°C) | 2001 | -1.2 | -1.4 | 0.8 | 7.3 | 12.2 | 13.8 | 19.5 | 18.4 | 12.0 | 10.5 | 2.2 | -4.6 |
| | 2002 | -1.1 | 2.9 | 3.5 | 7.3 | 16.1 | 15.9 | 19.3 | 19.8 | 12.5 | 6.4 | 3.1 | -6.6 |
| | 2003 | -3.8 | -5.2 | 1.4 | 6.1 | 14.2 | 16.5 | 18.9 | 17.3 | 13.7 | 4.8 | 5.0 | 1.3 |
| | (1961-2000) | -3.5 | -2.6 | 1.2 | 6.6 | 12.4 | 15.7 | 16.9 | 16.5 | 12.6 | 8.1 | 2.8 | -1.3 |
| Precipitation (mm) | 2001 | 13.3 | 30.2 | 43.5 | 45.5 | 31.3 | 48.8 | 135.5 | 81.8 | 99.3 | 35.1 | 47.5 | 24.1 |
| | 2002 | 41.8 | 54.5 | 37.0 | 10.0 | 90.1 | 72.5 | 43.2 | 87.3 | 60.5 | 143.5 | 28.2 | 6.8 |
| | 2003 | 14.1 | 6.0 | 11.8 | 23.6 | 78.6 | 60.7 | 118.2 | 34.9 | 19.1 | 66.1 | 39.4 | 48.6 |
| | (1961-2000) | 27.4 | 21.6 | 28.5 | 35.4 | 57.6 | 69.5 | 81.6 | 75.2 | 59.0 | 53.5 | 48.9 | 41.8 |

# Results and Discussion

## Main crop – spring barley

Various sowing rates and red clover forms had significant effect on the number of emerged plants and productive culms of spring barley while the interaction of the factors was not significant (Table 2). Diploid form Parada and the highest sowing rate of red clover significantly lowered the number of the two traits of spring barley.

Table 2
Number of emerged plants and number of productive culms of spring barley depending on cultivar and sowing rate of red clover

| Forms and cultivars of red clover | Sowing rate of red clover (kg · ha$^{-1}$) | | | | Means |
|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | |
| 1 | 2 | 3 | 4 | 5 | 6 |
| Spring barley – number of emerged plants (means from years) | | | | | |
| Krynia (2*n*) | 144 | 167 | 144 | 135 | 148 |
| Parada (2*n*) | 137 | 119 | 129 | 117 | 126 |
| Bona (4*n*) | 147 | 141 | 154 | 131 | 143 |
| Jubilatka (4*n*) | 167 | 133 | 151 | 134 | 146 |
| Mean | 149 | 140 | 144 | 129 | |
| HSD ($p < 0.05$) for: cultivars (*C*) 20<br>sowing rate (*S*) 15<br>C · S nsd | | | | | |

cont. Table 2

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| Spring barley – number of productive culms (mean from years) | | | | | |
| Krynia | 577 | 668 | 575 | 541 | 590 |
| Parada | 548 | 476 | 517 | 468 | 502 |
| Bona | 587 | 564 | 617 | 523 | 573 |
| Jubilatka | 669 | 532 | 603 | 537 | 585 |
| | 595 | 560 | 578 | 517 | |
| HSD ($p < 0.05$) for: cultivars ($C$) 79<br>sowing rate ($S$) 61<br>C · S nsd | | | | | |

The development of spring barley and red clover was balanced. Growing this cereal species with red clover had no effect on plant habit of both crops (Phot. 1).

Phot. 1. General view of a plot with red clover sown into spring barley – full maturity stage of spring barley (photo taken by K. Żuk-Gołaszewska)

ZAJĄC (1993) reported that the total plant and field area of spring barley grown with red clover depended primarily on development phase, and the values of leaf area index were high. Results of a three-year series of experi-

Table 3

Traits of spring barley grown as a main crop on various treatments of undersown red clover

| Sources of variation | Years of study, red clover treatments | Traits of spring barley ($\bar{x} \pm se$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | culm length (cm) | ear length (mm) | number of grains per ear | grain weight per ear (g) | thousand grain weight (g) | grain yield ($t \cdot ha^{-1}$) |
| Mean of experiment | | 53.4 ± 0.36 | 61.5 ± 0.41 | 18.5 ± 0.09 | 0.897 ± 0.009 | 49.8 ± 0.25 | 3.38 ± 0.08 |
| Years | 2001 | 52.7 ± 0.76 | 60.9 ± 0.85 | 18.5 ± 0.17 | 0.852 ± 0.010 | 52.5 ± 0.16 | 2.73 ± 0.05 |
| | 2002 | 56.2 ± 0.34 | 61.7 ± 0.66 | 18.3 ± 0.15 | 0.850 ± 0.013 | 45.6 ± 0.19 | 2.93 ± 0.12 |
| | 2003 | 51.2 ± 0.49 | 61.8 ± 0.58 | 18.5 ± 0.15 | 0.990 ± 0.012 | 51.4 ± 0.26 | 4.49 ± 0.07 |
| $HSD_{0.05}$ | | 4.17 | 4.17 | nsd | nsd | 2.04 | nsd |
| Cultivars | Krynia | 53.0 ± 0.71 | 61.7 ± 0.62 | 18.4 ± 0.20 | 0.898 ± 0.016 | 49.4 ± 0.52 | 3.32 ± 0.15 |
| | Parada | 53.0 ± 0.83 | 60.3 ± 0.60 | 18.5 ± 0.15 | 0.896 ± 0.017 | 49.7 ± 0.50 | 3.50 ± 0.14 |
| | Bona | 54.0 ± 0.69 | 61.4 ± 0.77 | 18.6 ± 0.19 | 0.903 ± 0.022 | 50.0 ± 0.51 | 3.29 ± 0.15 |
| | Jubilatka | 53.6 ± 0.61 | 62.3 ± 1.14 | 18.3 ± 0.17 | 0.891 ± 0.015 | 50.1 ± 0.46 | 3.42 ± 0.16 |
| $HSD_{0.05}$ | | nsd | nsd | nsd | nsd | nsd | nsd |
| Sowing Rate ($kg \cdot ha^{-1}$) | 4 | 52.8 ± 0.80 | 63.1 ± 1.17 | 18.7 ± 0.16 | 0.909 ± 0.021 | 50.0 ± 0.52 | 3.43 ± 0.16 |
| | 8 | 54.9 ± 0.87 | 60.3 ± 0.61 | 18.0 ± 0.16 | 0.879 ± 0.016 | 49.8 ± 0.51 | 3.49 ± 0.15 |
| | 12 | 51.9 ± 0.65 | 60.7 ± 0.50 | 18.6 ± 0.15 | 0.890 ± 0.015 | 49.7 ± 0.46 | 3.23 ± 0.14 |
| | 16 | 53.9 ± 0.66 | 61.8 ± 0.76 | 18.5 ± 0.22 | 0.911 ± 0.017 | 49.9 ± 0.51 | 3.38 ± 0.15 |
| $HSD_{0.05}$ | | 2.19 | nsd | nsd | nsd | nsd | 0.245 |

nsd – non-significant differences

ments show that the main reason for trait variability in spring barley were soil and climate conditions. Generally, the values of main crop traits were not affected by red clover cultivar or sowing rate (Table 3). However, significant differences (at the significance limit) were recorded in plant height and grain yield, which decreased from 54.9 cm to 51.9 cm and from 3.49 to 3.23 respectively when sowing rate was increased from 8 to 12 kg · ha$^{-1}$. This suggests that the optimum grain yield of spring barley grown with red clover may be achieved at a sowing rate of 8 kg · ha$^{-1}$ red clover.

A further increase in sowing rate, to 12 or 16 kg · ha$^{-1}$ red clover, resulted in a significant decrease in the grain yield of spring barley. Only the interaction year x sowing rate of red clover was significant what suggest the different effects in spring barley yield on red clover sowing rate depending on the climatic conditions of the year of study (Fig. 1).

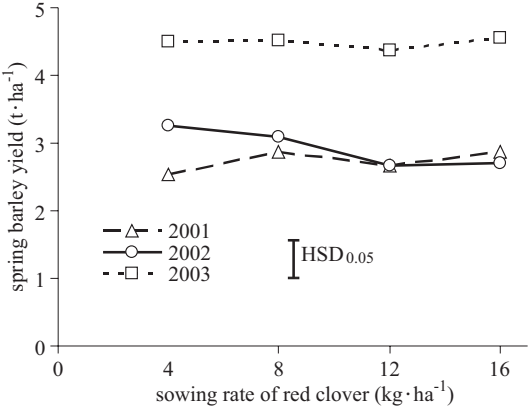


Fig. 1. Grain yield of spring barley grown as a cover crop in years of studies in treatments with various sowing rates of red clover

In 2001 and 2003 the grain yield of spring barley was almost identical in treatments with various sowing rates of red clover, but in 2002 the grain yield of spring barley displayed a falling tendency. This decrease, reflected by the value of the simple regression coefficient, was relatively low, i.e. a 5.3 kg reduction in the grain yield of spring barley per kg of red clover sown seeds ($100\,R^2 = 86\%$). The tendency of decreased yield of spring barley on increasing red clover sowing rate in 2002 can be attributed to high precipitation and generally optimal conditions for red clover growth and the effects of its competitiveness to spring barley in such conditions.

The results of the present study are consistent with those obtained by WILCZEK et al. (1999). Various sowing rates of red clover and different rates of

potassium fertilization in exact field trials had no significant effect on yield structure components and the grain yield of spring barley grown as a cover crop. Grain yield ranged from 3.68 to 3.98 $t \cdot ha^{-1}$ over the experimental period, and was considered fully satisfactory. A good grain yield of barley, grown with red clover and meadow fescue sown by five different techniques, was also attained by KANKANEN et al. (2001) – it amounted to 4.87 $t \cdot ha^{-1}$ in the first year, and to 3.03 and 3.24 $t \cdot ha^{-1}$ in subsequent two years. STEWARD et al. (1980) and OHLANDER et al. (1996) reported a reduction in barley grain yield. In the latter case yield decrease was 5% and 2% for the cultivars tested. Moreover, grain yield level depended upon the development phase of the cover crop into which red clover and two grass species were sown. Early harvest of main crops assures better wintering and yielding in the year of full utilization of undersown crops. In order to reduce the risk of yield decrease, cereals may be harvested at the milk stage (OHLANDER et al. 1996) or at the soft-dough stage (NICKEL et al. 1990).

Table 4 presents relationships between barley traits and grain yield, measured as the values of simple correlation coefficients and path coefficients. The relationships were generally non-significant in years, with exception of correlation and direct effect of ear length and simple correlation with thousand grain weight. The low values of coefficient of determination, 0.50, 012 and 0.30 for years 2001, 2002 and 2003, respecitvely indicate on relatively low part of yield variation explained by the traits. The results seems to be obvious because the analysis was made on the data from various red clover treatments.

As for the red clover treatments the determination of spring barley yield was high but it resulted mainly from the strong correlations and direct effects of grain weight per ear. The exceptions were significant negative correlations between culm length and yield in the treatment where the sowing rate of red clover was 8 $kg \cdot ha^{-1}$, between ear length and grain yield in the treatment with the lowest sowing rate.

Different significant estimates for coefficient of correlation and path coefficient points out on indirect effects of the other traits in the set that blur and mask the real interdependence of a given trait with yield. In our study only for diploid forms cvs. Krynia and Parada it was stated specific relationship between traits, distinctly different from tetraploid forms. Significant correlation between grain weight per ear and yield was accompanied non-significant direct effect what can be attributed to indirect effects of other traits. Non-significant coefficient of correlation and significant path coefficient estimated for interrelationships between ear length, thousand grain weight and grain yield can be explained by masking indirect effects of other traits. To generalize the interrelationship analysis it can be stated that only good predictor of spring barley yield when grown with red clover as intersown crop can be grain yield per ear.

Table 4

Relationships between the biometric characters and grain yield of barley in relation to year of study, cultivar and sowing rate of red clover (simple correlation coefficients $r$ and direct path coefficients $p_1$)

| Treatment | | Culm length | Ear length | Number of grains per ear | Grain weight per ear | Thousand grain weight | $R^2$ |
|---|---|---|---|---|---|---|---|
| 2001 | $r$ | 0.3997 | -0.6319* | -0.1586 | -0.0866 | 0.2669 | |
| | $p_1$ | 0.1377 | -0.5842* | -0.0966 | 0.1107 | 0.2482 | 0.50 |
| 2002 | $r$ | 0.1337 | 0.1175 | 0.0451 | -0.1922 | -0.1608 | |
| | $p_1$ | 0.2070 | 0.0242 | 0.1543 | -0.3353 | -0.1077 | 0.12 |
| 2003 | $r$ | -0.0008 | -0.2712 | -0.2488 | -0.2221 | 0.4692* | |
| | $p_1$ | 0.0912 | -0.1295 | -0.1776 | -0.0543 | 0.4432 | 0.30 |
| Krynia | $r$ | -0.5020 | -0.1301 | -0.4217 | 0.6608 | 0.1825 | |
| | $p_1$ | -0.2744 | -0.1455 | -0.5910* | 0.9060* | -0.3512* | 0.94 |
| Parada | $r$ | -0.4091 | 0.4472 | 0.0215 | 0.8193* | 0.4239 | |
| | $p_1$ | 0.0260 | 0.7626* | -0.4221 | 0.3191 | 0.7744* | 0.91 |
| Bona | $r$ | -0.3496 | 0.1861 | 0.1320 | 0.7783* | 0.2296 | |
| | $p_1$ | 0.0614 | 0.3405 | -0.4825 | 0.9015* | 0.2657 | 0.74 |
| Jubilatka | $r$ | -0.3532 | -0.1423 | 0.4018 | 0.8450* | 0.1664 | |
| | $p_1$ | -0.0595 | -0.1378 | -0.5060 | 1.2315* | -0.1102 | 0.86 |
| 4 | $r$ | -0.2247 | -0.5534* | -0.1455 | 0.7696* | 0.0211 | |
| | $p_1$ | -0.1211 | -0.2623 | -0.0125 | 0.7391* | -0.3474 | 0.74 |
| 8 | $r$ | -0.8731* | 0.5316* | 0.1861 | 0.7514* | 0.2187 | |
| | $p_1$ | -0.5332 | 0.3121 | -0.3415 | 0.3570 | 0.0301 | 0.71 |
| 12 | $r$ | -0.4541 | 0.4195 | -0.0234 | 0.7415* | 0.3194 | |
| | $p_1$ | -0.8100 | 0.0358 | -0.3943 | 0.8081* | -0.4980 | 0.82 |
| 16 | $r$ | -0.4865 | 0.2402 | 0.1674 | 0.8951* | 0.4128 | |
| | $p_1$ | -0.0153 | 0.0067 | -0.3030 | 0.9950* | 0.1347 | 0.90 |

* significant at $p < 0.05$

## Undersown crop – red clover

The seed germination of cvs Bona, Jubilatka, Parada and Krynia was in the range 88-90%, 85-90%, 81-95% and 73-80%, respectively. In the experiments the number of emerged red clover plants when grown with spring barley depended on sowing rates (Table 5). Increasing sowing rates resulted in a higher number of plants per area unit but this effect was depended on cultivars.

Cultivar Bona had only tendency because the differences in the number of plants at the following sowing rates were not significant while in comparison with the lowest sowing rate in the study, 8 kg · ha$^{-1}$, cultivar

Parada significantly increased the number of plants up to 12 kg · ha$^{-1}$ and cultivars Krynia and Jubilatka up to the highest sowing rate 16 kg · ha$^{-1}$. The increasing number of emerged red clover plants on increasing rate of sowing was also observed by ŚCIBOR and BAWOLSKI (1994).

Table 5

Number of emerged red clover plants per m$^2$ when sown with spring barley

| Forms and cultivars of red clover | Sowing rate (kg · ha$^{-1}$) | | | | Means |
|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | |
| Krynia (2*n*) | 272.2 | 416.0 | 445.5 | 617.0 | 437.7 |
| Parada (2*n*) | 314.0 | 468.5 | 532.0 | 598.0 | 478.1 |
| Bona (4*n*) | 314.5 | 406.0 | 505.0 | 473.0 | 424.5 |
| Jubilatka (4*n*) | 255.7 | 373.5 | 455.5 | 669.5 | 438.6 |
| Means | 289.1 | 416.0 | 485.5 | 589.3 | |

HSD ($p < 0.05$) for: cultivars – nsd; sowing rates – 71.00; cultivars x sowing rates 194.53

Among the factors analyzed only sowing rate affected the growth characteristics of red clover (Table 6). Increased sowing rates caused a significant decrease in the number of branches and the dry matter content of the aboveground parts of red clover. In treatments with the lowest sowing rate (4 kg · ha$^{-1}$) the plants had more branches and formed a more abundant rosette. The dry matter content of aboveground parts was 1.41 g, and was significantly higher than in treatments with higher sowing rates (8, 12 and 16 kg · ha$^{-1}$). In addition, these plants had the thickest root crowns (13.87 mm) and the highest dry matter content of roots (11.1 g). The plant habit of red clover sown into spring barley was similar to the plant habit in a study conducted by ZAJĄC et al. (1999). Sowing rates and cultivars with various chromosome sets did not differentiate chlorophyll concentration (SPAD).

In 2002 stubble-clover yield amounted to 7.25 t · ha$^{-1}$, and was determined by rainfall. This relationship was also confirmed by WILCZEK et al. (1999), who achieved the highest stubble-clover yield in a year of favorable weather conditions. Stubble-clover yield was related to the year of studies rather than the sowing rate of red clover.

To recapitulate, the analysis of red clover growth characteristics measured in the autumn showed that the differences between forms are not significant and only sowing rates can differentiate the number of branches and dry matter of aboveground parts of plant.

Table 6

Traits of red clover and stubble-clover yield when sown with spring barley

| Sources of variation \ Treatments | | Traits of red clover $\bar{x} \pm se$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | number of branches | root crown thickness (mm) | chlorophyll concentration SPAD | dry matter of aboveground parts (g) | dry matter content of roots (g) | stubble-clover yield ($t \cdot ha^{-1}$) |
| Mean for experiment | | 9.10 ± 0.41 | 12.31 ± 0.38 | 34.64 ± 0.91 | 1.02 ± 0.08 | 0.90 ± 0.06 | 7.25 ± 0.29 |
| Cultivars | Krynia | 8.75 ± 0.59 | 11.40 ± 0.64 | 37.25 ± 2.40 | 0.88 ± 0.09 | 0.73 ± 0.14 | 8.18 ± 0.60 |
| | Parada | 8.75 ± 0.90 | 13.03 ± 0.89 | 32.86 ± 1.99 | 1.01 ± 0.19 | 0.94 ± 0.14 | 6.41 ± 0.50 |
| | Bona | 8.25 ± 0.83 | 11.80 ± 0.79 | 33.01 ± 1.17 | 0.94 ± 0.14 | 0.82 ± 0.10 | 6.76 ± 0.48 |
| | Jubilatka | 9.68 ± 0.93 | 13.01 ± 0.65 | 35.45 ± 1.38 | 1.24 ± 0.17 | 1.09 ± 0.11 | 7.64 ± 0.63 |
| $HSD_{0.05}$ | | 3.14 | nsd | nsd | 0.50 | nsd | nsd |
| Sowing rate ($kg \cdot ha^{-1}$) | 4 | 11.06 ± 0.90 | 13.87 ± 0.72 | 34.65 ± 1.49 | 1.41 ± 0.21 | 1.11 ± 0.14 | 7.86 ± 0.89 |
| | 8 | 9.00 ± 0.83 | 11.84 ± 0.76 | 35.35 ± 1.95 | 0.91 ± 0.07 | 0.89 ± 0.12 | 7.54 ± 0.61 |
| | 12 | 7.87 ± 0.76 | 12.17 ± 0.78 | 35.06 ± 1.15 | 0.91 ± 0.13 | 0.76 ± 0.12 | 6.75 ± 0.46 |
| | 16 | 8.50 ± 0.81 | 11.35 ± 0.68 | 33.51 ± 2.53 | 0.84 ± 0.13 | 0.83 ± 0.11 | 6.84 ± 0.48 |
| $HSD_{0.05}$ | | 3.14 | nsd | nsd | 0.50 | nsd | nsd |

nsd – non-significant difference

## Conclusions

1. The yield of spring barley grown with red clover as an undersown crop depends on the climate and soil conditions prevailing in particular years of the study, and may vary in a wide range of 2.7 to 4.5 $t \cdot ha^{-1}$.

2. The plant habit and the yield of spring barley do not depend on undersown di- or tetraploid forms of red clover. The sowing rate of red clover should not exceed 8 $kg \cdot ha^{-1}$, since a higher sowing rate may significantly reduce the yield of spring barley.

3. The interdependences between the biometric traits of spring barley are similar regardless of the undersown form of red clover and the rate of sowing. The yield of spring barley is significantly correlated with the grain yield per ear, for di- and tertaploid forms of red clover and the sowing rates analyzed, but this relationship is not observed when the analysis is made for years.

4. Differences in the growth characteristics of red clover, as dependent on di- and tetraploid forms, are non-significant, while the sowing rate differentiates the number of branches and the dry matter content of the aboveground parts of plants.

Translated by Aleksandra Poprawska

Accepted for print 12.01.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 135-145, Y. 2006

# EFFECTS OF MINERAL FERTILIZERS AND GROWTH REGULATORS ON THE GROWTH DYNAMICS AND ABOVEGROUND PART BIOMASS OF PEA

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K e y w o r d s: *Pisum sativum*, fertilization, growth regulators, growth dynamics, plant productivity.

A b s t r a c t

The fertilizers applied in the experiment had a significant effect on the height of pea plants, the number of pods per plant and the number of seeds per pod. NPK fertilization (simple fertilizers – ammonium nitrate, triple superphosphate, potassium salt) and Polifoska 6 were found to be most efficient. Fertilization with Polifoska 6 considerably differentiated plant height, regardless of pea form. Plants of cv. Poker and cv. Venus set the greatest number of pods when fertilized with Amofoska 3 + triacontanol and Polifoska 6 respectively. The pea cultivars tested in the study yielded at a similar level, irrespective of fertilization variant. The dry matter of particular plant parts was at a level comparable with the control treatment, or slightly higher. Pea fertilization with Amofoska 3 and growth regulators reduced the proportion of seed biomass.

## WPŁYW NAWOŻENIA MINERALNEGO I REGULATORÓW WZROSTU NA DYNAMIKĘ WZROSTU I KSZTAŁTOWANIE SIĘ MASY NADZIEMNYCH ORGANÓW GROCHU SIEWNEGO

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S ł o w a k l u c z o w e: *Pisum sativum*, nawożenie, regulatory wzrostu, dynamika wzrostu, produkcyjność roślin.

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Abstrakt

Zastosowane w doświadczeniu nawożenie wpływało istotnie na wysokość roślin grochu, liczbę strąków na jednej roślinie oraz liczbę nasion w strąku. Najskuteczniejsze okazało się nawożenie NPK (nawozy pojedyncze – saletra amonowa, superfosfat potrójny, sól potasowa) oraz nawożenie Polifoską 6. Nawożenie Polifoską 6 istotnie różnicowało wysokość roślin, niezależnie od badanej formy grochu. Odmiana Poker zawiązywała największą liczbę strąków pod wpływem Amofoski 3 z dodatkiem triacontanolu, a odmiana Wenus w wyniku nawożenia Polifoską 6. Testowane odmiany grochu siewnego plonowały na zbliżonym poziomie, niezależnie od wariantu zastosowanego nawożenia. Sucha masa poszczególnych elementów rośliny kształtowała się na poziomie roślin kontrolnych lub była tylko nieznacznie wyższa. Nawożenie Amofoską 3 łącznie z regulatorami wzrostu zmniejszało udział nasion w gromadzeniu biomasy.

## Introduction

Plant yield is closely related to nutrient abundance in the soil. It follows that adequate nutrient supply is indispensable for the optimum development and yielding of plants (HENRY et in. 1995, PODLEŚNA 1999). Phosphorus and potassium deficiency may reduce photosynthesis rate in pea (PSZCZÓŁKOWSKA et al. 2002). According to KOCOŃ (2002b) and PODLEŚNA (1999), potassium deficiency in the soil may be followed by a decrease in biomass and seed yield in legumes.

Another factor affecting plant productivity may be exogenous growth regulators, whose effects depend on their type and dose (ARSHAD, FRANKENBERGER 1991, BANGERTH 1989, KING 1988, PRUSIŃSKI, BOROWSKA 2002, WIERZBOWSKA, SIENKIEWICZ 2004). DARLEN et al. (2004) demonstrated that auxin plays a key role during pea leaf development, in a period from the process initiation in the apical meristem to the appearance of first leaves on the stem. Other authors pointed to the crucial role of auxin (IAA) in pea stem elongation (RICCI et al. 1995). In an experiment performed by TRAMONTANO et al. (1989) cytokinins impacted both cell division and protein synthesis in pea seeds. According to ARSHAD, FRANKENBERGER (1991) and KING (1988), the hormones directly related to blossom fall are 3-indoleacetic acid (IAA) and cytokinins. An effective way of supplying exogenous growth regulators is to introduce their precursors – L-tryptophan and adenine – into the soil, since soil microorganisms can synthesize auxin and cytokinins from them (KUCHARSKI, NOWAK 1994, NIETO, FRANKERBERGER 1990).

Growth regulators can be applied to improve crop yielding. The studies conducted so far focused primarily on the foliar application of growth promoters at certain developmental stages. Seeds can be also soaked in solutions of growth regulators, but this requires the use of organic solvents which may negatively affect germination. Thus, research was launched on the combined

application of growth regulators and fertilizers, to simplify the procedure and reduce the dose of growth promoters. Another advantage of this method of application of growth-promoting substances is the fact hat they are synthesized not only by higher plants, but also by some soil microorganisms, which enables to use not only phytohormones, but their precursors as well.

The purpose of the present study was to determine the effects of traditional NPK fertilization (simple fertilizers) and multi-component fertilizers combined with growth regulators on the growth dynamics, plant habit and dry matter accumulation in the aboveground parts of various forms of pea.

## Materials and Methods

A two-factorial pot experiment was performed in a greenhouse of the University of Warmia and Mazury in Olsztyn, in a completely randomized design, in three replications. Pea was grown in modified Kick-Braukmann pots filled with 10 kg light soil composed of heavy loamy sand. The soil had a slightly acid reaction (pH = 5.52 in 1 M KCl) and was abundant in available nutrients (P – 170, K – 207 and Mg – 100 mg · $kg^{-1}$).

The first experimental factor was pea cultivar, i.e. traditional sugar form Poker and narrow-leaved form Venus. The second experimental factor was fertilization variant: simple fertilizers (NPK) and multi-component fertilizers Polifoska 6 (P6), Amofoska 3 (A3) which – due to nutrient proportions – may be used for legume fertilization. They were applied alone or in combinations with growth regulators: IBA ($\beta$-indolebutyric acid) – (A3 + IBA), NAA ($\alpha$-naphthylacetic acid) – (A3 + NAA), tria (triacontanol) – (A3 + tria), try (L-tryptophan) – (A3 + try), ade (adenine ) – (A3 + ade), BA (benzyladenine) – (A3 + BA). Pea grown without mineral fertilizers served as control treatment (C).

NPK fertilization was applied at a rate of 0.3 g N in the form of ammonium nitrate (34% N), 0.44 g P in the form of triple superphosphate (20.1% P) and 2.33 g K in the form of potassium salt (50% K) per pot. Polifoska 6 and Amofoska 3 were applied at a rate of 6 g (0.36 g N, 0.52 g P and 1.50 g K) and 10 g (0.30 g N, 0.44 g P and 2.33 g K) per pot respectively. Growth regulators were supplied using acrylamide gel as carrier. Amofoska 3 was coated with this gel immediately before sowing. The fertilizers were applied at points before seed sowing. The growth regulators were administered at the following doses: IBA and NAA – 10 mg per pot, tria – 5.2 mg per pot, try – 90 mg per pot, ade – 40 mg per pot, BA – 30 mg per pot.

12 pea seeds were sown per pot. Soil moisture content was 60% of field water capacity of the soil. The plants were collected at the full maturity stage,

and the following parameters were determined: plant height, the height of first pod setting, specific leaf weight, the number of pods per plant, the number of seeds per pod, dry matter accumulation in particular vegetative organs, and seed mass per plant.

The results of the experiment were analyzed statistically by an analysis of variance for two-factorial experiments in a completely randomized design, using STATISTICA software. The significance of differences was verified by the Tukey test at a significance level $\alpha = 0.01$.

## Results and Discussion

During plant growth and development mean temperature in the greenhouse ranged from 10°C in April to 30°C in July. The period of plant emergence lasted on average for 20 days. First plants emerged in the control treatment. The process was slower and plant growth was inhibited in its initial phase in fertilized treatments, especially when fertilizers were applied together with growth regulators. However, afterwards the plants in these treatments developed more rapidly, reaching a height comparable with the control or higher – especially in the case of simple fertilizers NPK and Polifoska 6 (Figure 1).

An analysis of regression showed highly significant linear changes in plant height at successive developmental stages. Plant height was also considerably affected by mineral fertilization (Table 1).

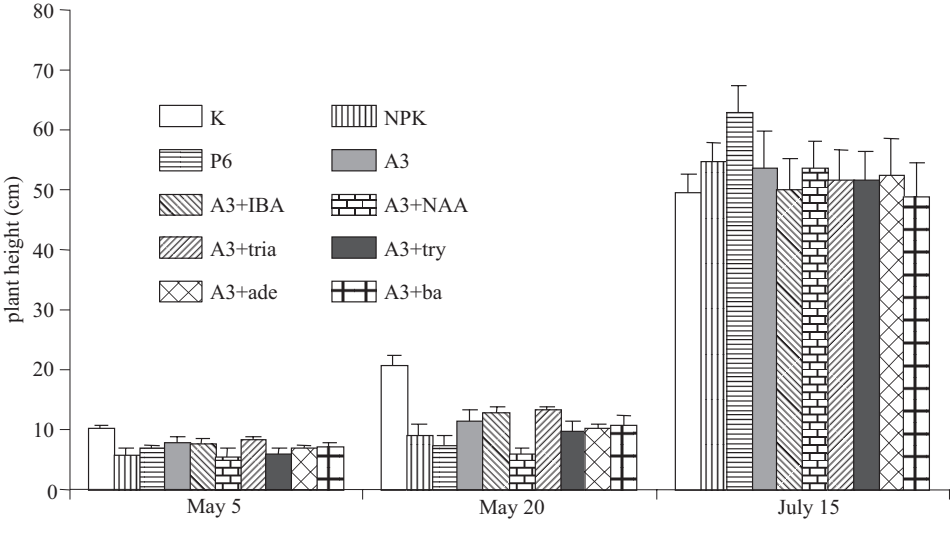


Fig. 1. Height dynamics of pea plants (cm) over the growing season

Table 1

Height of pea plants depending on fertilization variant and date of measurement

| Treatment | Poker | | | Wenus | | | Regression analysis | |
|---|---|---|---|---|---|---|---|---|
| | May 5 | May 20 | July 15 | May 5 | May 20 | July 15 | $b_{y/x}$ | $R^2$ |
| Control | 9.6 | 21.4 | 42.2 | 10.9 | 19.8 | 56.8 | 1.818 | 0.995 |
| NPK | 4.2 | 6.9 | 48.6 | 7.0 | 11.3 | 60.4 | 1.347 | 0.984 |
| Polifoska 6 | 5.2 | 4.8 | 54.2 | 8.1 | 10.0 | 71.4 | 1.133 | 0.969 |
| Amofoska 3 | 5.6 | 6.8 | 41.1 | 10.0 | 15.5 | 65.7 | 1.445 | 0.985 |
| Amofoska 3+IBA | 6.0 | 10.5 | 38.6 | 9.2 | 14.6 | 61.3 | 1.593 | 0.993 |
| Amofoska 3+NAA | 2.6 | 3.4 | 44.7 | 8.1 | 8.1 | 62.3 | 1.317 | 0.967 |
| Amofoska 3+tria | 6.4 | 12.2 | 41.6 | 9.7 | 14.1 | 61.7 | 1.548 | 0.993 |
| Amofoska 3+try | 5.0 | 7.6 | 41.4 | 7.0 | 11.4 | 61.7 | 1.450 | 0.985 |
| Amofoska 3+ade | 5.2 | 8.7 | 40.0 | 8.2 | 11.8 | 65.0 | 1.442 | 0.986 |
| Amofoska 3+BA | 6.2 | 9.8 | 36.2 | 8.0 | 11.9 | 61.0 | 1.603 | 0.988 |
| Mean | 5.6 | 9.2 | 42.9 | 8.6 | 12.9 | 62.7 | | |
| LSD ($p < 0.01$) for interactions date x cultivar = 3.33<br>date x cultivar x fertilization = 14.26 | | | | | | | | |

$b_{y/x}$ – linear regression coefficient

Due to the fact that the pea cultivars examined represented various morphological types, they differed in height in particular periods, but these differences were confirmed statistically as late as at the full maturity stage (Table 1). At this stage plants of the traditional cultivar Poker were by about 32% shorter than plants of the narrow-leaved cultivar Wenus. At the full maturity stage, the tallest plants were recorded in the treatment fertilized with Polifoska 6, but for cv. Wenus this difference was statistically significant only when compared with the control. Plants of cv. Poker fertilized with Polifoska 6 produced significantly longer stems than pea plants fertilized with Amofoska 3 in combination with cytokinin (BA) and synthetic auxin (IBA).

Regression analysis revealed that control plants were characterized by the fastest growth rate, which was reflected by a high value of the regression coefficient $b_{y/x}$ 1. 818). In the other treatments the mean daily height increment ranged between 1.133 cm (Polifoska 6) and 1.603 cm (Amofoska 3 + BA).

Depending on the rate of photosynthesis, the same leaf weight may produce various biomass. The mean leaf weight ratio in cv. Poker was 0.41 $g \cdot g^{-1}$ and was significantly lower than in cv. Wenus (0.55 $g \cdot g^{-1}$) – Table 2. Fertilization had no significant effect on the value of this ratio, but responses of both cultivars were varied. The narrow-leaved cultivar Wenus responded to the application of mineral fertilizers, especially combined with cytokinin (BA), by an increase in the proportion of leaves in the aboveground part biomass. Also TAUB (2002) reported that increasing nitrogen fertilization generally contributed to an increase in the value of the leaf weight ratio in C3 plants.

Table 2

Morphological characters and yield components of pea

| Cultivar | Fertilizer | Height of the first pod setting (cm) | Number of pods per plant | Number of seeds per pod | Weight of a single seed (g) | Leaf weight ratio (LWR) ($g \cdot g^{-1}$) | Seed weight per plant (g) |
|---|---|---|---|---|---|---|---|
| Poker | Control | 31.10 | 2.61 | 3.87 | 0.18 | 0.39 | 1.88 |
| | NPK | 31.57 | 2.55 | 5.26 | 0.17 | 0.39 | 2.37 |
| | Polifoska 6 | 35.65 | 3.81 | 5.24 | 0.14 | 0.39 | 2.83 |
| | Amofoska 3 | 26.81 | 2.65 | 4.26 | 0.16 | 0.40 | 1.76 |
| | Amofoska 3 + IBA | 27.77 | 2.49 | 3.37 | 0.16 | 0.41 | 1.70 |
| | Amofoska 3+NAA | 25.50 | 2.52 | 5.60 | 0.16 | 0.44 | 2.28 |
| | Amofoska 3+ tria. | 29.18 | 2.96 | 4.51 | 0.15 | 0.40 | 2.07 |
| | Amofoska3+L-tryp | 27.29 | 2.71 | 4.37 | 0.19 | 0.32 | 2.13 |
| | Amofoska 3+ade. | 25.98 | 2.41 | 3.89 | 0.13 | 0.45 | 1.26 |
| | Amofoska 3+ BA | 45.98 | 2.20 | 4.05 | 0.16 | 0.43 | 1.47 |
| Mean | | 31.14 | 2.69 | 4.55 | 0.16 | 0.41 | 1.97 |
| Wenus | Control | 45.64 | 2.37 | 2.76 | 0.25 | 0.49 | 1.65 |
| | NPK | 49.67 | 2.61 | 3.93 | 0.23 | 0.58 | 2.10 |
| | Polifoska 6 | 51.98 | 3.07 | 3.86 | 0.21 | 0.60 | 2.51 |
| | Amofoska 3 | 59.07 | 2.54 | 4.03 | 0.23 | 0.57 | 2.34 |
| | Amofoska 3 + IBA | 54.34 | 2.23 | 3.51 | 0.21 | 0.54 | 1.66 |
| | Amofoska 3+NAA | 49.71 | 2.67 | 3.30 | 0.22 | 0.47 | 1.84 |
| | Amofoska 3+ tria. | 51.67 | 2.46 | 3.55 | 0.24 | 0.55 | 1.72 |
| | Amofoska3+L-tryp | 53.13 | 2.08 | 3.85 | 0.23 | 0.53 | 1.59 |
| | Amofoska 3+ade. | 53.83 | 2.62 | 3.35 | 0.22 | 0.53 | 1.95 |
| | Amofoska 3+ BA | 51.31 | 2.28 | 2.26 | 0.24 | 0.63 | 1.77 |
| Mean | | 52.47 | 2.49 | 3.49 | 0.22 | 0.55 | 1.91 |
| Mean for fertilization | control | 41.89 | 2.49 | 3.29 | 0.22 | 0.44 | 1.77 |
| | NPK | 41.76 | 2.58 | 4.60 | 0.20 | 0.49 | 2.37 |
| | Polifoska 6 | 47.37 | 3.44 | 4.55 | 0.18 | 0.49 | 2.67 |
| | Amofoska 3 | 40.58 | 2.60 | 4.14 | 0.19 | 0.49 | 2.05 |
| | Amofoska 3 + IBA | 38.74 | 2.36 | 3.94 | 0.18 | 0.48 | 1.68 |
| | Amofoska 3+NAA | 39.59 | 2.59 | 4.45 | 0.19 | 0.45 | 2.06 |
| | Amofoska 3+ tria. | 39.60 | 2.71 | 4.03 | 0.20 | 0.48 | 1.90 |
| | Amofoska3+L-tryp | 40.21 | 2.38 | 3.91 | 0.21 | 0.43 | 1.86 |
| | Amofoska 3+ade. | 39.91 | 2.52 | 3.62 | 0.18 | 0.49 | 1.60 |
| | Amofoska 3+ BA | 48.80 | 2.24 | 3.65 | 0.20 | 0.53 | 1.61 |
| LSD ($p < 0.01$) for cultivars | | 4.397 | nsd | 0.372 | 0.017 | 0.034 | nsd |
| fertilizers | | nsd | 1.069 | 1.220 | nsd | nsd | nsd |
| interaction | | nsd | nsd | nsd | nsd | nsd | nsd |

nsd – non-significant difference

The pea forms tested in the study differed significantly in plant habit and yield components (Table 2), which most probably resulted from their morphological types. In cv. Poker pods were set significantly lower than in cv. Wenus (31.14 and 52.47 cm respectively). However, the differences were not confirmed statistically. Fertilizers and growth regulators modified the height

of first pod setting. Plants of the narrow-leaved cultivar Wenus treated with mineral fertilizers and growth promoters set the first pod higher. A different situation was observed in plants of the traditional cultivar Poker fertilized with Amofoska 3, which set the first pod lower. Among growth regulators, only cytokinin (BA) made plants set the first pod higher, both in the control and experimental treatments. Fertilization with Polifoska 6 enabled to obtain the greatest number of pods per plant. Their number was significantly higher when compared with fertilization with Amofoska 3 combined with BA and IBA. A low number of pods, observed especially in the case of cytokinin (BA), results most probably from a higher position of the first pod on the plant. The number of seeds per pod and the weight of a single seed are varietal features, but can be modified – to a certain degree – by growth conditions. In cv. Poker the number of seeds per pod set was significantly higher (by approx. 30%) than in cv. Wenus, but they were smaller. Fertilization with Polifoska 6 and simple fertilizers (NPK) contributed to an increase in the number of seeds per pod. In the case of Amofoska 3 only the addition of auxin (NAA) allowed to achieve a number of seeds comparable with that recorded in pea plant fertilized with Polifoska 6 and simple fertilizers.

Seed weight per plant was similar in both pea cultivars. Seed mass was not considerably differentiated by fertilization. However, both cultivars displayed a tendency towards increased seed mass when fertilized with simple fertilizers (NPK) and Polifoska 6, as well as with Amofoska 3 in the case of cv. Wenus. The effects of growth regulators varied depending on pea cultivar. In the case of cv. Poker, NAA, triacontanol and L-tryptophan enabled to increase seed mass by 10.1 to 21.3%, in comparison with the control treatment, and by 17.6 to 29.5%, as compared with Amofoska 3 applied alone. This cultivar responded negatively to the combination Amofoska 3 + cytokinin (BA) and its precursor – adenine, which contributed to a decrease in seed mass by 21.8 to 48.4% when compared with the control plants, and by 16.4 to 28.4% when compared to the plants fertilized with Amofoska 3.

The results obtained by numerous authors show that mineral fertilization substantially affects the yield-forming potential of legumes. According to KOCOŃ (2002a), both yield height and utilization in pea depend on potassium supply. Pea plants grown under conditions of severe potassium deficiency in the soil produce fewer flowers, pods per plant and seeds per pod, which results in a lower yield and less developed seeds. In a study carried out by PODLEŚNA et al. (1997) faba bean plants fertilized with a higher potassium rate (1.59 g $K \cdot pot^{-1}$) produced more seeds. Mineral nitrogen fertilization had no effect on the yield potential of this species, expressed as the number of flowers per plant (KOCOŃ et al. 1997). Phosphorus fertilization of faba bean resulted in a higher seed yield at the highest rate (200 kg) of superphosphate (ABDALLA 2002). YEMANE,

SKJELVAG (2003) reported that phosphorus fertilization had a positive effect on pea seed yield.

Nutrients may be introduced into the soil both in the form of simple (NPK) and multi-component fertilizers. STĘPIEŃ, MERCIK (2001), who conducted long-term field trials with multi-component fertilizers, attained seed yield at a level similar to that achieved in the treatment with simple NPK fertilizers. In an experiment with fiber flax, complex forms of mineral fertilizers and bioactive substances (Fenomelan) significantly increased the yields of seeds and straw (KUKRESH, KHODYANKOVA 2001).

The tested cultivars differed significantly in the level of dry matter accumulation in aboveground vegetative organs (Figure 2). The application of NPK (simple fertilizers) and Polifoska 6 had a positive effect on dry matter accumulation in leaves, especially in cv. Wenus (increase by 12.5 to 14.5%), as well as in pod-shells. The application of Amofoska 3 to cv. Poker was followed

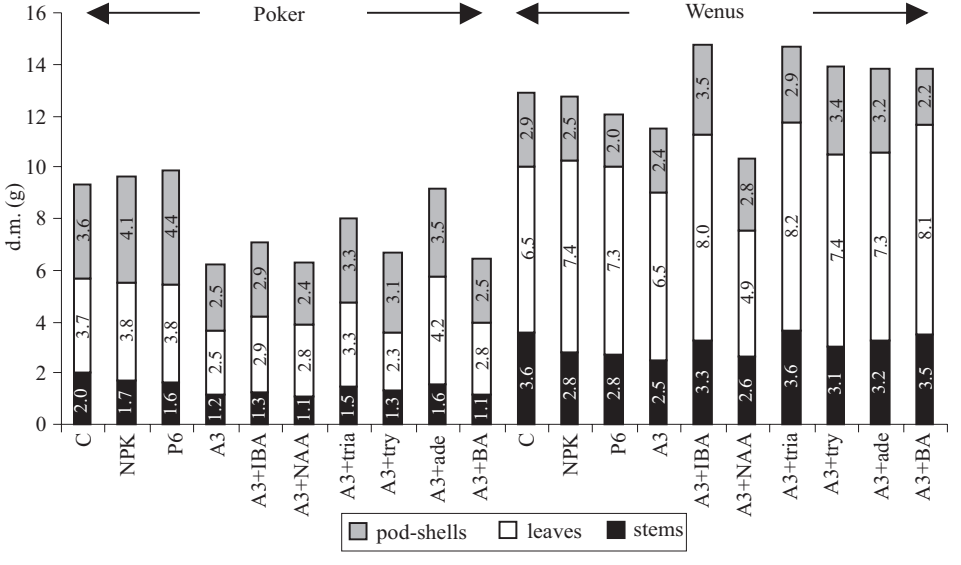


Fig. 2. Accumulation of dry matter of leaves, stems and pod shells in pea plants for different variants of mineral fertilization and bioregulators

by a 33% reduction in leaf mass, when compared with unfertilized plants. In addition, a decrease in stem mass (40% in cv. Poker and 30% in cv. Wenus) and pod-shell mass (30% and 17% respectively) was recorded in both cultivars. The pea cultivars differed also in their response to growth promoters applied together with Amofoska 3. In cv. Wenus, all growth promoters except NAA had a positive effect when combined Amofoska 3, thus increasing the accumulation

of vegetative dry matter, when compared with both the control treatment and the treatment fertilized only with Amofoska 3. The combination of IBA and tracontanol increased dry matter accumulation in aboveground vegetative organs by about 30% and 13-15%, in comparison with Amofoska 3 applied alone and with the control treatment, respectively. In cv. Poker growth regulators applied with Amofoska 3 stimulated plant growth, but vegetative mass accumulation was at a much lower level than in the control plants.

Also KOCOŃ (2002a) reported that pea seed yield is closely related to cultivar, fertilization and weather conditions. According to BENEDYCKA, NOWAK (1995), nitrogen fertilization of faba bean plants had a significant effect on root mass and aboveground part mass. In a precise trial performed by YEMANE, SKJELVAG (2003) higher phosphorus rates differentiated plant biomass, LAI and the number of branches.

Pea cultivars of different morphological types differed also in the distribution of aboveground part biomass (Figure 3). In the control treatment the traditional cultivar Poker accumulated 63.5% biomass in seeds, 14.3% in pod shells and leaves, and 7.8% in stems. The narrow-leaved cultivar Venus

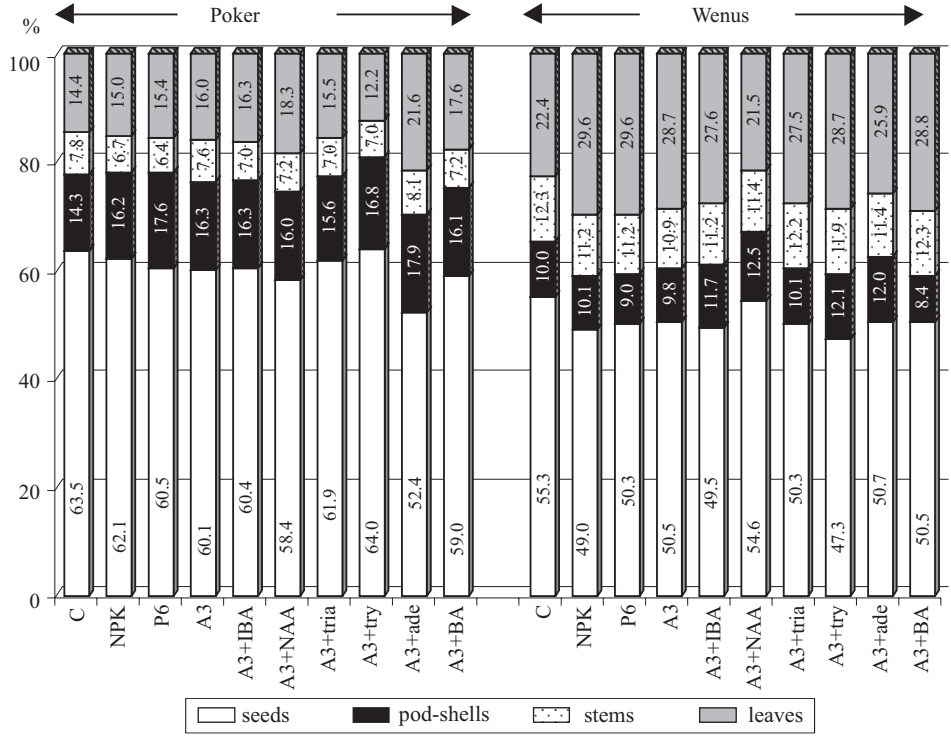


Fig. 3. Accumulation of biomass in organs of pea

accumulated 55.3% biomass in seeds, 22.4% in leaves, 12.3% in stems and 10.0% in pod shells.

The fertilizers applied in the study reduced the proportion of seed biomass. Among the growth-promoting substances tested in our experiment only Amofoska 3 applied with L-tryptophan slightly increased the percentage of seed biomass in the traditional cultivar Poker (+0.5%), as compared with the control treatment. A particularly negative effect was observed in cv. Poker fertilized with Amofoska 3 + adenine. This combination resulted in a 11.1% decrease in biomass accumulation in seeds, as compared with the control treatment. A similar response (a decrease by 8.0%) was recorded in cv. Wenus fertilized with Amofoska 3 + L-tryptophan. The application of all mineral fertilization variants and bioregulators caused an increase in biomass accumulation in pod-shells and – to a lower degree – in leaves in cv. Poker. In cv. Wenus an increase was noted primarily in the accumulation of aboveground mass in leaves.

## Conclusions

1. The morphological characters of both pea forms were modified to a higher degree by genotype than by fertilization.

2. The greatest mass per plant was obtained applying simple fertilizers NPK and Polifoska 6, as well as Amofoska 3 in cv. Wenus. The combination of Amofoska 3 and growth regulators had no significant effect on seed yield.

3. Mineral fertilizers and bioregulators decreased biomass accumulation in seeds. The pea cultivars tested in the study responded differently to the combination of Amofoska 3 and L-tryptophan. In cv. Poker biomass accumulation in seeds increased slightly, when compared with the control treatment, whereas in cv. Wenus treated with L-tryptophan the lowest amount of biomass was accumulated in seeds.

Translated by Aleksandra Poprawska

Accepted for print 7.04.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1):147-154, Y. 2006

# CHEMICAL COMPOSITION AND PHYSICOCHEMICAL PROPERTIES OF COLOSTRUM AND MILK OF WIELKOPOLSKA MARES*

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K e y w o r d s: mares milk, colostrum, chemical composition, Wielkopolska horses.

A b s t r a c t

Basic chemical composition, density, active acidity and free fatty acid contents were investigated in colostrum and milk of Wielkopolska mares during 21 weeks of lactation. Colostrum in comparison to milk contained significantly more total protein and casein and was characterized by higher dry matter contents and density. It contained less fat, lactose and free fatty acids. Mean total protein content in milk was 2.00%, decreasing from the 1st (3.78%) to the 12$^{th}$ (1.54%) week of lactation. In the period between week 13 and 21 of lactation the level of total protein was similar, within the range from 1.62 to 1.72%. Contents of lactose, casein, fat, dry matter and free fatty acids in milk were 6.23%, 1.34%, 2.41%, 11.33% and 0.30 $\mu Eq \cdot cm^{-3}$, respectively. The smallest fluctuations in the investigated lactation period were found in the contents of free fatty acids. Lactose content was relatively stable. Starting from the 8$^{th}$ to the 21$^{st}$ week of lactation it was similar, within the range from 6.25 to 6.50%.

## SKŁAD CHEMICZNY I WŁAŚCIWOŚCI FIZYKOCHEMICZNE SIARY I MLEKA KLACZY WIELKOPOLSKICH

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S ł o w a k l u c z o w e: mleko klaczy, siara, skład chemiczny, klacze wielkopolskie.

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* The research was supported by grant 2 P06Z 070 27 from the Polish State Committee for Scientific Research.

Abstrakt

Określono podstawowy skład chemiczny, gęstość, kwasowość czynną oraz zawartość wolnych kwasów tłuszczowych w siarze i mleku klaczy wielkopolskich w okresie 21 tygodni laktacji. Siara, w porównaniu z mlekiem, zawierała istotnie więcej białka ogólnego i kazeiny oraz miała wyższą zawartość suchej substancji i większą gęstość, natomiast mniej tłuszczu, laktozy i wolnych kwasów tłuszczowych. Zawartość białka ogólnego w mleku klaczy wynosiła średnio 2%, zmniejszając się od 1. (3.78%) do 12. (1.54%) tygodnia laktacji. Między 13. a 21. tygodniem laktacji jego zawarość była na zbliżonym poziomie i wynosiła 1.62 – 1.72%. Zawartość laktozy, kazeiny, tłuszczu, suchej substancji i wolnych kwasów tłuszczowych w mleku wynosiła odpowiednio: 6.23%, 1.34%, 2.41%, 11.33% i 0.30 $\mu Eq \cdot cm^{-3}$. Najmniejsze wahania w analizowanym okresie laktacji stwierdzono w zawartości wolnych kwasów tłuszczowych. W miarę stabilna była również zawartość laktozy od 8. do 21. tygodnia laktacji, wynosiła 6.25 – 6.50%.

## Introduction

Milk of various mammals has been consumed by man since time immemorial. Egyptians, Greeks, Romans, Mongols, the Chinese, as well as German tribes, apart from cow milk consumed milk of donkeys, sheep, goats, mares or even camels (SEIDL, DERLER-TÖCHTERLE 2002). Research studies conducted by Russian and German scientists revealed valuable nutritive and therapeutic properties of mare milk (LAMEK, KOCH 1997). In Germany interest in this type of milk has been observed for approximately 40 years, since Dr. Rudolf Storch found scientific evidence proving a positive effect of mare milk on the human organism. In Switzerland, Austria and Germany farms have been established, on which from mare milk obtained by mechanical milking premium fresh, frozen, freeze-dried and fermented milk is produced, along with various types of cosmetics (LAMEK, KOCH 1997, MARCONI, PANFILI 1998). In the former Soviet Union and in Mongolia, and recently also in Europe, mare milk has been used to produce a fermented drink – kumis, used in holistic medicine in the treatment of tuberculosis and numerous allergies (DI CAGNO et al. 2004, KNY 1998, RIELAND 1997).

Chemical composition of mare milk differs significantly from milk of other domestic animal species (WÓJTOWSKI 2005) and it resembles rather the composition of human milk (JAHREIS et al. 1999, MALCARNE et al. 2002). Both mare and human milk is poor in casein and rich in albumins and globulins. Contents of lactose (approximately 55-65% dry matter) and vitamins are similar, except for vitamin C, the content of which is higher in mare milk. Lysozyme content – two times higher than that in human milk – explains the effectiveness of mare milk in the treatment of thrush, aphtae, inflammation of nasal passages, rhinitis and whooping cough (CSAPÓ et al. 1995, JOHNSTON et al. 1970, HÖFFKEN 2002).

In Poland production of mare milk for human consumption practically does

not exist (WÓJTOWSKI 2005). However, the relatively large population of horses in our country makes it possible to produce and utilize it in the diet of the elderly and children allergic to cow milk (WÓJTOWSKI 2005).

The aim of this study was to determine the basic composition and physicochemical properties of milk coming from mares of the Wielkopolska breed.

## Material and Methods

Material for the study consisted of milk collected from 10 Wielkopolska mares, which foaled in January, February and March 2005 in the stud farm of the Jarosławiec Farm located near Środa Wielkopolska. Mares were aged from 6 to 9 years. All the animals were kept under identical environmental conditions and fed in accordance with the feed requirement of nursing mares (CHACHUŁOWA 2004). Colostrum was collected twice: for the first time 18 to 24 h after foaling and the second time 42-48 h post-partum. Milk for analyses in the amount of 200-250 $cm^3$ was collected daily on the 3rd, 4th, 5th and 6th day of lactation, and then in 6-7 day intervals for the following 21 weeks of lactation. It was always milked at the same time of the day, i.e. between 8 and 9 a.m., after previous separation of foals from their mothers for the period of 1 hour. During milking foals remained in eye and touch contact with their mothers.

In the collected 20 samples of colostrum and 250 samples of milk the protein, fat, lactose and dry matter were determined using a Milkoscan 133B device, and that of casein by the Walker technical method (Polish Standard 1968). Density measurements were taken with a lactodensimeter at fiducial temperature of 20°C using the areometric method (Polish Standard 1968). Active acidity was measured with a CP-315 pH-meter by Elmetron (Polish Standard 1968). The content of free fatty acids was determined using the Dole extraction-titrimetric method as modified by DOETH and FITZ-GERALD (1976).

Statistical analysis of data was carried out using a analysis of variance (SAS® 1996). The following effects were included in the model: the effect of the type of milk (colostrum, milk) and stage of lactation (day or week of lactation).

## Results and Discussion

Colostrum, in comparison to milk, contained significantly more protein and casein and was characterized by a higher dry matter content and higher density ($p \leq 0.01$). Percentages of total protein and casein in colostrum

collected on the first day after foaling were 1.75 times higher than analogous indexes determined in colostrum produced on the second day after parturition (Figure 1). In turn, percentages of fat, lactose and free fatty acids in colostrum were lower than those found in milk ($p \leq 0.01$). Also active

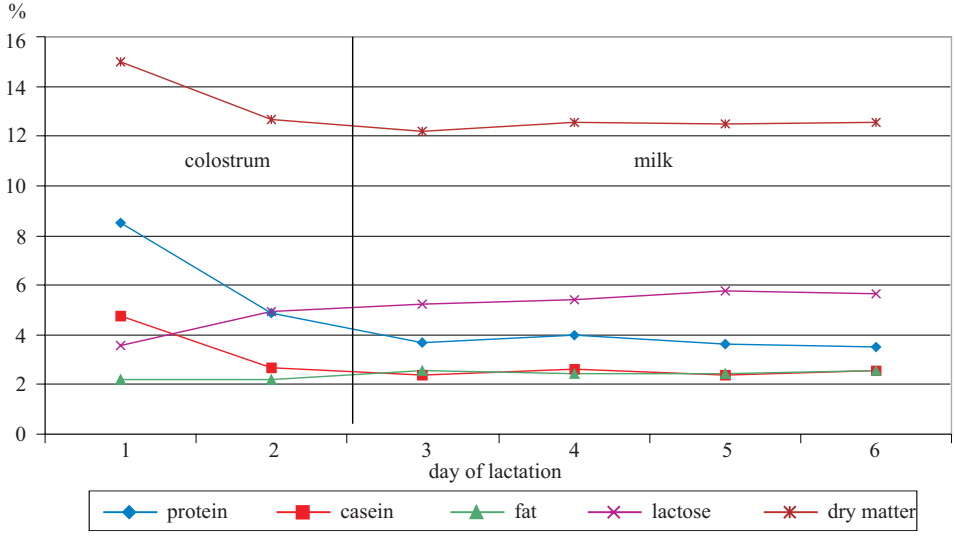


Fig. 1. Basic chemical composition of mares' colostrum and milk during first 6 days

Table 1

Physiochemical properties of mares' colostrum and milk during first 6 days of lactation (means and standard deviations)

| Day of lactation | Active acidity (pH) | | Density ($g \cdot cm^{-3}$) | | Free fatty acids ($\mu Eq \cdot cm^{-3}$) | |
|---|---|---|---|---|---|---|
| | $x$ | SD | $x$ | SD | $x$ | SD |
| Colostrum | | | | | | |
| 1 | 6.60 | 0.25 | $1.0527^{A}$ | 0.0064 | 0.21 | 0.13 |
| 2 | 6.56 | 0.06 | $1.0407^{B}$ | 0.0010 | 0.25 | 0.10 |
| Milk | | | | | | |
| 3 | $6.57^{a}$ | 0.04 | 1.0348 | 0.0018 | 0.32 | 0.03 |
| 4 | 6.59 | 0.08 | 1.0350 | 0.0001 | 0.30 | 0.02 |
| 5 | 6.62 | 0.03 | 1.0354 | 0.0003 | 0.29 | 0.01 |
| 6 | $6.62^{b}$ | 0.07 | 1.0359 | 0.0008 | 0.34 | 0.11 |
| Colostrum – milk | ** | | ** | | ** | |

*A,B,a,b* – means denoted witch different letters differ significantly
capital letters – $p \leq 0.01$, small letters – $p \leq 0.05$
** – $p \leq 0.01$

acidity of colostrum was statistically significantly higher in comparison to milk (Table 1). Obtained values are consistent with literature data and confirm reports by foreign authors that the period of colostrum secretion by the mare is much shorter than the analogous period observed in cattle, whereas significant differences observed in the basic composition of colostrum and milk are observed only in the period up to 48 h after foaling (CASPÓ et al. 1995, RIELAND 1997). The biggest differences between colostrum and milk, similarly as in this study, were observed by other authors in contents of protein, lactose and dry matter (NESENI et al. 1958, CSAPÓ-KISS et al. 1995, ). In contrast, differences in fat percentage, similarly to this experiment, were much smaller (CSAPÓ et. al. 1995).

Total protein content in milk analyzed in this study was 2.00%. Its lowest level was found in milk samples collected in the 16th and 21st weeks of lactation (Figure 2). Similarly to the total protein content, also casein level decreased along with the duration of lactation from 1.48% (the 4th weeks) to 1.10% (from the 16th to 21st weeks, $p \leq 0.01$). Mean casein ratio in total milk protein was 0.66, which is consistent with the results of chemical analyses of milk coming from Arab, Małopolska and Hucul horses (KULISA 1977).

Mean fat content in milk of the investigated Wielkopolska mares was 2.41%. The highest level of this trait, i.e. 3.11%, was reported in the fifth week of lactation, after which it was observed to decrease gradually, on average to the level of 2.20% (Figure 2, $p \geq 0.05$). The obtained values and the observed trend are consistent with those reported in professional literature (JAWORSKI et al. 1982, KULISA 1980, SUMMER et al. 2004).

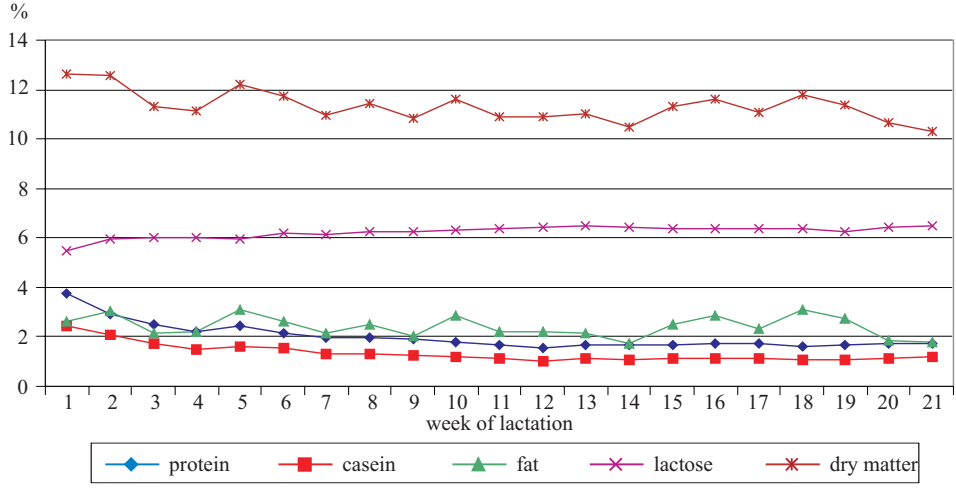


Fig. 2. Basic chemical composition of mares; milk during 21 weeks of lactation

In this experiment lactose concentration in milk was 6.23% and was the lowest in the first week of lactation (5.50; $p \leq 0.01$). Starting from the 8th week of lactation until the time of the completion of this study its level in milk was relatively stable and amounted to 6.25 – 6.50% (Figure 2). The concentration of lactose in milk of Wielkopolska mares found in this study did not differ from the results obtained in other horse breeds (BOUWMAN, van der SCHEE 1978, KUBIAK et al. 1991, KULISA 1977, SUMMER et al. 2004), and it was very similar to the concentration of this component in human milk (JAHREIS 1999, RIELAND 1997).

Mean density of milk analyzed in this study was 1.0335 $g \cdot cm^{-3}$ and it was the highest in the first week of lactation ($p \leq 0.05$). Starting from the 12th week until the end of the observations it remained on a stable, almost identical level of 1.0330 $g \cdot cm^{-3}$ (Table 2). Mean active acidity for the 21-week period of lactation was 6.78. Significantly higher acidity (6.60) was found for milk

Table 2

Physiochemical properties of mares' milk during 21 weeks of lactation (means and standard deviations)

| Week of lactation | Active acidity (pH) | | Density ($g \cdot cm^{-3}$) | | Free fatty acids ($\mu Eq \cdot cm^{-3}$) | |
|---|---|---|---|---|---|---|
| | $x$ | SD | $x$ | SD | $x$ | SD |
| 1 | 6.60 | 0.05 | 1.0352 | 0.0010 | 0.31 | 0.04 |
| 2 | 6.63 | 0.18 | 1.0346 | 0.0009 | 0.31 | 0.07 |
| 3 | 6.75 | 0.06 | 1.0339 | 0.0009 | 0.28 | 0.05 |
| 4 | 6.81 | 0.14 | 1.0343 | 0.0008 | 0.31 | 0.03 |
| 5 | 6.80 | 0.01 | 1.0342 | 0.0011 | 0.33 | 0.02 |
| 6 | 6.81 | 0.05 | 1.0338 | 0.0012 | 0.31 | 0.03 |
| 7 | 6.83 | 0.10 | 1.0337 | 0.0013 | 0.30 | 0.02 |
| 8 | 6.82 | 0.06 | 1.0338 | 0.0015 | 0.30 | 0.06 |
| 9 | 6.77 | 0.08 | 1.0333 | 0.0015 | 0.29 | 0.04 |
| 10 | 6.81 | 0.09 | 1.0329 | 0.0010 | 0.30 | 0.07 |
| 11 | 6.84 | 0.05 | 1.0331 | 0.0023 | 0.26 | 0.10 |
| 12 | 6.79 | 0.05 | 1.0334 | 0.0015 | 0.29 | 0.01 |
| 13 | 6.80 | 0.09 | 1.0310 | 0.0014 | 0.27 | 0.03 |
| 14 | 6.84 | 0.02 | 1.0328 | 0.0009 | 0.28 | 0.02 |
| 15 | 6.74 | 0.13 | 1.0343 | 0.0004 | 0.31 | 0.03 |
| 16 | 6.77 | 0.09 | 1.0320 | 0.0009 | 0.33 | 0.03 |
| 17 | 6.78 | 0.04 | 1.0332 | 0.0006 | 0.30 | 0.04 |
| 18 | 6.75 | 0.12 | 1.0325 | 0.0024 | 0.33 | 0.04 |
| 19 | 6.77 | 0.10 | 1.0330 | 0.0010 | 0.28 | 0.05 |
| 20 | 6.83 | 0.01 | 1.0333 | 0.0005 | 0.29 | 0.01 |
| 21 | 6.82 | 0.01 | 1.0343 | 0.0013 | 0.28 | 0.08 |

produced in the first week of lactation ($p \leq 0.01$, table 2). Levels of both density and pH of milk did not differ from analogous values determined in milk of other farm animal species, such as cows, goats and sheep (DANKÓW et al. 1996). A lack of available literature data makes it impossible to compare contents of free fatty acids (FFA) in colostrum and milk of mares with data obtained by other authors. The FFA level in individual weeks of lactation was very uniform and the observed statistical differences were non-significant (Table 2). In comparison to milk of other species the FFA level in mare milk is slight. For example in cow milk it is 1.36 – 1.90 $\mu Eq \cdot cm^{-3}$, goat milk it is 1.32 – – 1.90 $\mu Eq \cdot cm^{-3}$, while in sheep milk it is 2.56 – 3.86 $\mu Eq \cdot cm^{-3}$, respectively (SZYMCZAK 2002).

## Conclusions

1. The basic chemical composition and analyzed physicochemical properties of mare colostrum differed significantly from the composition and analogous properties of milk.

2. Levels of most analyzed traits were significantly affected by the stage of lactation.

3. The level of free fatty acids found in mare milk was low and independent from the stage of lactation.

Accepted for print 12.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 155-162, Y. 2006

# NUTRIENT DIGESTIBILITY AND NITROGEN BALANCE IN FINISHING PIGS FED DIETS CONTAINING GROUND WHEAT STRAW*

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Key words: pigs, feeding, fiber, ground wheat straw, apparent digestibility, nitrogen balance.

Abstract

Three complete diets fed to finishing pigs were analyzed. Control diet (I) was made of soybean meal, ground barley, ground wheat and vitamin-mineral supplements. Experimental diets II and III were supplemented with 10% and 15% of ground wheat straw respectively. Nutrient digestibility and nitrogen balance were determined by the balance method on 12 Polish Landrace fatteners with average initial body weights of 102.3 kg, divided by the analogue method into three feeding groups, each of four pigs. The experiment was divided into a 10-day preliminary period and 5-day experimental period.

Experimental diet supplementation with ground wheat straw resulted in an increase in crude fiber content, from 2.76% (diet I – control) to 5.36% (diet II) and 8.69 (diet III). It was found that a higher crude fiber concentration in diets, caused by the addition of 15% of ground wheat straw (group III), significantly reduced the digestibility of crude protein, NDF and hemicellulose. A statistically significant decrease in the digestibility of N-free extractives and gross energy was observed in the case of both the diet with 10% and 15% of ground wheat straw (groups II and III respectively). No significant differences were found in the digestibility of crude fat, crude fiber, ADF, cellulose, and in daily nitrogen retention in fatteners.

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* This work was conducted as a part of project no 3 PO6Z 01924 financed by the State Committee for Scientific Research

**STRAWNOŚĆ SKŁADNIKÓW POKARMOWYCH I BILANS AZOTU U ŚWIŃ ŻYWIONYCH W II OKRESIE TUCZU MIESZANKAMI Z UDZIAŁEM ZMIELONEJ SŁOMY PSZENNEJ**

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S ł o w a k l u c z o w e: świnie, żywienie, włókno, zmielona słoma pszenna, strawność pozorna, bilans azotu.

A b s t r a k t

Badano 3 mieszanki pełnoporcjowe przeznaczone dla tuczników na II okres tuczu. W skład mieszanki kontrolnej (I) wchodziły: śruta poekstrakcyjna sojowa, śruta jęczmienna, śruta pszenna oraz dodatki mineralno-witaminowe. Do mieszanek doświadczalnych (II i III) dodano kolejno 10 i 15% zmielonej słomy pszennej. Strawność składników pokarmowych i bilans azotu badano metodą bilansową na 12 wieprzkach Polish Landrace o średniej początkowej masie ciała 102,3 kg, podzielonych metodą analogów na 3 grupy żywieniowe po 4 szt. w każdej. Okres wstępny trwał 10 dni, a właściwy 5 dni.

Wprowadzenie do mieszanek doświadczalnych słomy pszennej wpłynęło na zwiększenie poziomu włókna surowego z 2,76% (mieszanka kontrolna – I) do 5,36% (mieszanka II) i 8,69% (mieszanka III). Stwierdzono, że zwiększenie zawartości włókna surowego w dietach dla tuczników przez dodatek 15% słomy pszennej (gr. III) istotnie zmniejszyło strawność białka surowego, NDF i hemicelulozy. Statystycznie istotne zmniejszenie strawności związków bezazotowych wyciągowych i energii brutto obserwowano zarówno w przypadku diety z 10%, jak i 15% udziałem słomy (gr. II i III). Nie stwierdzono natomiast istotnych różnic w strawności tłuszczu surowego, włókna surowego, ADF, celulozy oraz dobowej retencji azotu u badanych tuczników.

## Introduction

The results of numerous experiments showed that the intake of complete diets for fattening pigs, balanced in terms of energy and protein content, may be higher than provided for in the relevant standards. This concern especially the second stage of fattening, when animals compete for feed, and when diets with a low crude fiber content cannot satiate their appetite. Under such circumstances feeding *ad libitum* may lead to excessive fatness. Therefore, the problem of diet supplementation with fibrous feed in pig nutrition is still widely discussed. The analysis concerns the effects of both the level and type of fiber added to diets on productivity, nutrient digestibility, metabolism, carcass quality and behavior patterns (DOHERTY et al. 2002, FALKOWSKI et al. 2004, FALKOWSKA et al. 2004, GALASSI et al. 2004, MEUNIER-SALAUN et al. 2001, MOORE et al. 1988, PASCHMA et al. 2005).

HAKANSSON et al. (2000) reduced the concentrations of energy and protein in diets for fatteners through adding 8 and 16% of ground wheat straw, which

allowed to decrease backfat thickness and improve carcass meatiness. However, digestibility tests revealed that an increase in crude fiber content from 4.5% (control diet) to 7.3% (diet containing 8% of ground wheat straw) had no influence on total protein digestibility, and significantly decreased gross energy digestibility.

O'DOHERTY et al. (2002) found that an increase in crude fiber content from 4.9% to 7.2% resulting from the addition of soybean husks significantly reduced total protein digestibility. Our previous studies (FALKOWSKA et al. 2004, FALKOWSKI et al. 2004) showed that diet supplementation with 10%, 15% or 20% of wheat bran did not cause a significant decrease in the apparent digestibility of crude protein and the level of daily nitrogen retention in fatteners at the second stage of fattening.

This indicates that the results of balance and digestibility tests are ambiguous. Thus, the aim of the present study was to determine nutrient digestibility and nitrogen balance in fatteners fed diets containing 10% and 15% of ground wheat straw at the second stage of fattening.

## Materials and Methods

Three complete diets fed to fatteners at the second stage of fattening were analyzed. Control diet (I) was made of soybean meal, ground barley, ground wheat and vitamin-mineral supplements, according to *Pig Nutrient Requirements* (1993). Experimental diets II and III were supplemented with 10% and 15% of ground wheat straw respectively (Table 1).

Nutrient digestibility and nitrogen balance were determined by the direct balance method on 12 Polish Landrace fatteners with average initial body weights of 102.3 kg, divided by the analogue method into three equal feeding

Table 1

Composition of experimental diets (%)

| Specification | Diets | | |
|---|---|---|---|
| | I | II | III |
| Ground wheat | 30.00 | 27.00 | 25.50 |
| Ground barley | 49.03 | 44.13 | 41.66 |
| Soyabean meal | 14.00 | 12.60 | 11.90 |
| Wheat straw meal | – | 10.00 | 15.00 |
| Dicalcium phosphate | 1.50 | 1.35 | 1.28 |
| Limestone | 1.50 | 1.35 | 1.28 |
| Premix | 1.50 | 1.35 | 1.28 |
| Salt | 0.30 | 0.27 | 0.26 |
| Rapeseed oil | 2.00 | 1.80 | 1.70 |
| DL- lysine (99%) | 0.17 | 0.15 | 0.14 |

groups. The experiment was divided into a 10-day preliminary period and 5-day experimental period. The daily ration was identical in all groups – 3 kg/day/head. The fatteners were fed twice a day and had free access to water.

The nutrient content of diets and feces samples was determined by the Weende method. Nitrogen concentration was determined in fresh feces samples preserved with concentrated sulfuric acid, and in urine samples preserved with 20% sulfuric acid. The other nutrients, including fiber fractions and energy content, were determined in samples of partly dried feces. The gross energy content of diets and feces samples was determined using an adabatic bomb calorimeter. Fiber fractions were determined by the detergent extraction method (VAN SOESTA, WINE, 1967) using a Fibertec System M apparatus (Tecator). The level of metabolizable energy in diets was calculated according to the equation developed by Hoffmann and Schiemann (*Pig Nutrient Requirements*, 1993), based on chemical composition and digestibility coefficients determined in the study.

The results of the study were verified statistically by a one-factor analysis of variance using the Duncan's test. The calculations were performed using Statistica for Windows software.

## Results and Discussion

Experimental diet supplementation with ground wheat straw resulted in a slight decrease in protein content, from 15.71% (group I) to 15.24% and 14.73% (groups II and III respectively), as well as in an increase in crude fiber content, from 2.76% (diet I – control) to 5.36% (diet II) and 8.69 (diet III). The levels of fiber fractions, i.e. NDF, ADF, hemicellulose, cellulose and lignins, increased in diets II and III (Table 2).

The gross energy content of diets I, II and III was comparable, and amounted to 16.92, 16.93 and 16.91 MJ/kg respectively. The level of metabolizable energy, calculated based on chemical composition and digestibility coefficients determined in the study, was by about 6% and 13% lower in groups II and III, respectively, as compared with group I – control (Table 2). HAKANSSON et al. (2000) added 8% and 16% of ground wheat straw to complete diets containing 12.5 MJ/kg ME, fed to fatteners at the second stage of fattening, and noted a decrease in metabolizable energy content, by 6.4% and 14.4% respectively. Just (1982) added 30% of ground barley straw to diets for fatteners, which enabled to increase crude fiber content threefold (4.8% to 14% d.m.). As a result, this author observed a decrease in metabolizable energy concentration by about 15% (from 14.75 MJ/kg to 11.04 MJ/kg d.m.).

Table 2

Chemical composition and nutritive value of experimental

| Specification | Diets | | |
|---|---|---|---|
| | I | II | III |
| Chemical composition, % | | | |
| Dry matter | 88.27 | 88.09 | 88.06 |
| Crude ash | 5.37 | 5.39 | 5.56 |
| Organic matter | 82.90 | 82.70 | 82.50 |
| Crude protein | 15.71 | 15.24 | 14.73 |
| Crude fat | 3.01 | 2.50 | 2.71 |
| Crude fiber | 2.76 | 5.36 | 8.69 |
| N-free extraction | 61.42 | 59.60 | 56.37 |
| NDF | 18.16 | 27.71 | 28.32 |
| ADF | 4.34 | 8.71 | 11.28 |
| ADL | 0.83 | 1.06 | 1.80 |
| Hemicellulose | 13.82 | 19.00 | 17.04 |
| Cellulose | 3.51 | 7.65 | 9.48 |
| Gross energy, MJ/kg | 16.92 | 16.93 | 16.91 |
| Digestible energy, MJ/kg | 14.50 | 13.63 | 12.59 |
| Metabolisable energy, MJ/kg | 13.16 | 12.37 | 11.44 |

The increase in the crude fiber content of diets supplemented with ground wheat straw affected nutrient digestibility (Table 3). The increase in crude fiber levels was accompanied by reduced digestibility of organic matter, total protein, N-free extractives and gross energy.

The coefficients of total protein digestibility, recorded in groups I, II and III, were relatively high, i.e. 84.41%, 83.16% and 80.13% respectively. A two-fold increase in crude fiber content, from 2.76% (group I) to 5.36% (group II) had no significant effect on a decrease in total protein digestibility. A significant decrease in total protein digestibility was observed in group III, which received a diet with a threefold higher level of crude fiber, in comparison with group I. HAKANSSON et al. (2000) added 8% and 16% of ground wheat straw to diets for fatteners, and noted a significant decrease in total protein digestibility in the latter case. The coefficients of total protein digestibility reported by HAKANSSON (75%, 70%, 68%) were much lower than those recorded in our study. Just (1982) increased the crude fiber content of diets for fattening pigs threefold, and observed a decrease in total protein digestibility from 79% to 61%. GALASSI et al. (2004) analyzed nutrient digestibility in fatteners with body weights of 85 kg, fed diets supplemented with crude fiber from various sources, including wheat bran and dried beet pulp. The crude fiber content of diets was 3.3%, 4.3% and 5.4%. A significant effect of crude fiber level on a decrease in crude protein digestibility was noted.

A significant reduction in the digestibility of N-free extractives and gross energy was observed in the groups fed diets containing 10% and 15% of ground

Table 3

Apparent digestibility and N balance in pigs

| Specification | | Diets | | |
|---|---|---|---|---|
| | | I | II | III |
| Digestibility coefficients, % | | | | |
| Organic matter | $\bar{x}$ | $87.0^{Aa}$ | $82.0^{Ab}$ | $76.3^{B}$ |
| | $s$ | 0.98 | 1.66 | 1.83 |
| Crude protein | $\bar{x}$ | $84.4^{A}$ | $83.1^{a}$ | $80.1^{Bb}$ |
| | $s$ | 2.17 | 2.00 | 0.35 |
| Crude fat | $\bar{x}$ | 82.8 | 80.20 | 79.0 |
| | $s$ | 0.73 | 3.38 | 2.98 |
| Crude fiber | $\bar{x}$ | 21.4 | 20.0 | 24.9 |
| | $s$ | 6.09 | 2.73 | 12.24 |
| N-free extractions | $\bar{x}$ | $89.1^{Aa}$ | $86.4^{Ab}$ | $81.4^{B}$ |
| | $s$ | 0.98 | 1.06 | 1.27 |
| Gross energy | $\bar{x}$ | $85.7^{Aa}$ | $80.5^{Ab}$ | $74.5^{B}$ |
| | $s$ | 2.34 | 1.97 | 1.79 |
| NDF | $\bar{x}$ | $68.4^{a}$ | $63.6^{a}$ | $52.9^{b}$ |
| | $s$ | 2.71 | 3.51 | 4.12 |
| ADF | $\bar{x}$ | 32.7 | 31.3 | 28.0 |
| | $s$ | 5.95 | 8.69 | 7.43 |
| Hemicellulose | $\bar{x}$ | $79.6^{A}$ | $78.4^{A}$ | $69.4^{B}$ |
| | $s$ | 4.06 | 2.66 | 4.82 |
| Cellulose | $\bar{x}$ | 32.4 | 30.5 | 27.4 |
| | $s$ | 5.54 | 7.68 | 9.11 |
| Nitrogen balance, g | | | | |
| N intake | $\bar{x}$ | 72.63 | 73.14 | $70.70^{B}$ |
| N in faeces | $\bar{x}$ | 11.40 | 12.31 | 14.13 |
| | $s$ | 2.30 | 1.46 | 0.18 |
| N digested | $\bar{x}$ | $61.23^{a}$ | $60.83^{a}$ | $56.58^{b}$ |
| | $s$ | 3.38 | 1.46 | 0.18 |
| N in urine | $\bar{x}$ | 26.86 | 28.06 | 25.69 |
| | $s$ | 0.63 | 4.99 | 2.85 |
| N retention | $\bar{x}$ | 34.37 | 32.77 | 30.88 |
| | $s$ | 3.23 | 6.37 | 3.02 |
| N retained/N intake, % | $\bar{x}$ | 47.26 | 44.79 | 43.67 |
| | $s$ | 0.94 | 8.71 | 4.28 |
| N retained/N digested, % | $\bar{x}$ | 56.04 | 53.70 | 55.14 |
| | $s$ | 2.44 | 9.25 | 5.88 |

*a,b* – $P \leq 0.05$
*AB* – $P \leq 0.01$

wheat straw. An increased crude fiber concentration had no significant influence on the digestibility of crude fat and crude fiber. GALASSI et al. (2004) and FALKOWSKI et al. (2004) did not found significant differences in the digestibility of crude fat and crude fiber resulting from an increased crude fiber content of diets fed to fatteners at the second stage of fattening, either. JUST (1982) increased the crude fiber content of diets for fattening pigs (from 4.8% to 14% d.m.) and analyzed nutrient digestibility three times (at b.m. of ≈ 25, 50

and 80 kg). This author observed a decrease in the digestibility of crude fat and crude fiber, from 64% to 50% and from 38% to 25% respectively.

An increase in crude fiber levels in diets II and III had no significant effect on the digestibility of ADF and cellulose. A significant decrease in the digestibility of NDF and hemicellulose was observed in group III, which received a diet with 15% of ground wheat straw (Table 3).

MOORE et al. (1988) added 15% of oat husks to a diet for fattening pigs, and recorded a significant decrease in the digestibility of NDF, ADF, cellulose and hemicellulose. The addition of 15% soybean husks had no negative influence on the digestibility of particular fiber fractions. GALASSI et al. (2004) reported that the addition of 24% of wheat bran to a complete diet for fatteners with body weights of 85 kg resulted in a significant decrease in the digestibility of both NDF and ADF. FALKOWSKI et al. (2004) added 10% and 20% of wheat bran to diets fed to fatteners at the second stage of fattening, and observed a significant decrease in the digestibility of NDF, ADF, hemicellulose and cellulose.

Table 3 presents the results of daily nitrogen balance in fattening pigs. The pigs of group III, fed a diet with 15% of ground wheat straw, excreted more nitrogen in the feces and less in the urine, as compared with the pigs of groups I and II. The amount of digested nitrogen was significantly lower in group III than in groups I and II. However, daily nitrogen retention and the utilization of nitrogen taken up were comparable in all groups.

GALASSI et al. (2004) found no differences in nitrogen retention in pigs fed a diet containing 24% of wheat bran (4.7% of crude fiber), when compared with the control group (3.3% of crude fiber), but at the same time recorded a significantly higher level of nitrogen excretion in the experimental group. MOORE et al. (1988) fed fatteners diets with an increased fiber content (oat husks, soybean husks, alfalfa meal) and did not observe significant differences in nitrogen retention, either. Increased nitrogen excretion in feces in pigs fed diets with elevated fiber levels was also reported by MORGAN and WHITTEMORE (1988). According to these authors, this was related to higher microbiological activity in the blind gut. BACH KNUDSEN et al. (1991) demonstrated that carbohydrates, especially non-starch polysaccharides, are the main source of energy during microbiological fermentation in the large intestine, and that these compounds enhance microbiological nitrogen synthesis.

## Conclusions

An increased crude fiber concentration in complete diets fed to finishing pigs, caused by the addition of 10 and 15% of ground wheat straw, resulted in:

1) a significant decrease in the digestibility of crude protein, NDF and hemicellulose (diet with 15% of ground wheat straw), and

2) a significant decrease in the digestibility of N-free extractives and gross energy (diets with 10% and 15% of ground wheat straw).

3) No significant differences were found between the groups in the digestibility of crude fat, crude fiber, ADF, cellulose and daily nitrogen retention.

Translated by Aleksandra Poprawska

Accepted for print 26.04.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 163-174, Y. 2006

# THE BREEDING USE OF COLD-BLOODED STALLIONS FROM THE KĘTRZYN STATE STUD FARM IN BREEDING CENTRES IN THE NORTH-EAST OF POLAND

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K e y w o r d s: cold-blooded stallions, breeding centres, use in reproduction, number of covered mares.

A b s t r a c t

This study analyses the parameters of use for reproduction of stallions from the State Stud Farm (PSO) in Kętrzyn in breeding centres in the north-west of Poland. Included in the study were cold-blooded stallions, both those used for breeding in the past and presently, in the register of PSO Kętrzyn between 1.01.1970 and 31.12.2000.

325 copulation centres were found to exist in the 21 breeding centres in 5 provinces. During the whole period of study, 494 cold-blooded studs covered 190.712 mares, 12.8% of which were licensed female horses. The average employment of a stud during a reproductive season amounted to 52.3 mares, with the effectiveness of 2.09 jumps needed to cover a mare.

The most intensive use of studs from PSO Kętrzyn was recorded in the breeding centres of the Province of Podlasie, with those in Sokółka, Mońki, Augstów and Grajewo playing the dominant role.

Cold-blooded studs used as studhorses in the area serviced by PSO Kętrzyn stayed a little less than 2 seasons at the same breeding stations.

It is noteworthy that the breeding centres serviced by PSO Kętrzyn in the Province of Podlasie managed the breeding capacity of the stud horses in their use very reasonably, as was visible in the value of the correlation coefficient between the number of covered mares and the number of mounts (0.83). Considering the total number of covered mares (148,619) the relationship is favourable, as with such a high number of the covered mares, over-intensive use of stallions would not be desired.

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# WYKORZYSTANIE ROZPŁODOWE OGIERÓW ZIMNOKRWISTYCH Z PAŃSTWOWEGO STADA OGIERÓW W KĘTRZYNIE W OŚRODKACH HODOWLANYCH W PÓŁNOCNO-WSCHODNIEJ POLSCE

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S ł o w a k l u c z o w e: ogiery zimnokrwiste, ośrodki hodowlane, użytkowanie rozpłodowe, liczba pokrytych klaczy.

A b s t r a k t

Przeanalizowano parametry wykorzystania rozpłodowego reproduktorów z Państwowego Stada Ogierów (PSO) w Kętrzynie w ośrodkach hodowlanych na terenie północno-wschodniej Polski. Badaniami objęto ogiery zimnokrwiste, tzw. archiwalne, i aktualnie użytkowane w rozrodzie, znajdujące się w ewidencji PSO Kętrzyn w okresie od 1.01.1970 do 31.12.2000 r.

Stwierdzono funkcjonowanie 325 punktów kopulacyjnych na terenie 21 ośrodków hodowlanych zlokalizowanych w 5 województwach. W całym okresie badań 494 reproduktory zimnokrwiste pokryły łącznie 190 712 klaczy, wśród których 12,8% stanowiły samice licencjonowane. Średnie obciążenie ogiera w sezonie rozrodczym wynosiło 52,3 klacze, skuteczność krycia – 2,09 skoków potrzebnych na pokrycie samicy.

Najintensywniejsze użytkowanie reproduktorów z PSO Kętrzyn odnotowano w ośrodkach hodowlanych województwa podlaskiego, w którym pod względem hodowlanym dominują: sokólski, moniecki i augustowsko-grajewski.

Ogiery zimnokrwiste użytkowane rozpłodowo na terenie działalności PSO Kętrzyn przebywały średnio niecałe 2 sezony w jednym punkcie kopulacyjnym.

Na terenie oddziaływania PSO Kętrzyn na uwagę zasługuje rozsądne gospodarowanie możliwościami rozpłodników użytkowanych w województwie podlaskim, widoczne w wielkości współczynnika korelacji między liczbą pokrytych klaczy a liczbą oddanych skoków (0,83). Biorąc pod uwagę ogólną liczbę klaczy pokrytych (148 619 szt.), odnotowana relacja jest korzystna, gdyż w związku z tak dużą ich liczbą nadmierna eksploatacja ogierów byłaby niepożądana.

## Introduction

The Stud Farm in Kętrzyn was established in 1877 and at that time it conducted its activities in the south-eastern part of East Prussia (ZDANOWICZ 1952). During the period of the farm's history, it was initially dominated by Trakehnen and east Prussian studhorses and a small number of cold-blooded ones. Near the end of World War II all the stallions were evacuated to Germany. The breeding region of the Polish Stallion Herd, established on 1 March 1947 was quite different from the region covered by its German predecessor and its aim was to provide services to breeding centres in the Province of Białystok. The change in the serviced region entailed changes in the breed profile of the stud horses kept at the farm. With time, the proportion

of cold-blooded stallions in the herd increased. Currently, the area where PSO Kętrzyn conducts its activities covers the Province of Podlasie, Province of Warmia and Mazury and the Province of Lublin.

Because of the low profitability of studhorse breeding, stallion herds are subsidised worldwide. It is the task of the PSO to raise the level of mass breeding nationwide by directly influencing the production of high quality horses (JAWORSKI 1986). State Stallion Herds purchase the best, specially selected male material (GRZYBOWSKI 1993).

From an economic point of view, the centres do not bring any immediate profit but they do so indirectly by improving the herd quality and its utility value. For many years, when breeding centres provided service only to horse breeders from their catchment area, herds played the dominant role in shaping horse types and breeds (PIOTROWSKI 1960).

Deploying studhorses in breeding stations is not easy as, according to KOWNACKI and JASZCZAK (1968), numerous factors have to be taken into account, the main ones being the quality of horses in a region, the breeders; tastes and the environmental conditions. The appropriate deployment of studhorses in breeding stations could level out the horse population and consolidate the regional horse types (JAWORSKI 1986).

A progressive decrease in the number of horses in Poland, both in private hands and in state stud farms, affects the intensity of the breeding use of studhorses. A decrease in the number of mares covered during a season by one stallion conditions the number of studhorses kept at stud farms (BUDZYŃSKI et al. 1987).

Considering the breeding aspects and the economy of studhorse use, as well as the intensity of PSO Kętrzyn stallion use in the region where the Farms conducts its activities, it was appropriate to analyse the parameters of the studhorse use for breeding in breeding centres in the north-east of Poland.

## Materials and Methods

Cold-blooded stallions, so called archival stallions, and the ones currently used for breeding, on record at the State Stud Farm in Kętrzyn from 1 January 1970 to 31 December 2000 were included in the study. Materials for analysis were obtained from sources owned by the State Stud Farm in Kętrzyn, the Provincial Union of Horse Breeders in Białystok (WZHK) and the Union of Horse Breeders of Warmia and Mazury in Olsztyn (W-MZHK). The analysed documents included: stallion cards, protocols of breeding seasons, lists of state owned and recognised stallions and herd books (*Acta... a, b, c; Stud book...* 1964, 1972, 1978, 1986, 1993 ).

The area where the Kętrzyn Stud Farms conducts its activities was analysed, taking into account the provinces established by the administrative reform of 1999. Illustrating the range of the PSO Kętrzyn activities on a map, the areas of various communes where cold-blooded stallions were kept in the years 1970-2000 are shown with different colours. For the purpose of this study, a marked area, called a breeding area, was divided into breeding centres, in which the intensity of studhorses use was analysed.

During the detailed analysis of the use of cold-blooded stallions from PSO Kętrzyn for breeding, the following were calculated:

- the number of breeding stations where studhorses from Kętrzyn are used, divided into those run by PSO Kętrzyn employees and the leased ones, run by private breeders,
- the service time of the breeding stations, expressed as the number of breeding seasons,
- the number of studhorses,
- the time during which a studhorse is used at a breeding station, expressed as the number of breeding seasons,
- the number of covered mares: overall at particular breeding centres and the average number of mares covered by a stallion in a season,
- the proportion of licensed mares in the mating service,
- the effectiveness of covering by studhorses, expressed by the number of mounts needed to cover a mare.

The collected data are presented both in tables and graphically. Figures were analysed statistically with the use of tests with the Statistica (Statsoft) software pack, calculating:

- the weighted averages of the mean number of mares covered by a stallion during a breeding season and the number of jumps made by a stallion in order to cover a mare,
- the coefficients of variation, concerning the average number of mares covered by a stallion in a breeding season in a selected breeding centres,
- the rank correlation coefficients between the mean number of covered mares and the number of mounts in order to check the effectiveness of mares covering by studhorses.

In order to show the differences in the number of covered mares, an analysis of variance was conducted of the mean number of mares covered by a stallion during a breeding season in the provinces under study.

## Results and Discussion

The area where PSO Kętrzyn conducts its activities covers the north-east of Poland with part of the Provinces of Mazovia and of Lublin. For the purpose of

this study the area serviced by the PSO stallions was divided among the breeding centres, shown in Figure 1.

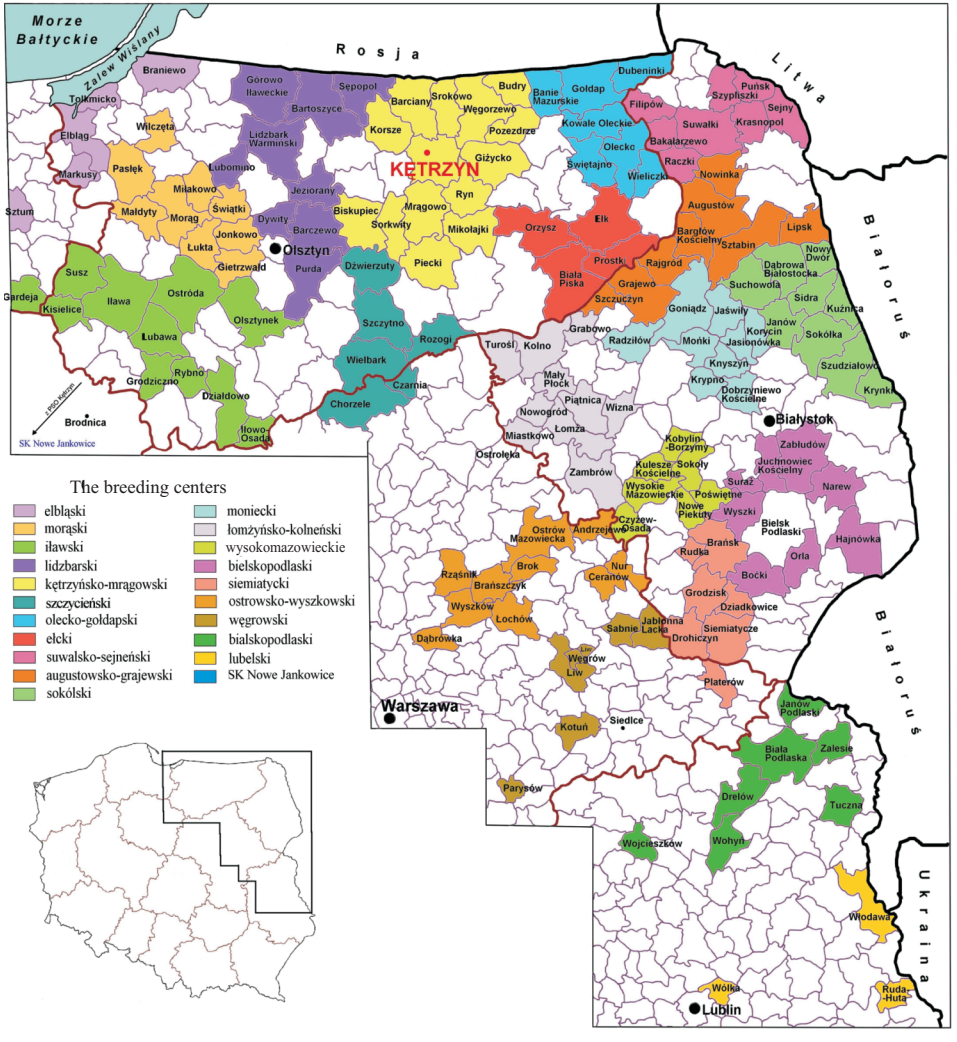


Fig. 1. The breeding centres located in the active territorz of the Kętryzn State Stud

21 breeding centres were selected from 5 provinces: Podlasie – 8, Warmia and Mazury – 8, Mazovia – 2, Lublin – 2 and the breeding station at the Nowe Jankowice Stud Farm in the Province of Kujawy and Pomorze.

The results of mating service of cold-blooded stallions from PSO Kętrzyn in 21 selected breeding centres are shown in Table 1. The detailed analysis of the

Table 1

Use in reproduction of the cold-blooded stallions from the Kętrzyn State Stud in the breeding centres

| Breeding centre | Breeding stations | | | Breeding stallions | | Number of covered mares | | | | | Number of mounts to cover one mare | Rank correlation coefficient[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | number | | stationas' seasons acitivity | number | period of using stallion on station (breeding season) | total spec. | average by a stallion in a season | coefficient of variation (%) | licensed | | | |
| | PSO[1] | leased[2] | | | | | | | spec. | (%) | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Suwałki – Sejny | 3 | 11 | 6.1 | 42 | 2.2 | 6937 | 63.6 | 44.3 | 389 | 5.6 | 2.05 | 0.90** |
| Augustów – Grajewo | 5 | 22 | 10.1 | 172 | 1.9 | 24 740 | 57.4 | 42.6 | 3305 | 13.4 | 2.15 | 0.77** |
| Mońki | 15 | 16 | 9.7 | 170 | 1.8 | 28 954 | 58.0 | 47.1 | 4403 | 15.2 | 1.91 | 0.75** |
| Sokółka | 14 | 28 | 10.1 | 205 | 2.0 | 42 455 | 60.0 | 41.3 | 8261 | 19.5 | 1.95 | 0.76** |
| Łomża – Kolno | 5 | 8 | 4.2 | 63 | 1.5 | 6428 | 58.9 | 41.1 | 312 | 4.9 | 2.28 | 0.84** |
| Wysokie Mazowieckie | 4 | 12 | 5.8 | 66 | 1.9 | 9599 | 61.9 | 45.9 | 783 | 8.2 | 2.10 | 0.87** |
| Bielsk Podlaski | 4 | 8 | 6.3 | 98 | 1.6 | 9994 | 57.4 | 49.0 | 509 | 5.1 | 2.10 | 0.93** |
| Siemiatycze | 8 | 12 | 7.4 | 156 | 1.6 | 19 512 | 58.4 | 49.8 | 2285 | 11.7 | 2.19 | 0.92** |
| **Province of Podlasie – Total** | **58** | **117** | **8.3** | **421** | **1.8** | **14 8619** | **59.0[A]** | **44.8** | **20 247** | **13.6** | **2.05** | **0.83**** |
| Olecko- Gołdap | 6 | 2 | 3.9 | 31 | 1.8 | 3490 | 60.2 | 41.1 | 173 | 5.0 | 2.15 | 0.72** |
| Ełk | 3 | 8 | 3.1 | 41 | 1.5 | 3224 | 45.4 | 49.1 | 328 | 10.2 | 2.14 | 0.91** |
| Kętrzyn – Mrągowo | 10 | 17 | 4.8 | 180 | 1.8 | 8538 | 22.9 | 107.5 | 638 | 7.5 | 2.18 | 0.99** |
| Szczytno | – | 10 | 4.1 | 47 | 1.7 | 3811 | 44.8 | 50.0 | 274 | 7.2 | 2.41 | 0.90** |
| Lidzbark Warmiński | 3 | 14 | 3.7 | 45 | 1.8 | 3274 | 39.4 | 58.9 | 365 | 11.1 | 2.04 | 0.90** |
| Morąg | 3 | 14 | 2.2 | 30 | 1.4 | 1555 | 31.7 | 57.2 | 255 | 16.4 | 1.76 | 0.84** |
| Iława | 3 | 12 | 2.2 | 27 | 1.4 | 1511 | 38.7 | 59.1 | 147 | 9.7 | 2.13 | 0.93** |
| Elbląg | 2 | 3 | 1.0 | 7 | 1.0 | 147 | 21.0 | 50.2 | 95 | 64.6 | 2.44 | 0.93** |
| **Province of Warmia and Mazury – Total** | **30** | **80** | **3.4** | **285** | **1.7** | **25550** | **33.4[B]** | **78.7** | **2275** | **8.9** | **2.14** | **0.96**** |
| Ostrów Mazowiecka – Wyszków | 9 | 9 | 4.1 | 88 | 1.5 | 7324 | 43.9 | 50.7 | 704 | 9.6 | 2.16 | 0.93** |
| Węgrów | 1 | 8 | 4.8 | 50 | 1.8 | 5511 | 58.0 | 39.3 | 524 | 9.5 | 2.28 | 0.79** |

cont. Table 1

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Province of Mazovia – Total** | **10** | **17** | **4.3** | **110** | **1.6** | **12835** | **49.0[C]** | **47.8** | **1228** | **9.6** | **2.20** | **0.89**** |
| Biała Podlaska | – | 9 | 3.7 | 19 | 1.7 | 1836 | 45.9 | 49.9 | 78 | 4.2 | 2.07 | 0.80** |
| Lublin | – | 3 | 7.0 | 16 | 1.8 | 1418 | 50.6 | 47.9 | 127 | 9.0 | 1.68 | 0.86** |
| **Province of Lublin – Total** | **–** | **12** | **4.5** | **35** | **1.7** | **3254** | **47.9C[D]** | **48.9** | **205** | **6.3** | **1.91** | **0.81**** |
| **Province of Kujawy and Pomorze** | **–** | **1** | **10.0** | **13** | **2.4** | **454** | **14.6[E]** | **75.5** | **454** | **100.0** | **2.85** | **0.97**** |
| **Total** | **98** | **227** | **6.2** | **494** | **1.8** | **190712** | **52.3** | **53.9** | **24409** | **12.8** | **2.09** | **0.83**** |

[1] – the Kętrzyn State Stud stations
[2] – private stations with stallions rented from the Kętrzyn State Stud
[3] – correlation coefficient rang between number of covered mares by a stallions in a season and effectiveness denote number of mounts needed on mating mare, ** correlation significance at a level $\alpha = 0.01$
rank correlation coefficient between the number of mares covered by a stallion in a season and the effectiveness, expressed as the number of mounts needed to cover a mare

selected centres was started with the Province of Podlasie, where stallions from PSO Kętrzyn covered the largest number of mares and, having been used for breeding in the area for a long time they have contributed to a significant progress in the quality of cold-blooded horses. In 1970-2000 the studhorses from PSO Kętrzyn were used altogether in 175 breeding centres, majority of which were run by leased establishments (117). The average time of operation of breeding centres which is a measure of stability of the breeding process in the Province of Podlasie exceeded 8 breeding seasons, with the longest times at the breeding stations in the centres in Augustów-Grajewo, Sokółka, Mońki. 421 studhorses in the analysed province covered 148,619 mares altogether, with a seasonal employment of each stallion with 59 mares on average, including 13.6% licensed ones. The average time of a stallion at a breeding centres was 1.8 seasons in the Province of Podlasie, the longest such time found in the centre of Suwałki-Sejny – 2.2 seasons.

According to ZWOLIŃSKI (1976) the period was too short to consolidate the horse population in the region. Similarly, KOWNACKI and JASZCZAK (1969) examined the length of time of PSO Białka, Łąck and Sieraków stallions at a breeding centre and found it to lie within the range from 1.86 to 2.56 seasons, which they considered to be too short. In order to establish the regional horse types, the cited authors suggested that two studs related to each other be used at a breeding centre as well as one with the genotype close to them but not related. JANISZEWSKA et al. (1993) and KAPROŃ et al. (1985) recorded a longer time of cold-blooded stallions at breeding centres – 4 and 3.1 seasons, respectively.

In terms of the intensity of use of the stallions from PSO Kętrzyn, the dominating breeding centres in the Province of Podlasie are: Sokółka, Mońki and Augustów-Grajewo (Table 1).

The Sokółka centres has for many years been considered one of the best cold-blooded horse breeding centres in Poland. It covers 9 communes with 42 breeding stations. Altogether, 205 stallions covered 42.455 mares, with an average employment with 60 mares in a season and a variation of the characteristic slightly exceeding 40% (Table 1). The high level of breeding in the Sokółka centre is confirmed by the 20% proportion of licensed mares in all the communes of the area.

The Mońki centre is another of the active ones in the region; in the 9 communes there are 31 breeding stations, using 170 studhorses altogether. These covered 28.954 mares altogether, 58 per one stallion during a season. 4403 licensed mares were brought to the mating service in the Mońki centre, which accounts for 15.2% of the mares covered in the region. The cold-blooded stallions in Mońki were the most effective in covering, needing only 1.91 mounts to cover a mare. The effectiveness, confirmed by the statistical

analysis, may be proof of their fitness and of good conditions in which they were kept and which indirectly influenced their breeding potential.

Another region where breeding activities are quite intense is the Augustów-Grajewo centre. The state owned studhorses (172) were used in 8 communes where 27 breeding stations were situated (Table 1). Cold-blooded stallions in the centre covered 24,740 mares altogether, 57.4 mares in a season on average. The level of breeding activities is constantly increasing in the region (KARPETA 1998), which is partly linked to the fact that a stallion by the name of 698 Villor has been kept at the Pomiany – Bargłów Kościelny breeding station since 1988; among the mares he covered were 63% licensed ones. In the years 1970-2000 the stallions from PSO Kętrzyn covered 13.4% breed mares.

CHRZANOWSKI et al. (1989) examined the use of cold blooded stallions in breeding in Poland in the years 1977-1984 and found them to have been used with the greatest intensity in the provinces of Białystok, Łomża and Suwałki, which is corroborated by our study (Table 1).

There are some reports of studies showing that cold-blooded stallions used in other herds in the country had achieved worse results of mating, e.g.: in PSO Klikowa – 36.8 mares covered by a stallion (JANISZEWSKA et al. 1993) or in PSO Łobez – 18.1 mares per stallion (PIKUŁA et al. 1994).

The use of cold-blooded stallions in the province of Warmia and Mazury (25,550 covered mares) was lower as compared to the total number of mares covered in the whole area serviced by PSO Kętrzyn and accounted for about 13%. In the years 1970-2000, the 285 breed stallions covered altogether 25.550 mares at 110 breeding stations, maintaining an average of 1.7 season at a point. The average number of mares covered by a stallion during each of the seasons under study was 33.4, with the highest observed variation of the characteristic equal to 78.7%. The proportion of licensed mares was 8.9% and was lower than in the whole analysed areas where cold-blooded stallions from PSO Kętrzyn were used.

The highest number of covered mares (8538) was found at the Kętrzyn-Mrągowo centre, where 180 stallions were kept and used. The significantly higher number of stallions used in the centre as compared to other centres in the Province of Warmia and Mazury was linked to the presence of the breeding station at the Stud Farm itself. The highest variation of the number of mares covered by a stallion during a season (107.5%) was found in the region under study, which may be proof of a lack of stability of cold-blooded horse breeding in the centre. On the other hand, a significant correlation was observed in the Kętrzyn-Mrągowo region, which is a proof of a decreasing effectiveness of stallions with an increasing number of mares covered in a season (Table 1). The fact may have been influenced by a number of reasons, i.e. old age of a stallion, lack of stamina, caused by improper preparation of a studhorse to the mating service or his excessive use.

Among other breeding centres in the Province of Warmia and Mazury, the most intensive use of stallions was recorded at the Olecko-Gołdap centre, where each of the 31 stallions covered over 60 mares during a season (Table 1).

The largest proportion of licensed mares – 64.6% – was recorded in the Elbląg centre. The stallions in the centre were used with greatest intensity and they needed 2.44 mounts on average to cover a mare (Table 1).

In the Province of Mazovia, in the two centres (Ostrów Mazowiecka-Wyszków and Węgrów) 110 stallions covered 12.835 mares (Table 1). Both centres started to use studhorses from PSO Ketrzyn in the 1980s. However, in 2000 the area covered by PSO Kętrzyn was reduced and it no longer includes the province of Mazovia; the breeding centres in the region use stallions from the Łąck Stud Horse.

An analysis of the breeding use of stallions from the Kętrzyn Stud Horse in the Province of Mazovia revealed that the average number of mares covered in a season amounted to 49.0 and was significantly higher than the examined characteristic in the Province of Warmia and Mazury and in the Stud Farm in Nowe Jankowice. The proportion of licensed mares in the total population of all the mares covered in the Province of Mazovia was 9.6% and was close to that found in both of the analysed centres.

Compared to both of the analysed breeding centres, situated in the Province of Podlasie, Warmia and Mazury, and Mazovia, the number of stallions used in the Province of Lublin was the lowest (35); they were deployed in 12 leased breeding centres (Table 1). The centres included in the analysis started their activities in the 1980s and 1990s. Earlier breeding stations were established in the Province of Biała Podlaska. The stallions covered a total of 3,254 mares, with an average of 47.9 per stallion in a breeding season. The proportion of licensed mares in the region is the lowest (6.3%) compared to the numbers of breed mares in the other provinces which use the stallions from PSO Kętrzyn.

The last of the analysed points is situated in the Stud Farm in Nowe Jankowice in the Province of Kujawy and Pomorze, with a rather small territorial range (Table 1). In the establishment, which has been using stallions from PSO Kętrzyn since 1990, 13 studhorses covered 454 mares, which makes 14.6 female horses per breeding season; the variation of the examined characteristic amounted to 75.5%. Compared to the whole of the breeding area, where stallions from PSO Kętrzyn were used, the proportion of licensed mares in Nowe Jankowice was the highest and equalled 100.0%. The result was influenced by the fact that the stallions in the centre covered the mares gathered in the cold-blooded horse farm, where all the mares are licensed. It is also noteworthy that the time of using cold-blooded stallions at this station was the longest – 2.4 reproductive seasons on average – and was

longer than the average time that cold-blooded stallions from the Łobez Stud farm stayed at the same breeding point (FEDORSKI, KILIŃSKI 1987).

The analysis of the intensity of use of cold-blooded stallions in all the breeding centres in the area covered by the Kętrzyn Stud Horse revealed a highly significant correlation between the number of mares covered in a season and the number of jumps made for an effective covering to take place (Table 1). According to RUSZCZYC (1978) the correlation coefficients of the studhorse effectiveness obtained in the study (over 0.5) confirms the relationship between the examined characteristics.

It is noteworthy that the breeding centres serviced by PSO Kętrzyn in the Province of Podlasie managed the breeding capacity of the stud horses in their use very reasonably, which was visible in the value of the correlation coefficient between the number of covered mares and the number of mounts (0.83). Considering the total number of covered mares (148.619) the relationship is favourable, as with such a high number of the covered mares, over-intensive use of stallions would not be desired.

## Summary and Conclusions

An analysis of the use for breeding of cold-blooded stallions from the Stud Farm in Kętrzyn in the breeding centres of the north-eastern Poland produced the following findings:

– there are 325 breeding stations in 21 breeding centres in 5 provinces;

– the average time of a stallion at a breeding station was 1.8 seasons;

– 494 cold-blooded studhorses covered 190,712 mares altogether, 12.8% of which were licensed mares;

– the average employment of stallions from PSO Kętrzyn was 52.3 mares covered during a breeding seasons, with a mating effectiveness of 2.09 mounts needed to cover a mare;

– the stallions from PSO Kętrzyn were used the most intensely in the Province of Podlasie, with the dominant centres in Sokółka, Mońki and Augustów-Grajewo.

Translated by JOANNA JENSEN

Accepted for print 11.04.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 175-184, Y. 2006

# ALTERNATIVES FOR FLAVOMYCIN IN BROILER CHICKEN NUTRITION

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K e y w o r d s: broiler chickens, flavomycin, probiotics, symbiotic, acidifier, enzymatic preparation.

A b s t r a c t

In order to determine the efficiency of application of potential substitutes of antibiotic growth promoters, a feeding experiment was carried out on 480 one-day old broiler Ross 308 male-chicks. All experimental birds were divided into six nutritional groups. Only chickens from the control group (C) were fed on diet containing antibiotic growth promoter (AGP) – flavomycin (flavophospholipol). The diet used in the second (N) group, was composed without any growth promoters. Birds from group three (P) were fed the feed supplemented with a probiotic (*Enterococcus faecium* ATCC 53519, *Enterococcus faecium* ATCC555 93). Birds from group four (S) were fed diet supplemented with a symbiotic which consisted of the probiotic from group P and whey as a source of lactose. The Diet from group five (SA) was composed of a symbiotic and an acidifier (Salacid balance dry®). A mixture of symbiotic, acidifier and enzymatic preparation (Avizyme 1500®) was used in the last group (SAE). The applied additives in groups P, S, SA and SAE, influenced positively the feed conversion ratio value (FCR 1.49 $kg \cdot kg^{-1}$ of body weight, 1.56 $kg \cdot kg^{-1}$ of body weight, 1.53 $kg \cdot kg^{-1}$ of body weight, 1.50 $kg \cdot kg^{-1}$ of body weight) and also the body weight gain (BWG) (1773.3 g, 1796.5 g, 1854.8 g, 1803.6 g). FCR and BWG results from the above-mentioned groups differed statistically significantly ($P$«0.05) when compared with the results obtained in groups C and N. No statistically significant differences in BWG results were observed between groups P, S, SA and SAE. The applied acidifier and probiotic decreased the pH of the crop as well as the gizzard (in the group P – pH 4.69, and S – pH 4.79, $P \leq 0.05$), although no differences in pH were observed in the case of the ileum and the caecum.

## ZAMIENNIKI FLAWOMYCYNY W ŻYWIENIU KURCZĄT BROJLERÓW

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S ł o w a k l u c z o w e: kurczęta brojlery, flawomycyna, probiotyk, symbiotyk, zakwaszacz, preparat enzymatyczny.

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Abstrakt

W celu określenia efektywności stosowania potencjalnych zamienników antybiotykowych promotorów wzrostu przeprowadzono doświadczenie żywieniowe na 480 jednodniowych kogutkach towarowych Ross 308. Kurczęta podzielono losowo na 6 grup. Tylko 1. grupa, kontrolna (C), zawierała antybiotyk paszowy – flawomycynę (flavophospholipol). Paszę zastosowaną w grupie 2. (N) skomponowano bez stymulatorów wzrostu. W grupie 3. (P) zastosowano probiotyk (*Enterococcus faecium* ATCC 53519, *Enterococcus faecium* ATCC555 93), w grupie 4. (S) – symbiotyk, w którego skład wchodziły probiotyk z grupy P oraz serwatka jako źródło laktozy, w grupie 5. (SA) – mieszaninę symbiotyku oraz zakwaszacza – Salacid balance dry®, w grupie 6. (SAE) – symbiotyk, zakwaszasz oraz preparat enzymatyczny Avizyme 1500®.

W grupach P, S, SA i SAE użyte dodatki wpłynęły pozytywnie na współczynnik wykorzystania paszy (1.49 kg · kg$^{-1}$ masy ciała, 1.56 kg · kg$^{-1}$ masy ciała, 1.53 kg · kg$^{-1}$ masy ciała, 1.50 kg · kg$^{-1}$ masy ciała), a także na przyrost (1773.3 g, 1796.5 g, 1854.8 g, 1803.6 g), na poziomie istotności $P \leq 0.05$. Różnice zaobserwowano w stosunku do grupy C oraz N. U kurcząt z grup P, S, SA, SAE nie zauważono istotnych różnic w przyrostach masy ciała podczas całego okresu odchowu, tj. od 1. do 35. dnia ($P \leq 0.05$).

Wykazano, że użycie probiotyku oraz mieszaniny kwasów organicznych wpłynęło na obniżenie pH wola (grupa P – pH 4.69, S – 4.79, $P \leq 0.05$).

W grupie kontrolnej (C) żaden z zastosowanych dodatków paszowych nie obniżył pH żołądka mięśniowego ($P \leq 0.05$). Najniższą wartość pH treści żołądka mięśniowego zaobserwowano w grupie SAE (pH 3.60), lecz nie była to różnica istotna statystycznie ($P \leq 0.05$). We wszystkich grupach nie odnotowano różnic istotnych statystycznie ($P \leq 0.05$) w przypadku wartości pH treści jelita cienkiego oraz jelit ślepych.

## Introduction

The positive effects of AGPs (antibiotic growth promoters) on the performance of broiler chickens were discovered in the second half of 1940s (STOKESTAD, JUKES 1949, 1950). During the experiment, in which broiler chickens were fed on by-products from tetracycline production, it was observed that these birds grew faster and the feed was converted better in comparison with the control group. Originally those by-products were used as a source of $B_{12}$ vitamin; however it turned out that BWGs and FCRs improved not because of vitamin $B_{12}$ contained in the by-products but due to the remains of tetracycline in these wastes (STOKESTAD, JUKES 1949, 1950).

Antibiotic growth promoters (AGPs) were thought to be responsible for suppressing gastrointestinal tract microflora (TOMKE, ELWINGER 1997). The first experiments demonstrated that germ-free chickens grew on average about 20% faster than standard chickens (FORBES, PARK 1959, EYSSEN, DE SOMER 1967). The latter revealed that microflora status of the host gastrointestinal tract may inhibit the absorption of nutrients. Germ-free chickens fed on diets with AGP did not grow faster in comparison with the germ-free chickens fed on diets without AGP (FORBES, PARK 1959, EYSSEN, DE SOMER 1967). On the basis of these results, researchers concluded that chickens kept in clean and disinfected rooms grew faster and converted feed better, in comparison with the birds kept in worse sanitary conditions (ROURA et al. 1992).

Throughout the period of application of antibiotic growth promoters, attention was paid to the possibilities of the occurrence of cross resistance in bacteria. Bacterial antibiotic resistance phenomenon is based on the transfer of plasmids between two cells of bacteria.

The observed high number of bacteria resistant to antibiotic (*Salmonella* spp., *E. faecium, E. faecalis*) was the reasons for the division of antibiotics into two groups. The first group comprised antibiotics used in the health care of people, while the second group included all antibiotics which were applied in animal feeds (*Committee Swann...* 1969). In 1980's. the Swedish parliament banned completely the use of antibiotic growth promoters. Soon, Portugal and Denmark joined the Swedish model of animal feeding. The European Union introduced a total ban on the use of avoparcin and vancomycin in 1997. In 1998 the above list was expended by more items such as: tylosinphosphate, zinc bacitracin, spiramycin and virginiamycin. As of 01. 01 2006, no AGP's are allowed in animal production in any of the EU member states. This means that the four remaining AGPs; flavophospholipol, avilamycin, monensin and salinomycin have been also removed from animal production. It should be emphasized that antibiotics will still be used to treat animal diseases. Also ionophoric antibiotics will be still used in order to prevent coccidiosis.

Many alternatives for AGPs have recently appeared on the market of animal feed additives. It is the answer of feed additive producers to the market niche which developed after the removal of AGPs from animal production. Alternatives for AGPs include: probiotics, prebiotics, acidifiers and multienzymatic and herbal preparations (FULLER 1989, SCHREZENMEIR, DE VRESE 2001, SCHNEITZ et al. 1992).

Unfortunately, the effectiveness of the above preparations is still subject to the reviews and experiments (CAVAZZONI et al., 1998, JÓZEFIAK et al. 2002, JÓZEFIAK, RUTKOWSKI 2005).

The objective of the experiment was to study the influence of potential alternatives of AGPs, on the performance of broiler chickens as well as the pH in the crop, gizzard, ileum and caeca.

## Materials and Methods

In order to determine the efficiency of the application of potential substitutes of antibiotic growth promoters, a feeding experiment was carried out on 480 one-day old broiler Ross 308 male-chicks. The birds were kept in metal cages with wire flooring and the density rate was 16 birds per 1 $m^2$. Cages were grouped together in 6 rows. Each row consisted of one dietary group, and individual cages containing eight chickens, constituted replications. Average chick weight was established at the level of 41.2 g.

The total of six feeding groups were used in this experiment. The first group, control – C, contained the antibiotic growth promoter (AGP) – flavophospholipol (flavomycin). Groups N, P, S, SA and SAE, were fed on diets without AGP, while birds from group three (P) were given diets containing probiotics. The applied probiotics contained the following bacterial strains: *Enterococcus faecium* ATCC 53519, *Enterococcus faecium* ATCC 55593. Chickens from group four (S) were fed on diets with symbiotic, which consisted of the probiotic from group P and whey as a source of lactose. In group SA the feed composed of symbiotic and an acidifier – Salacid balance – dry® was used. The diet of group six (SAE) contained a symbiotic and acidifier, the same as in group SA and a multi-enzymatic preparation Avizyme 1500® which, contained the following enzymes: protease of 4000 $U \cdot g^{-1}$ activity, alfa-amylase of 400 $U \cdot g^{-1}$ activity, xylanase of 300 $U \cdot g^{-1}$ activity and pectinase of 25 $U \cdot g^{-1}$ activity. All diets contained the same 1% premix (Table 1). The applied premix contained 60 ppm coccidiostat (salinomycin).

Table 1

Composition of diets (%) and nutritional value for day 1 to day 14 (starter) and for day 15 to day 35 (Grower)

| Components | For day 1 to day 14 | | For day 15 to day 35 | |
|---|---|---|---|---|
| | groups | | groups | |
| | C,NC,P | S,SA,SAE | C,NC,P | S,SA,SAE |
| Maize | 48.5 | 47.65 | 58.3 | 57.5 |
| Soybean meal 43% | 40.05 | 39 | 31.5 | 30.5 |
| Rape seed oil | 6.6 | 7.0 | 5.7 | 6.1 |
| Calcium phosphorus | 1.6 | 1.6 | 1.5 | 1.5 |
| L-lysine 20% | 0.2 | 0.2 | 0.2 | 0.15 |
| DL-methionine 20% | 0.95 | 0.95 | 0.7 | 0.7 |
| NaCl | 0.3 | 0.3 | 0.3 | 0.3 |
| Limestone | 0.7 | 0.7 | 0.7 | 0.65 |
| $NaHCO_3$ | 0.1 | 0.1 | 0.1 | 0.1 |
| Premix starter* | 1.0 | 1.0 | – | – |
| Premix grower** | – | – | 1.0 | 1.0 |
| Dry whey | – | 1.5 | – | 1.5 |
| Nutritional value: | | | | |
| Metabolic energy (MJ/kg) | 12.80 | 12.80 | 13.00 | 13.00 |
| N x 6.25 (%) | | | | |
| Crude protein (%) | 22.50 | 22.50 | 19.5 | 19.5 |
| Lisin (%) | 1.30 | 1.30 | 1.07 | 1.07 |
| Methionine (%) | 0.54 | 0.54 | 0.45 | 0.45 |
| Calcium (%) | 0.99 | 1.01 | 0.95 | 0.94 |
| Available phosphorus (%) | 0.44 | 0.45 | 0.40 | 0.41 |

* Premix starter A 12 500 IU; $D_3$ 4000 IU; E 150 mg; $K_3$ 3 mg; $B_1$ 3 mg; $B_2$ 8 mg; $B_6$ 5 mg; $B_{12}$ 0.025 mg; niacin 50 mg; D-pantothenic acid 10 mg; folic acid 2 mg; biotin 0.15 mg; C 150 mg; choline 400 mg. Salinomycin 60 ppm

** Premix grower A 10 000 IU; $D_3$ 2500 IU; E 30 mg; $K_3$ 2 mg; $B_1$ 2 mg; $B_2$ 6 mg; $B_6$ 2.5 mg; $B_{12}$ 0.015 mg; niacin 25 mg; D-pantothenic acid 9 mg; folic acid 1.2 mg; biotin 0.10 mg; C 100 mg; choline 300 mg. Salinomycin 60 ppm

The feed intake and body weight were controlled every week, and the feed conversion ratios (FCR) and body weight gains (BWG) were calculated. The pH in the content of all gastrointestinal segments (crop, gizzard, small intestine and caecum) was measured with a combined glassreference electrode (ElMetron CP 40). The ileum was defined as the small intestinal segment caudal to the Meckels diverticulum.

The dietary concentration of dry matter, crude protein and crude fat, ash and mineral content was determined using the AOAC procedure (1990). The obtained results were processed statistically using the statistical computer program package SAS (1996), SAS®STAT. One way analysis of variance was made and its statistical significance was established at $P \leq 0.05$.

## Results

The performance results of the broiler chickens are shown in Table 1. From the 1st to 14th day of life, chickens from group C, P, S, SA, and SAM did not differ statistically significantly with respect to the body weight gain (BWG) ($P \leq 0.05$). The only group in which BWGs were statistically significantly lower was group N ($P \leq 0.05$).

The use of the symbiotic (group S) reduced slightly feed consumption during the entire period of experiment in relation to the control group (C). With regard to the FCR and BWG, the applied additive (symbiotic) improved

Table 2

Mean values of BWG (g), FCR ($kg \cdot kg^{-1}$) and pH values for individual segments of the gastrointestinal tract

| | Groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | N | P | S | SA | SAE | SEM |
| BWG | | | | | | | |
| Days 1-14 | 333.0 | $293.1^{b*}$ | 338.0 | 333.0 | 354.2 | 339.2 | 5.70 |
| Days 15-35 | $1434.9^{ab}$ | $1428.7^{b}$ | $1435.3^{ab}$ | $1463.5^{ab}$ | $1500.6^{a}$ | $1464.4^{ab}$ | 8.86 |
| Days 1-35 | $1767.9^{b}$ | $1753.0^{b}$ | $1773.3^{b}$ | $1796.5^{ab}$ | $1854.8^{a}$ | $1803.6^{ab}$ | 10.52 |
| FCR | | | | | | | |
| Days 1-14 | $1.41^{a}$ | $1.35^{a}$ | $1.32^{a}$ | $1.36^{a}$ | $1.19^{b}$ | $1.16^{b}$ | 0.02 |
| Days 15-35 | $1.64^{a}$ | $1.67^{a}$ | $1.53^{c}$ | $1.61^{ab}$ | $1.61^{ab}$ | $1.58^{cd}$ | 0.01 |
| Days 1-35 | $1.60^{a}$ | $1.61^{a}$ | $1.49^{cd}$ | $1.56^{ab}$ | $1.53^{bc}$ | $1.50^{cd}$ | 0.01 |
| pH value in individual segments of the gastrointestinal tract | | | | | | | |
| Crop | $5.41^{a}$ | $5.21^{ab}$ | $5.31^{a}$ | $4.69^{c}$ | $4.79^{cb}$ | $5.04^{abc}$ | 0.06 |
| Gizzard | $4.03^{ab}$ | $4.30^{a}$ | $4.24^{a}$ | $3.87^{ab}$ | $4.02^{ab}$ | $3.60^{b}$ | 0.06 |
| Ileum | 6.08 | 6.14 | 6.19 | 6.07 | 6.10 | 6.15 | 0.04 |
| Caeca | 6.75 | 6.79 | 6.80 | 6.49 | 6.42 | 6.50 | 0.05 |

* Values designated with the same letters in a row or not designated with a letter do not differ significantly at the level of $P \leq 0.05$ (SEM – statistical error).

slightly these parameters, but the differences were not statistically significant (Table 2). From the 1st to 14th day of life, group N (without AGP) demonstrated the worst growth, the highest growth values were observed in chickens from group SA (symbiotic plus acidifier). However, they were not statistical significantly different ($P \leq 0.05$). During the entire experimental period (1st to – 35th) the similar results were observed in case of body weight gains. However, values observed in group SA differed statistically ($P \leq 0.05$) in comparison with group C and N, and were by 4.9% and 5.7% higher.

During the starter period, birds from group SAE had lower feed utilisation per 1 kg of body weight gain (FCR) (1.16 $kg \cdot kg^{-1}$) in comparison with the remaining groups. Taking into account the entire experiment, the worst FCR value were found in the birds from group N and C and the best in birds from the group on fed the diet with lyophilised cultures of probiotic bacteria (P).

In groups S and SA, a statistically significant pH reduction of the crop ingesta was observed, whereas in groups SA and SAE – a trend towards lower pH in the gizzard contents was stated. Irrespective of the diet, there were no changes in the pH value in the ileum and caecum ($P \leq 0.05$). However, a tendency to lower pH of the caecum chime was found in group SE, however difference did not differ statistically ($P \leq 0.05$).

## Discussion

Throughout the entire experiment, the group with the antibiotic (C) and the negative group without additives (N) did not differ from each other with respect to the body weight gains and feed utilisation per 1 kg of body weight gain. It appears that the obtained results can be attributed to very good environmental conditions (management without litter, on wire flooring) as well as to the microbiological conditions of the experimental area. In general, the positive effect of alternatives for antibiotics and AGPs becomes most apparent in worse environmental conditions (PATTERSON, BURKHOLDER 2003, ROURA et al. 1992) where organism is exposed to pathogenic microflora. FORBES, PARK (1959) and EYSSEN, DE SOMER (1967) reported that germ-free chickens grew on average about 20% faster than chickens keep in standard conditions. But growth depression was not observed only when pathogenic microflora appeared in GIT (gastrointestinal tract). Germ-free chickens which were fed on the diet with *Enterococcus faecalis* had also the worst production results in comparison with other germ-free chickens. When AGP was used, production results were similar to those observed in the germ-free chickens (LEV, FORBES 1959, ESSEN, DE SOMER 1967).

When the experimental probiotic *Enterococcus faecium* was used, during the entire period of experiment feed conversion ratio value was lower in relation to other experimental groups. The observed value, in comparison with group C (flavomycin), was lower by 6.87%. However, it should be noted that body weight gains stated in group P were the worst in relation to other experimental groups. Only in case of group N, slightly lower body weight gains were observed. Other authors (JIN et al. 2000, ABDULRAHIM et al. 1999, SEO et al. 2002); reported similar results when lyophilised cultures of probiotic bacteria were used. However, it should be mentioned that the effectiveness of the probiotic application is sometimes questioned (CAVAZZONI et al. 1998). The growth depression when lyophilized cultures of probiotic bacteria were used is not explained. Some researchers explained it is effect of bile salts deconiugation by: *Lactobacillus* spp. (DE SMET et al. 1995, GRILL et al. 1995, LUNDEEN et al. 1992), *Bifidobacterium* (GRILL et al. 2000) and *Enterococcus* (KNARREBORG et al. 2002). Bile salt hydrolases (BSH) are enzymes which hydrolyze bile salts. An increased activity of these enzymes was reported when growth depression in broiler chickens was observed (FEIGHNER, DASHKEVICZ 1987). It is well documented that deconjugated bile salts lost their emulsifying properties and this decreased fat digestibility.

The use of the experimental symbiotic reduced slightly feed consumption during the entire period of experiment in relation to the control group (C). With regard to the FCR and BWG, the applied additive (symbiotic) improved slightly these parameters but the differences were not statistically significant. Birds do not have lactase to digest lactose and therefore lactose is fermented in the lower parts of GIT (HUME et al. 1992), mainly to lactic and propionic acids. The growth of pathogenic bacteria is inhibited by these compounds. Consequently, the concentrations of *Clostridium* sp. and *Salmonella typhimurium* decrease (OYARZABAL, CONNER 1995, 1996). The length and also the surface area of caeca increase significantly, but at the same time their thickness decreases when lactose is applied as probiotics (ORBAN et al. 1997). Chickens receiving the symbiotic had slightly higher BWG, but FCR value was lower after 35 days, in comparison with groups C and N. Similar results were observed by GÜLSEN (2002) but he used 2.5% lactose. Many other authors (PETTERSON, BURKHOLDER 2003, ORBAN et al. 1997) show that oligosaccharides may have a positive influence on the microflora of the gastro intestinal tract and in general on birds condition. On the other hand TERADA et al. (1994) and WALDROUP et al. (1993) reported that the application of oligosaccharides did not improve the production results (FCR, BWG). However, according to CORRIER et al. (1990) despite the absence of the effect of application of the lactose on the production results, it is believed that lactose metabolism products may have bacteriostatic effect

on pathogenic bacteria. The substitution of AGPs by lactose and probiotic improved slightly production results in relation to group (P). In addition, the reduced pH value in the crop and the gizzard could have been caused by microflora activity in the upper parts of the GIT. As the result of the activity of microflora lactic, propionic or acetic acids were produced. It seems necessary to carry out more microbiological and physiological investigations in order to recognize and fully understand the lactose effect on the microflora of the GIT.

Birds fed an symbiotic supplemented diets and mixtures of organic acids and their salts were the heaviest among the all experimental groups. After 35 days birds from group SE were by 4.8% and 5.8% heavier than those from the control and negative group. SKINNER et al. (1991) and RICKE (2003), pointed to the positive influence of organic acids on the production results of broiler chickens. It should be emphasized that the addition of organic acids did not influence the pH of the ileum and caeca. GELINAS and GOULET (1983) observed that organic matter reduced the antimicrobial activity of organic acids. The status of the intestinal lumen will obviously have an effect on the digestion and absorption of any nutrient. FREEMAN (1969) indicates that digesta pH can influence fat digestion in that acidic conditions reduce micellar solubilization. In rats, fat digestibility is reduced when the diet contains lactic acid. Usually pH of the broiler gut after *ad libitum* feeding is observed at level: crop 5.5, proventriculus and gizzard 2.5-3.5, duodenum 5-6, jejunum 6.5-7 and ileum 7-7.5. The effectiveness of the application of multienzymatic preparations is well documented (ELWINGER, TEGLÖF 1991). However, it should by emphasized that majority of published experiments on multienzymatic preparations concern the use of these compounds to degrade non-starch polysaccharides. Birds fed on the multienzymatic preparation diet (SAE) were characterized by better FCR value in comparison with the control and negative groups. It is very hard to observe the positive influence of this multienzymatic preparation because in comparison with group SA differences were not statistically significant.

Compensative effect of the applied preparations was not observed in the present experiment. This may suggest that using a combinations of different feed additives in broiler chicken nutrition may actually rise the cost of the feed and not necessarily improve birds performance in terms of body weight gains or feed utilization.

Translated by ZBIGNIEW ŚNIATAŁA

Accepted for print 15.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 185-191, Y. 2006

# COMPARISON OF PHYSICOCHEMICAL MEAT PROPERTIES OF WILD BOAR (*Sus scrofa ferus*) X DUROC WITH POLISH LARGE WHITE X POLISH LANDRACE CROSSES

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K e y w o r d s: Wild boar x Duroc, PLW (Polish Large White) x PL (Polish Landrace), meat quality.

A b s t r a c t

In this research there were a number of physicochemical properties determined, which are characteristic of the quality and technological suitability of meat, produced by the European wild boar x Duroc sows ($n$ = 32) and fattener PLW x PL ($n$ = 58) crosses. The latter were represented by fatteners without the stress susceptibility gene, producing meat of a high standard. It was shown that nearly all the traits of meat quality produced by the wild boar x Duroc crosses have exceeded the quality of meat from Polish Large White (PLW) and Polish Landrace fattener (PL) crosses. However, both types of meat were of high quality. Both had comparatively high pH1 (6.21 and 6.49) which has confirmed the proper post mortem processes; a low value of electric conductivity $EC_1$ (3.00 and 3.94 $mS \cdot cm^{-1}$); high water holding capacity (19.63 and 22.18%) and a reasonably small meat juice drip loss (1.16 and 4.69%). Meat colour, which was determined to a high degree of accuracy, indicated a suitable and desirable lightness, saturation and redness. The contents of water, protein, and fat in meat of both groups were normal. The results obtained in this work indicate the high quality of meat that was produced through crossbreeding the European wild boar (*Sus scrofa ferus*) with Duroc sows.

## PORÓWNANIE FIZYKOCHEMICZNYCH CECH MIĘSA MIESZAŃCÓW DZIKA (*Sus scrofa ferus*) x DUROC Z WBP x PBZ

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S ł o w a k l u c z o w e: świniodziki, wbp x pbz, jakość mięsa.

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Abstrakt

Wykonano oznaczenia właściwości fizykochemicznych charakteryzujących jakość i przydatność technologiczną mięsa mieszańców dzika europejskiego z lochami duroc ($n$ = 32) oraz tuczników ras wielkiej białej polskiej i polskiej białej zwisłouchej (wbp x pbz, $n$ = 58) prezentujących wysoki standard tuczników wyskomięsnych pozbawionych genu wrażliwości na stres. Wykazano, że mięso mieszańców dzika z lochami duroc pod względem prawie wszystkich badanych cech jakości przewyższało mięso tuczników mieszańców ras wbp i pzb. W obu porównywanych grupach charakteryzowało się jednak wysokimi walorami jakości. Miało odpowiednie wysokie pH1 (6,21 i 6,49) świadczące o prawidłowym przebiegu przemian *post mortem*, niską wartość przewodnictwa elektrycznego $EC_1$ (3,00 i 3,94 $mS \cdot cm^{-1}$), wysoką wodochłonność (19,63 i 22,18%) i niewielki wyciek soku z mięsa (1,16 i 4,69%). Bardzo szczegółowo określona barwa mięsa wskazywała na odpowiednią i pożądaną jasność, nasycenie i udział czerwieni. Zawartość wody, białka i tłuszczu w mięsie z obu grup była prawidłowa. Uzyskane wyniki świadczą o wysokich walorach surowca otrzymanego przez krzyżowanie dzika europejskiego (*Sus scrofa ferus*) z lochami rasy duroc.

## Introduction

The ease of crossbreeding domestic pigs with wild boar (*Sus scrofa ferus*) makes possible the production of new quality pork, that fulfils the conditions and criteria of natural pro-health food. Several years of selective pig breeding, leading towards maximizing production, has forced a shift in the balance of several metabolic processes which control low tissue fat and high muscle contents in a carcass. This is usually accompanied by large daily body mass increases, shortening the fattening period and achieving the body slaughter weight at a much younger age (KAPELANSKI et al. 1999, PETERSEN et al. 1996).

However, together with progress in breeding, the quality of meat in the area of its consumption properties, as well as its technological and processing value, have significantly deteriorated. (KAPELANSKI et al. 1999, OKSBJERG et al. 1997, PETERSEN et al. 1997). Large losses in meat mass during the carcass processing, portioning, packaging and sales, caused by an insufficient ability to retain tissue fluids, also lead to significant decreases in product mass (POSPIECH et al. 1998). This also causes the meat to lose its valuable soluble fraction, containing sarcoplasmic protein, mineral components and low molecular weight organic substances which constitute precursors of the smell and taste senses (CORNET, BOUSSET 1999). Meat quality, in a major way, is also determined by genetically conditional susceptibility of pigs to stress, as well as a negative correlation between the quality and quantity of meat (KAPELANSKI et al. 2000, KORTZ et al. 2000).

In the majority of European countries pork consumption is very high but it does not show tendencies to increase further. At the same time, the so called civilization illnesses associated with diabetes, progressing atherosclerosis, heart disease and obesity are on the increase, which draws consumer attention

to healthier, safer and more nutritious, attractive and tastier food products. It seems that meat from domestic pig and wild boar crossbreeds is able to fulfill consumer expectations and requirements concerning food safety and its culinary attractiveness.

The aim of this work was the evaluation of physicochemical meat properties of Duroc sows and the European wild boar crossbreeds and their comparison to the meat of crossbreeds of the two most common breeds, PLW and PL, not carriers of the stress susceptibility gene.

## Materials and Methods

The research involved two groups of crossbreeds $F_1$ bred along the following pattern:

1. ♂ European Boar (*Sus scrofa ferus*) x ♀ Duroc – 32 animals
2. ♂ Polish Large White (PLW) x ♀ Polish Landrace (PL) – 58 animals

The fatteners from crossbreeding 1 have descended from 5 Duroc sows covered by a single boar *Sus scrofa ferus*, whilst in crossbreeding 2 there were progenies of 15 PL sows covered by 3 PLW boars qualified for this research. The PL sows and PLW boars used for reproduction were free of the $RYR1^T$ gene which meant that all the progenies in this group were stress resistant (genotype $RYR1^{CC}$). In both groups the animal gender proportions were 1:1. Animals were slaughtered at about 101 kg live weight.

Slaughter was conducted under conditions prescribed by the regulations relevant to the Polish meat plant. Meat quality was determined in samples obtained from 1-3 lumbar vertebrae of *Longissimus lumborum* (*LL*) muscle. Value of $pH_1$ was determined using a pistol pH-meter (R. Matthaus), and the $pH_u$ was measured in minced meat diluted with water, 48 hours after slaughter. Muscle electric conductivity ($EC_1$) was determined with the LF-STAR apparatus (R. Matthaus) in the same place as pH1.

Subjective colour and wateriness evaluations were conducted on fresh meat samples by a panel of judges using a five point scale, where a score of 3 was considered an optimal value (CLAUSEN, THOMSEN 1956). Water holding capacity (WHC) as determined with the filter press method (GRAU, HAMM 1952) modified by POHJA, NIINIVAARA (1957). The result was expressed in percentage of loose water in the meat. Plasticity of meat was evaluated on basis of surface size of a pressed meat sample (300 mg) used for WHC measurement (GRAJEWSKA et al. 1998). Drip loss measurement was carried out on meat slices according to the method described by HONIKEL (1987).

Meat colour was measured twice, the first time with a Spekol 11 spectrophotometer armed with a reflective attachment and by using regression equations (ROZYCZKA et al. 1968) in order to determine the parameters of

colour such as: dominant wavelength, saturation and lightness and for the second time with a Minolta CR 310 photometer delivering values L, a*, b* in accordance with CIE (1976) system.

The basic meat chemical components were also determined: water, total protein, intramuscular fat and ash content (AOAC 1990).

Statistical analyses such as: arithmetic mean ($x$), standard deviation ($s$), significance of differences between the groups ($T$ student test), were conducted with the program Statistica 5.5 PL (2000).

## Results and Discussion

Meat properties analysed in this work cover the basic physical and chemical traits that shape its suitability for consumption and commercial and technological usefulness. Results of meat evaluation in compared animal groups are shown in Tables 1, 2 and 3. In the vast majority of cases the quality parameters which determine a culinary and processing value of tested meat samples proved to favour the European wild boar and Duroc crossbreeds. Differences have been confirmed to be statistically highly significant or significant.

Table 1

Physicochemical properties of meat

| Trait | Wild boar x Duroc | PLW x PL |
|---|---|---|
| Number, $n$ | 32 | 58 |
| $pH_1$ | $6.21^A \pm 0.41$ | $6.49^B \pm 0.33$ |
| $pH_u$ | $5.49^A \pm 0.06$ | $5.45^B \pm 0.07$ |
| $EC_1$ (mS · cm$^{-1}$) | $3.00^A \pm 0.67$ | $3.94^B \pm 0.71$ |
| WHC (% loose water | $19.63^A \pm 2.89$ | $22.18^B \pm 2.61$ |
| Drip loss (%) | $1.16^A \pm 1.03$ | $4.69^B \pm 2.09$ |
| Plasticity (cm$^2$) | $2.40^A \pm 0.24$ | $2.17^B \pm 0.19$ |
| Meat colour, score | $3.0^A \pm 0.32$ | $2.7^B \pm 0.32$ |
| Wateriness, score | $2.9^a \pm 0.24$ | $2.8^b \pm 0.24$ |
| Consistency, score | $3.0^a \pm 0.20$ | $2.8^b \pm 0.23$ |

*A, B – P* < 0.01; *a, b – P* < 0.05

Table 2

Meat colour

| Trait | Wild boar x Duroc | PLW x PL |
|---|---|---|
| Dominant wavelength (nm) | $587.4^A \pm 2.85$ | $584.4^B \pm 1.61$ |
| Saturation (%) | $23.40^A \pm 3.39$ | $21.15^B \pm 2.60$ |
| Lightness (%) | $19.38^A \pm 2.39$ | $23.52^B \pm 2.50$ |
| Minolta CIE values | | |
| L* | $46.70^A \pm 2.59$ | $52.66^B \pm 2.00$ |
| a* | $15.44^A \pm 0.97$ | $13.38^B \pm 0.66$ |
| b* | $1.66^a \pm 1.11$ | $2.10^b \pm 0.78$ |

*A, B – P* < 0.01; *a, b – P* < 0.05

Table 3

Basic chemical composition of meat

| Trait | Wild boar x Duroc | PLW x PL |
|---|---|---|
| Water content (%) | 73.94 ± 0.63 | 74.23 ± 0.64 |
| Total protein (%) | $22.99^{a}$ ± 0.77 | $22.70^{b}$ ± 0.60 |
| Fat (%) | 1.72 ± 0.55 | 1.87 ± 0.55 |
| Ash (%) | $1.11^{A}$ ± 0.02 | $1.16^{B}$ ± 0.05 |

*A, B* – $P < 0.01$; *a, b* – $P < 0.05$

The range and rate of glycolytic process was evaluated on the basis of muscle acidity 45 minutes after slaughter ($pH_1$) and after 48 hours ($pH_u$). These values, in meat of both wild boar x Duroc and WPL x PL crossbreeds, were classified as being within normal range of good quality meat (KORTZ 2001), despite the fact that mean values for each group differed highly significantly ($P < 0.01$) – Table 1. The consequence of irregularities in glycolysis in post mortem meat can be an increased electric conductivity (EC), which is due to a higher cell membrane permeability and an altered positioning of Ca, K and Na ions within the tissue structure. The values that characterize muscle electric conductivity in the first hour after slaughter (3.00 and 3.94 mS/cm) were indicative of good quality meat in both animal groups (GRZESKOWIAK et al. 2004), although the differences between them were highly significant and were in favour of crossbreeds with the European wild boar.

Water holding capacity is yet another trait that determines the consumer and technological quality of meat. A relatively high ability to hold endogenous water in meat guaranties lower mass losses during meat processing, as well as a higher production yield and a better quality of the finished product. Meat from both animal groups showed high water holding capacity and, at the same time, a low loose water content (19.63% and 22.18%). Drip loss in meat of crossbreeds during a 72 hour storage period was very small for the wild boars (1.16%) and much greater for PLW x PL crossbreeds (4.69%). Raw meat plasticity is considered a good indicator for predicting what tenderness of meat will be after cooking (GRAJEWSKA, BOCIAN 2005). High plasticity values are desirable and values of this trait were high for both pig groups but it was significantly higher for the wild boar x Duroc crossbreeds ($P < 0.01$).

Meat colour is considered as one of the more important quality indicators and it constitutes main criteria of consumer preference for both fresh meat and finished meat products. Our analyses have taken into consideration a wide range of colour characteristics. This included visual evaluation, as well as more objective, equipment based tests conducted in order to determine the individual colour parameters, such as dominant wavelength of reflected

light, saturation, lightness and the values of reflection L*, a* (redness) and b* (yellowness).

Meat of both animal groups showed good values and desirable colour traits (Table 2). Meat of wild boar x Duroc crossbreeds, in comparison to PLW x PL, had a decidedly darker colour with more redness and (more desirable to the human eye) greater colorimetric purity, expressed by the colour saturation. This was confirmed by the results obtained from a Minolta photometer tests (L*, a*, b*).

The basic chemical composition of meat from the wild boar x Duroc crossbreeds (Table 3) did not differ from the meat of other pig breeds as far as its contents of water, protein, fat and ash are concerned (BOCIAN, KAPELANSKI 2004, KAPELANSKI et al. 2002, KORTZ et al. 2000) and this was also consistent with data quoted by other authors of literature concerning wild boar or its crossbreeds with domestic pigs (KORZENIOWSKI, ZMIJEWSKI 2001, LUNDSTRÖM et al. 1995, WALKIEWICZ et al. 2004). Protein and ash contents were slightly higher in meat of wild boar crossbreds than PLW fatteners x PL ($P < 0.05$).

It must be stressed that WLP x PL pig group used in this research consisted of elite material from parents without the mutated $RYR1^T$ gene. It is possible that this became an important factor resulting in the meat from these pigs being of high quality and free of the PSE defect. Nevertheless, in comparison to wild boar x Duroc crossbreeds, WLP x PL meat represented a significantly lower culinary and technological value.

## Conclusions

1. Results indicated a high culinary and technological value of meat obtained by crossbreeding the European Wild boar with Duroc sows.

2. The Wild boar x Duroc crosses were significantly better in respect of meat colour, water holding capacity and drip loss, when compared with meat quality of elite material PLW x PL pigs being free of mutated $RYR1^T$ gene.

Translated by authors

Accepted for print 12.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 193-203, Y. 2006

# FATTENING RESULTS OF CROSSBRED (POLISH LANDRACE X PIETRAIN) PIGS FED DIETS WITH A HIGH WHEAT STRAW CONTENT*

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K e y w o r d s: fattening from 70-110 kg, fattening value, slaughter quality, carcass evaluation, fatty acids, wheat straw, crude fiber.

A b s t r a c t

The experiment was performed on 48 crossbred pigs (♀ Polish Landrace x ♂ Pietrain), divided into three feeding groups: group I – fed a cereal-soybean diet containing 15% of total protein (1), group II – fed a diet containing 10% of wheat straw (2), group III – fed a diet containing 15% of wheat straw (3). The crude fiber content of the diets was as follows: (1) – 3.52%, (2) – 7.30%, (3) – 8.62%. The pigs were kept in litter pens (two animals per pen), fed *at libitum* and fattened from 70 to 110 kg of live weight.

Dietary supplementation with wheat straw at the second stage of fattening negatively affected daily gains and feed conversion ratio. Differentiated feeding had a significant effect on the fatty acid profile in *m. longissimus dorsi* samples. The addition of wheat straw to diets had no impact on the blood biochemical indices analyzed in the study.

## EFEKTYWNOŚĆ TUCZU ŚWIŃ MIESZAŃCÓW PBZ X PIETRAIN ŻYWIONYCH MIESZANKAMI O WYSOKIEJ ZAWARTOŚCI SŁOMY PSZENNEJ

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S ł o w a k l u c z o w e: tucz do 70-110 kg, cechy tuczne, wartość rzeźna, ocena tusz, kwasy tłuszczowe, słoma pszenna, włókno surowe.

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* This work was conducted as a part of project no 3P06Z 01924 financed by the State Committee for Scientific Research

Abstrakt

Badaniami objęto 48 świń mieszańców (♀ polska biała zwisłoucha x ♂ pietrain) podzielonych na 3 grupy żywieniowe: I (kontrolną) – żywioną mieszanką (1) zbożowo-sojową o zakładanej zawartości 15% białka ogólnego, II – mieszanką (2) z udziałem 10% słomy pszennej, III – mieszanką (3) z udziałem 15% słomy pszennej. Zawartości włókna surowego w mieszankach wynosiła odpowiednio: 3.52% (1), 7,30% (2), 8,62% (3). Tuczniki utrzymywano w kojcach ściołowych (po 2 szt. w kojcu), żywiono je do woli, a tucz trwał w przedziale od 70 do 110 kg masy ciała.

Tuczniki żywione w II okresie tuczu mieszankami z udziałem mączki ze słomy pszennej miały niższe tempo wzrostu oraz gorzej wykorzystywały paszę. Stwierdzono wpływ zróżnicowanego żywienia tuczników na profil kwasów tłuszczowych tłuszczu mięśnia najdłuższego grzbietu, natomiast nie stwierdzono wpływu zastosowanego żywienia na analizowane parametry biochemiczne surowicy krwi świń doświadczalnych.

## Introduction

Modern pig production systems are aimed toward producing excellent-quality carcasses, characterized by high meatiness and desirable chemical composition. One of the methods enabling to attain this goal is feeding based on diets with elevated crude fiber levels (PARTANEN et al. 2002, SHRIVER et al. 2003). Numerous authors, both in Poland and abroad, studied the applicability of diets with an increased content of this nutrient. Apart from an evaluation of fattening results (e.g. GLAPŚ et al. 1982, PARTANEN et al. 2002, ZIÓŁKOWSKI 1993), they focused on the effects of crude fiber on nitrogen balance and digestibility (FALKOWSKI et al. 2004, HÄKANSSON et al. 2000), as well as on the fatty acid composition of intramuscular fat or hypocholesterolemic properties (BARTNIKOWSKA 1993, HANCZAKOWSKI 1999, DAVIDSON, MCDONALD 1998).

However, available literature on the subject provides scant information about the effects of diets with an increased concentration of crude fiber on production results at the second stage of fattening. Therefore, the aim of the present study was to determine the fattening results of crossbred (♀ Polish Landrace x ♂ Pietrain) pigs fed diets with elevated levels of crude fiber at the second stage of fattening.

## Material and Methods

The experiment was performed at a experimental piggery of the Department of Pig Breeding, University of Warmia and Mazury in Olsztyn, on 48 crossbred fatteners (♀ Polish Landrace x ♂ Pietrain) with initial body weights of about 65 kg. The animals were divided into three experimental groups by the analogue method (on the base of litter of origin, sex and initial body weight):

group I – fed a cereal-soybean diet containing 15% of total protein (1),
group II – fed a diet containing 10% of ground wheat straw (2),
group III – fed a diet containing 15% of ground wheat straw (3).

The composition of the experimental diets is given in Table 1. Wheat straw was ground in an universal grinder. The diets were supplemented with synthetic amino acids, in accordance with *Nutrient Requirements of Pigs* (1993). The pigs were kept in litter pens (two animals per pen) and had free access to water from automatic drinkers. Feed consumption was monitored and recorded on a daily basis. The animals were weighed every two weeks. They were given pelleted feed *ad libitum*, from automatic feeders. About seven days prior to slaughter, blood was collected for biochemical analysis, including the determination of the serum levels of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides.

Table 1

Composition of experimental diets (%)

| Specification | Diets | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Ground wheat | 30.00 | 41.00 | 44.60 |
| Ground barley | 51.11 | 22.60 | 11.00 |
| Soybean meal | 15.00 | 18.50 | 20.50 |
| Wheat straw | – | 10.00 | 15.00 |
| Dicalcium phosphate | 1.00 | 1.00 | 1.00 |
| Limestone | 1.00 | 1.00 | 1.00 |
| Grower premix | 1.50 | 1.50 | 1.50 |
| NaCl | 0.30 | 0.30 | 0.30 |
| Soybean oil | – | 4.00 | 5.00 |
| L-lysine | 0.09 | 0.10 | 0.10 |

The pigs were slaughtered at body weights of approx. 110 kg. The carcasses were chilled for 24 hours at about 2°C, and then samples of *m. longissimus dorsi (m.l.d.)* were taken to determine: dry matter content – by drying to constant mass at 105°C, crude fat content – by the Soxhlet method, total protein content – by the Kiejdhal method, crude ash content – by ashing at 550°C.

The slaughter analysis was made according to the methodology developed at the Pig Performance Testing Station (RÓŻYCKI 1996). The weights of particular right half-carcass elements were determined exact to 0.01 kg using an electronic scale. Carcass meatiness was estimated with the ULTRA FOM apparatus.

Meat quality was determined based on acidity measurements performed 45 minutes ($pH_{45}$) and 24 hours ($pH_{24}$) postmortem on *m.l.d.* samples, with the

Dramiński Mini System. Color brightness was measured using a Specol spectrometer with a R-45/0 remission attachment, at a wavelength of 560 nm. The water holding capacity (WHC) was determined by the method proposed by GRAU, HAMM (1952), modified by POHJA, NINIVARA (1957). The ether extract of *m.l.d.* samples, obtained as described by Peisker (ŻEGARSKA et al. 1991), was analyzed to determine FA concentrations, using a HP 6890 gas chromatograph, with helium as carrier gas.

The results were verified statistically by universally accepted methods, using STATISTICA PL for Windows software.

## Results and Discussion

The complete diets used in the experiment were characterized by a similar energy value and their total protein content, determined at a laboratory, generally did not differ from that assumed in Methodology (Table 2), ranging between 15.92 (group III) to 16.07% (group I). Dietary supplementation with wheat straw resulted in an increase in the crude fiber concentration in diets 2 and 3, to a level of 7.30% and 8.62% respectively. The proportions of NDF and ADF also increased, to 17.34% and 8.33% in diet 2, and to 20.55% and 10.99% in diet 3. The crude fat content ranged from 1.48% in diet 1 to 6.01% in diet 3. The levels of the other nutrients were comparable in all experimental diets, and corresponded to the relevant standards.

Table 2

Chemical composition of experimental diets (%)

| Specification | Diets | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Dry matter | 88.29 | 89.27 | 90.29 |
| Crude protein | 16.07 | 15.93 | 15.92 |
| Crude fat | 1.48 | 4.52 | 6.01 |
| Crude ash | 5.53 | 5.77 | 6.37 |
| Crude fibre | 3.52 | 7.30 | 8.62 |
| N-freeextractives | 61.69 | 55.75 | 53.37 |
| NDF | 14.98 | 17.34 | 20.55 |
| ADF | 5.29 | 8.33 | 10.99 |
| Hemicellulose | 9.69 | 9.01 | 9.56 |
| Energy ($MJ \cdot kg^{-1}$) | 15.38 | 16.26 | 16.64 |

Table 3 presents the production results achieved in the study. At the beginning of the experiment the mean initial body weights of pigs were similar (on average 68.8 kg), with no significant differences between the groups. No

statistically confirmed differences were found in the final body weights of pigs on the last day of fattening, either. They ranged from 108.2 kg in group III to 110.7 kg in group II. The highest average daily gains were observed in group I, fed a cereal-soybean diet with the lowest concentration of crude fiber (1212 g), and the lowest – in group III (1061 g). The differences between the groups were confirmed statistically. Similar tendencies were observed by SHRIVER et al. (2003) – the addition of high-fiber components to diets for growing pigs was followed by a decrease in daily gains. Our results are better than those obtained by American and Canadian authors in older studies (BAIRD et al. 1970, 1975, KENNELLY, AHERNE 1980). In their studies daily gains in experimental groups given diets with a high crude fiber content did not exceed 810 g. In more recent Scandinavian studies (PARTANEN et al. 2002) daily gains of experimental pigs were at a level of 943 g.

Table 3

Fattening performance of experimental pigs

| Specification | Statistics | Groups | | |
|---|---|---|---|---|
| | | I | II | III |
| Average initial body weight (kg) | $x$ | 69.6 | 68.7 | 68.1 |
| | $s$ | 8.03 | 8.16 | 7.28 |
| Average final body weight (kg) | $x$ | 109.8 | 110.7 | 108.2 |
| | $s$ | 6.24 | 6.65 | 5.60 |
| Average daily gains (g) | $x$ | $1212^{A}$ | $1157^{a}$ | $1061^{Bb}$ |
| | $s$ | 110 | 79 | 123 |
| Average daily feed intake (kg) | $x$ | 4.12 | 4.06 | 4.02 |
| | $s$ | 0.36 | 0.25 | 0.22 |
| Feed/gain ratio ($kg \cdot kg^{-1}$) | $x$ | $3.40^{B}$ | $3.51^{b}$ | $3.79^{Aa}$ |
| | $s$ | 0.3 | 0.22 | 0.21 |

*a, b* – $\alpha = 0.05$; *A, B* – $\alpha = 0.01$

In the present experiment the increased concentration of crude fiber in diets had no significant effect on mean daily feed consumption, which ranged between 4.02 kg (diet 3) to 4.12 kg (diet 1). The best feed conversion ratio was observed in group I (3.40 $kg \cdot kg^{-1}$), and the worst – in group III, fed the diet with 15% of ground wheat straw (3.79 $kg \cdot kg^{-1}$). A similar tendency was reported by BAIRD et al. (1970). PARTANEN et al. (2002) recorded comparable feed utilization in all experimental groups. In the study conducted by GLAPŚ et al. (1982) feed conversion was better in the experimental groups than in the control group.

The physicochemical properties of meat from the experimental animals are shown in Table 4. No statistically significant differences were found between

the groups. The levels of total protein, dry matter and crude ash in *m.l.d.* samples were comparable in all groups. A tendency towards a decrease in the fat content of meat resulting from an increase in the crude fiber concentrations in diets was observed. It ranged from 1.10% (group III) to 1.16% (group I). However, the differences between groups were not confirmed statistically. Our results are consistent with those reported by GLAPŚ et al. (1982) and SZOSTEK (2004).

Table 4

Chemical and physico-chemical properties of meat

| Specification | Statistics | Groups | | |
|---|---|---|---|---|
| | | I | II | III |
| Chemical (%): | | | | |
| dry matter | $x$ | 25.18 | 25.01 | 24.97 |
| | $s$ | 0.69 | 0.62 | 0.46 |
| crude protein | $x$ | 22.70 | 22.71 | 22.72 |
| | $s$ | 0.48 | 0.39 | 0.38 |
| crude fat | $x$ | 1.16 | 1.14 | 1.10 |
| | $s$ | 0.32 | 0.31 | 0.32 |
| crude ash | $x$ | 1.16 | 1.17 | 1.17 |
| | $s$ | 0.03 | 0.02 | 0.04 |
| Physico-chemical: | | | | |
| water holding capacity (WHC) | $x$ | 7.49 | 7.30 | 7.31 |
| | $s$ | 0.91 | 0.99 | 1.00 |
| brilliance of colour | $x$ | 20.75 | 21.00 | 21.31 |
| | $s$ | 2.18 | 2.42 | 1.74 |
| $pH_{45}$ | $x$ | 5.96 | 6.05 | 6.04 |
| | $s$ | 0.25 | 0.17 | 0.25 |
| $pH_{24}$ | $x$ | 5.62 | 5.58 | 5.59 |
| | $s$ | 0.10 | 0.08 | 0.06 |

The lowest value of $pH_{24}$ (5.58 – 5.62) was noted in group II, fed the diet with 10% of ground wheat straw. According to meat classification based on $pH_{24}$, proposed by KORTZ (2001) and POŚPIECH (2000), meat from the experimental pigs in our study can be considered normal and characterized by good quality, since the levels of $pH_{24}$ were in the 5.5 – 5.8 range. The values of the other physicochemical parameters were similar in all experimental groups.

The data included in Table 5 show that diets with varied levels of ground wheat straw had no considerable effect on slaughter value parameters. The pigs of group III, fed the diet with the highest concentration of crude fiber, had the thinnest layer of backfat (on average 21.9 mm) and the largest loin eye area (55.19 $cm^2$), and were characterized by the highest meatiness (58.68%). The lowest values of these parameters were observed in the control group. BAIRD et al. (1970) demonstrated that an increase in crude fiber in diets was accom-

panied by a decrease in the meat percentage in carcasses. On the other hand, SZOSTEK (2004) who performed an experiment on Polish Landrace x Duroc pigs found that an increase in crude fiber in diets was accompanied by an increase in the loin eye area.

Table 5

Some slaughter parameters of experimental fatteners

| Specification | | Statistics | Groups | | |
|---|---|---|---|---|---|
| | | | I | II | III |
| Lean meat percentage | (%) | *x* | 57.51 | 58.65 | 58.68 |
| | | *s* | 3.96 | 3.20 | 2.37 |
| Loin eye area | ($cm^2$) | *x* | 49.84 | 54.14 | 55.19 |
| | | *s* | 5.34 | 6.59 | 6.48 |
| Length of carcass | (cm) | *x* | 83.6 | 83.9 | 83.62 |
| | | *s* | 1.89 | 2.09 | 2.19 |
| Backfat thickness | (mm) | | | | |
| over the shoulder | | *x* | 34.7 | 35.4 | 35.1 |
| | | *s* | 4.9 | 5.6 | 4.7 |
| on the back | | *x* | 24.7 | 24.6 | 23.8 |
| | | *s* | 5.1 | 4.6 | 3.6 |
| over the loin I | | *x* | 19.1 | 19.9 | 18.1 |
| | | *s* | 4.9 | 4.4 | 3.5 |
| over the loin II | | *x* | 17.4 | 16.9 | 15.1 |
| | | *s* | 4.6 | 3.7 | 3.4 |
| over the loin III | | *x* | 19.0 | 19.8 | 17.7 |
| | | *s* | 4.9 | 3.9 | 3.6 |
| Mean from 5 measurements | | *x* | 23.1 | 23.3 | 21.9 |
| | | *s* | 4.4 | 3.9 | 3.2 |

The data concerning the proportions of some carcass elements are given in Table 6. The carcasses of pigs of group II, fed the diet with 10% of ground wheat straw, had the highest weights and percentages of the majority of cuts (except for belly). Ham weight ranged between 10.36 kg in group III and 10.97 kg in group II, shoulder weight – between 6.20 in group III and 6.39 in group II, loin weight – between 4.41 in group III and 4.59 in group II, whereas the weight of neck – between 2.75 in group I and 2.79 in group II. The differences between the groups were found to be statistically significant in the case of ham weight only. In the study conducted by American authors (BAIRD et al. 1975) the carcasses of fatteners fed a diet with a high crude fiber content (approx. 8%) had lower percentages of ham, loin, shoulder and belly. The values obtained in our study are higher than those recorded by BĄK et al. (2001) and WAJDA et al. (1995), concerning the slaughter quality of fatteners purchased from various producers and characterized by different meatiness.

Table 6

Share of primary elements in carcasses of experimental fatteners

| Specification | Statistics | Groups | | |
|---|---|---|---|---|
| | | I | II | III |
| Ham (kg) | $x$ | 10.41[b] | 10.97[a] | 10.36[b] |
| | $s$ | 0.86 | 0.72 | 0.58 |
| (%) | $x$ | 24.23 | 25.20 | 24.67 |
| | $s$ | 1.17 | 1.03 | 1.24 |
| Shoulder (kg) | $x$ | 6.29 | 6.39 | 6.20 |
| | $s$ | 0.39 | 0.43 | 0.43 |
| (%) | $x$ | 14.66 | 14.68 | 14.74 |
| | $s$ | 0.80 | 0.79 | 0.76 |
| Loin (kg) | $x$ | 4.50 | 4.59 | 4.41 |
| | $s$ | 0.44 | 0.41 | 0.50 |
| (%) | $x$ | 10.49 | 10.54 | 10.47 |
| | $s$ | 1.00 | 0.96 | 0.80 |
| Neck (kg) | $x$ | 2.75 | 2.79 | 2.78 |
| | $s$ | 0.19 | 0.26 | 0.19 |
| (%) | $x$ | 6.42 | 6.39 | 6.60 |
| | $s$ | 0.57 | 0.58 | 0.34 |
| Belly (kg) | $x$ | 4.72 | 4.50 | 4.48 |
| | $s$ | 0.56 | 0.70 | 0.43 |
| (%) | $x$ | 10.99 | 10.34 | 10.64 |
| | $s$ | 1.01 | 1.42 | 0.70 |

*a, b* – $\alpha = 0.05$

Table 7 presents the fatty acid profile in *m.l.d.* samples. The addition of ground wheat straw, in the amount of 10% and 15%, to diets for pigs caused a significant decrease in the concentration of stearic acid (C18:0), from 11.30% (group I) to 10.47% (group III). A substantial decrease in the levels of monounsaturated fatty acids (MUFA) were also observed in groups II and III, as compared with the control group (51.16% and 49.59% vs. 54.18%). Elevated levels of crude fiber in groups II and III caused a significant increase in the percentages of linoleic acid and linolenic acid in *m.l.d.* samples, but had no considerable impact on the concentration of arachidonic acid. However, polyunsaturated fatty acids (PUFA) concentrations increased significantly in the groups fed diets containing wheat straw, to 7.28%, 11.15% and 13.03% respectively.

The mean values of some indices of the serum lipid profile are shown in Table 8. The concentrations of total cholesterol, its fractions and triglycerides were comparable in all experimental groups and slightly exceeded the reference values (WINNICKA 2004). Increased crude fiber levels in the experimental diets resulted in an increase in HDL cholesterol, which varied from 1.31 in group I to 1.44 mmol $\cdot$ dm$^{-3}$ in group III. The differences between means were found to be statistically non-significant. The above tendencies (the increase in

Table 7

Fatty acids composition in *longissimus dorsi* muscle fat (%)

| Fatty acids | Groups | | |
|---|---|---|---|
| | I | II | III |
| C-14:0 | 1.44 | 1.39 | 1.52 |
| C-16:0 | 25.14 | 24.58 | 24.96 |
| C-16:1 | 4.29 | 3.99 | 4.08 |
| C-17:0 | 0.21 | 0.22 | 0.23 |
| C-17:1 | 0.26 | 0.26 | 0.25 |
| C-18:0 | $11.30^{A}$ | $11.25^{A}$ | $10.47^{B}$ |
| C-18:1 | $48.87^{A}$ | $46.17^{B}$ | $44.59^{B}$ |
| C-18:2 | $5.97^{Bd}$ | $9.73^{bc}$ | $11.01^{Aa}$ |
| C-18:3 | $0.52^{BD}$ | $0.80^{BC}$ | $1.13^{A}$ |
| C-20:0 | 0.16 | 0.14 | 0.08 |
| C-20:1 | $0.76^{A}$ | $0.73^{a}$ | $0.66^{Bb}$ |
| C-20:4 | 0.79 | 0.98 | 0.89 |
| SFA | 38.24 | 37.59 | 37.27 |
| PUFA | $7.28^{B}$ | $11.15^{Ab}$ | $13.03^{Aa}$ |
| MUFA | $54.18^{A}$ | $51.16^{B}$ | $49.59^{B}$ |

*a, b* – $\alpha = 0.05$; *A, B* – $\alpha = 0.01$

the concentrations of C18:2 and C18:3, the decrease in the concentration of C18:0, the increase in the level of HDL cholesterol) seem to confirm the health properties of crude fiber, already demonstrated by other authors (BARTNIKOWSKA 1993, HANCZAKOWSKI 1999).

Table 8

Lipids profile of blood serum of experimental pigs

| Specification | | Statistics | Groups | | |
|---|---|---|---|---|---|
| | | | I | II | III |
| Total cholesterol | ($mmol \cdot dm^{-3}$) | *x* | 2.06 | 2.10 | 2.15 |
| | | *s* | 0.19 | 0.26 | 0.20 |
| HDL | ($mmol \cdot dm^{-3}$) | *x* | 1.31 | 1.38 | 1.44 |
| | | *s* | 0.16 | 0.14 | 0.17 |
| LDL | ($mmol \cdot dm^{-3}$) | *x* | 0.62 | 0.56 | 0.51 |
| | | *s* | 0.10 | 0.16 | 0.13 |
| Triglycerides | ($mmol \cdot dm^{-3}$) | *x* | 0.33 | 0.42 | 0.48 |
| | | *s* | 0.08 | 0.13 | 0.19 |

## Conclusions

The results of the study allow to formulate the following conclusions:

1. Elevated levels of crude fiber in diets for pigs negatively affected production results, but the carcasses were characterized by slightly higher meatiness and thinner backfat.

2. The addition of ground wheat straw to diets caused a significant decrease in the concentrations of monounsaturated fatty acids (MUFA) and a considerable increase in the levels of polyunsaturated fatty acids (PUFA) in *m. longissimus dorsi*.

3. Differentiated feeding had no impact on the blood biochemical indices analyzed in the experiment.

Translated by Aleksandra Poprawska

Accepted for print 20.06.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 205:215, Y. 2006

# FATTENING VALUE AND SLAUGHTER QUALITY OF CROSSBRED (POLISH LANDRACE X PIETRAIN) PIGS FED DIETS WITH AN INCREASED CRUDE FIBER CONTENT*

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K e y w o r d s: feeding, oat bran, fattening from 70 to 110 kg, carcass evaluation, fatty acids.

A b s t r a c t

The experiment was performed on 48 crossbred pigs (♀ Polish Landrace x ♂ Pietrain), divided into three feeding groups: group I – fed a cereal-soybean diet containing 15% of total protein (1), group II – fed a diet containing 15% of oat bran (2), group III – fed a diet containing 30% of oat bran (3). The crude fiber content of the diets was as follows: (1) – 3.30%, (2) – 6.22%, (3) – 9.69%. The pigs were kept in litter pens (two animals per pen), fed *ad libitum* and fattened from 70 to 110 kg of live weight.

Dietary supplementation with oat bran negatively affected daily gains and feed conversion ratio. Carcass meatiness was high in all experimental groups and was not differed statistically in groups. Crude fiber levels in diets had a significant effect on the concentrations of polyunsaturated fatty acids in *m. longissimus dorsi* samples. The addition of oat bran to diets resulted in an increase in the blood levels of HDL cholesterol and a decrease in the blood levels of LDL cholesterol.

## WARTOŚĆ TUCZNA I RZEŹNA ŚWIŃ MIESZAŃCÓW PBZ X PIETRAIN ŻYWIONYCH MIESZANKAMI O ZWIĘKSZONEJ ZAWARTOŚCI WŁÓKNA SUROWEGO*

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K e y w o r d s: żywienie, otręby owsiane, tucz do 70-110 kg, ocena tusz, kwasy tłuszczowe.

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* This work was conducted as a part of project no 3P06Z 01924 financed by the State Committee for Scientific Research.

Abstrakt

Badaniami objęto 48 świń mieszańców (♀polska biała zwisłoucha x♂ pietrain) podzielonych na 3 grupy żywieniowe: I – żywioną mieszanką (1) zbożowo-sojową o zakładanej zawartości 15% białka ogólnego, II – mieszanką (2) z udziałem 15% otrąb owsianych, III – mieszanką (3) z udziałem 30% otrąb owsianych. Zawartość włókna surowego w mieszankach wynosiła odpowiednio: 3.30% (1), 6,22% (2), 9,69% (3). Zwierzęta utrzymywano w kojcach ściołowych (po 2 szt. w kojcu), żywiono do woli, a tucz trwał w przedziale od 70 do 110 kg masy ciała.

Udział otrąb owsianych w dietach spowodował zmniejszenie dobowych przyrostów masy ciała tuczników oraz pogorszenie wykorzystania paszy. Mięsność tusz badanych świń była wysoka i nie różniła się istotnie między grupami żywieniowymi. Stwierdzono istotny wpływ poziomu włókna surowego w mieszankach na zawartość wielonienasyconych kwasów tłuszczowych w tłuszczu mięśnia najdłuższego grzbietu. Udział otrąb owsianych w mieszankach miał istotny wpływ na zwiększenie poziomu HDL i zmniejszenie zawartości LDL we krwi tuczników.

## Introduction

Diets with elevated levels of crude fiber, offered *ad libitum*, enable to restrict feed intake in fatteners as well as to reduce carcass fatness (GLAPŚ et al. 1982, ZIÓŁKOWSKI 1993). A growing interest in this nutrient is also related to its health properties, since crude fiber affects the blood lipid profile and increases the concentrations of polyunsaturated fatty acids in the final product (BARTNIKOWSKA 1993).

The studies conducted to date focused on the effects of various levels and types of crude fiber on nitrogen balance and digestibility, fattening results and carcass quality (FALKOWSKI et al. 2004, HAKANSSON et al. 2000, PARTANEN et al. 2002, ZIÓŁKOWSKI 1993).

The aim of the present study was to determine the production results of crossbred (♀ Polish Landrace x ♂ Pietrain) pigs fed diets with an increased concentration of crude fiber at the second stage of fattening.

## Material and Methods

The experiment was performed at on experimental piggery of the Department of Pig Breeding, University of Warmia and Mazury in Olsztyn, on 48 crossbred fatteners (♀Polish Landrace x ♂Pietrain) with initial body weights of about 65 kg. The animals were divided into three experimental groups by the analogue method (on the base of litter of origin, sex and initial body weight):

group I – fed a cereal-soybean diet containing 15% of total protein (1),

group II – fed a diet containing 15% of oat bran (2),

group III – fed a diet containing 30% of oat bran (3).

The composition of the experimental diets is given in Table 1. The diets were supplemented with synthetic amino acids, in accordance with *Nutrient*

*Requirements of Pigs* (1993). The pigs were kept in litter pens (two animals per pen) and had free access to water from automatic drinkers. Feed consumption was monitored and recorded on a daily basis. The animals were weighed every two weeks. They were given friable feed *ad libitum*, from automatic feeders. About seven days prior to slaughter, blood was collected for biochemical analysis, including the determination of the serum levels of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides.

Table 1

Composition of experimental diets (%)

| Specification | Diets | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Ground wheat | 40.00 | 20.00 | 20.00 |
| Ground barley | 41.94 | 40.95 | 21.91 |
| Soybean meal | 13.50 | 16.50 | 18.50 |
| Oat bran | – | 15.00 | 30.00 |
| Dicalcium phosphate | 1.50 | 1.50 | 1.50 |
| Limestone | 1.00 | 1.00 | 1.00 |
| Grower premix | 1.50 | 1.50 | 1.50 |
| NaCl | 0.30 | 0.30 | 0.30 |
| Soybean oil | – | 3.00 | 5.00 |
| L-lysine | 0.24 | 0.21 | 0.23 |
| DL-methionine | 0.02 | 0.04 | 0.06 |
| Nutritive value of diets: | | | |
| metabolizable energy (MJ) | 12.50 | 12.50 | 12.46 |
| crude protein (g) | 147 | 152 | 145 |
| crude fibre (%) | 3.30 | 6.22 | 9.69 |

The pigs were slaughtered at body weights of approx. 110 kg. The carcasses were chilled for 24 hours at about 2°C, and then samples of *m. longissimus dorsi (m.l.d.)* were taken to determine: dry matter content – by drying to constant mass at 105°C, crude fat content – by the Soxhlet method, total protein content – by the Kiejdhal method, crude ash content – by ashing at 550°C.

The slaughter analysis was made according to the methodology developed at the Pig Performance Testing Station (RÓŻYCKI 1996). The weights of particular right half-carcass elements were determined exact to 0.01 kg using an electronic scale. Carcass meatiness was estimated with the ULTRA FOM apparatus.

Meat qualty was determined based on acidity measurements performed 45 minutes ($pH_{45}$) and 24 hours ($pH_{24}$) postmortem on *m.l.d.* samples, with the Dramiński Mini System. Color brightness was measured using a Specol spectrometer with a R-45/0 remission attachment, at a wavelength of 560 nm.

The water holding capacity (WHC) was determined by the method proposed by GRAU, HAMM (1952), modified by POHJA, NINIVARA (1957). The ether extract of *m.l.d.* samples, obtained as described by Peisker (ŻEGARSKA et al. 1991), was analyzed to determine fatty acids concentrations, using a HP 6890 gas chromatograph, with helium as carrier gas.

The results were verified statistically by universally accepted methods, using Statistica PL for Windows software.

## Results and Discussion

The complete diets used in the experiment were characterized by a similar energy value and their total protein content, determined at a laboratory, generally did not differ from that assumed in Methodology (Table 1). Dietary supplementation with oat bran resulted in an increase in the crude fiber concentration in diets 2 and 3, to a level of 6.22 and 9.69 respectively. The levels of the other nutrients were comparable in all experimental diets, and corresponded to the relevant standards.

Table 2 presents the production results achieved in the study. At the beginning of the experiment the mean initial body weights of pigs were similar

Table 2

Fattening performance of experimental pigs

| Specification | Statistics | Groups | | |
|---|---|---|---|---|
| | | I | II | III |
| Average initial body weight (kg) | $x$ | 66.1 | 66.4 | 66.7 |
| | $s$ | 4.65 | 3.71 | 6.81 |
| Average final body weight (kg) | $x$ | 113.8 | 111.7 | 112.3 |
| | $s$ | 5.26 | 3.88 | 4.87 |
| Average daily gains (g) | $x$ | 1160 | 1080 | 1060 |
| | $s$ | 114 | 107 | 102 |
| Average daily feed intake (kg) | $x$ | $3.59^{B}$ | $4.15^{A}$ | $4.16^{A}$ |
| | $s$ | 0.29 | 0.22 | 0.37 |
| Feed/gain ratio ($kg \cdot kg^{-1}$) | $x$ | $3.10^{B}$ | $3.85^{A}$ | $3.91^{A}$ |
| | $s$ | 0.38 | 0.43 | 0.62 |

*a, b* – $\alpha = 0.05$; *A, B* – $\alpha = 0.01$

(on average 66.4 kg), with no significant differences between the groups. No statistically confirmed differences were found in the final body weights of pigs on the last day of fattening, either. They ranged from 111.7 kg in group II to 113.8 kg in group III. Over the fattening period (70-110 kg) the average daily

gains of the experimental pigs were 1160, 1080 and 1060 g in groups I, II and III respectively. The highest daily gains were observed in group I, fed a cereal-soybean diet with the lowest concentration of crude fiber. However, the differences between the groups were not confirmed statistically. The fattening results, which may be considered very good, were similar to those obtained in previous experiments performed in the same piggery (FALKOWSKI, KOZERA 1999, SZOSTEK 2004), and better than those reported by Canadian authors (KENNELLY, AHERNE 1980). In their study the highest daily gains were recorded in the control animals, fed the diet containing 4.10% of crude fiber.

In the present experiment the increased concentration of crude fiber in diets had a significant effect on mean daily feed consumption, which ranged between 3.59 kg (diet 1) to 4.16 kg (diet 3). The best feed conversion ratio was observed in group I (3.10 $kg \cdot kg^{-1}$), and the worst – in group III, fed the diet with 30% oat bran (3.91 $kg \cdot kg^{-1}$). A similar tendency was reported by BAIRD et al. (1970) – an increase in crude fiber levels (from 3.5 to 11.5%) had a negative effect on feed conversion ratio (3.51 vs. 3.72 $kg \cdot kg^{-1}$).

The physicochemical properties of meat from the experimental animals are shown in Table 3. No statistically significant differences were found between the groups. The levels of total protein and crude fat in *m.l.d.* samples were comparable in all groups, and varied from 22.66 (group II) to 22.72% (group III), and from 1.14 (group II) to 1.16 (group I) respectively. GLAPŚ et al. (1982) studied the use of fractionated alfalfa in pig fattening and

Table 3

Chemical and physico-chemical properties of meat

| Specification | Statistics | Groups | | |
|---|---|---|---|---|
| | | I | II | III |
| Chemical (%): | | | | |
| dry matter | $x$ | 25.74 | 25.58 | 25.70 |
| | $s$ | 0.59 | 0.42 | 0.64 |
| crude protein | $x$ | 22.67 | 22.66 | 22.72 |
| | $s$ | 0.69 | 0.53 | 0.60 |
| crude fat | $x$ | 1.29 | 1.234 | 1.36 |
| | $s$ | 0.42 | 0.50 | 0.69 |
| crude ash | $x$ | 1.16 | 1.14 | 1.15 |
| | $s$ | 0.11 | 0.12 | 0.14 |
| Physico-chemical: | | | | |
| water holding capacity (WHC): | $x$ | 7.86 | 7.50 | 7.41 |
| | $s$ | 1.30 | 0.82 | 1.01 |
| brilliance of colour | $x$ | 21.81 | 20.44 | 21.88 |
| | $s$ | 3.06 | 1.36 | 2.70 |
| $pH_{45}$ | $x$ | 5.92 | 5.82 | 6.00 |
| | $s$ | 0.22 | 0.27 | 0.21 |
| $pH_{24}$ | $x$ | 5.65 | 5.56 | 5.58 |
| | $s$ | 0.08 | 0.09 | 0.11 |

found that an increase in the crude fiber content of diets was followed by a decrease in the levels of total protein and fat in meat (by approx. 0.73% and 0.58% respectively). SZOSTEK (2004) conducted an experiment on cross-bred (♀ Polish Landrace x ♂ Duroc) pigs fed diets containing wheat bran and also observed a decrease in the fat content of meat along with an increase in the crude fiber content of diets.

The lowest values of both $pH_{45}$ and $pH_{24}$ (5.82 – 6.00 and 5.56 – 5.65 respectively) were recorded in group II, fed the diet with 15% of oat bran. According to meat classification based on $pH_{45}$ and $pH_{24}$, proposed by POŚPIECH (2000), meat from the experimental pigs in our study can be considered normal and characterized by good quality, since the levels of $pH_{45}$ and $pH_{24}$ were above 5.8 and in the 5.5-5.7 range respectively.

The water-holding capacity (WHC) is used to determine the technological suitability of meat. In this experiment meat from the pigs of group III, fed the diet with 30% of oat bran, was characterized by the lowest WHC value, whereas meat from the animals of the control group – by the highest.

Color brightness is one of the physical parameters that characterize meat color. The more watery the meat, the more brighter the color. The lowest value of color brightness was observed in group II, and the highest in group III, which received the diet containing 30% of oat bran. It ranged from 20.44 to 21.88.

The data included in Table 4 show that diets with varied levels of crude fiber considerably affected average backfat thickness measured at five points. The pigs of group III, fed the diet with the highest concentration of crude fiber, had the thinnest layer of backfat. The largest loin eye area (58.13 $cm^2$) was recorded in group I, fed a standard diet, and the smallest (54.51 $cm^2$) – in group II. SZOSTEK (2004), who conducted an experiment on Polish Landrace x Duroc pigs, demonstrated that an increase in crude fiber in diets was accompanied by an increase in the loin eye area. A similar correlation was also observed by ZIÓŁKOWSKI (1993), who studied diets with an increasing concentration of alfalfa meal, and by KENNELY & AHERNE (1980). In the latter study the experimental diets contained 4.1, 9.8, 9.6 and 10.2% of crude fiber. Somewhat different results were obtained by BAIRD et al. (1975). In their study feeding high-fiber diets to fatteners caused an increase in the carcass dressing percentage and backfat thickness, as compared with the control group.

There were no statistically significant differences between the groups in the mean meat content of a carcass. Carcass meatiness was generally very good, between 56.51 and 58.10%, and reached the highest level in group I. Also BAIRD et al. (1970) demonstrated that an increase in the crude fiber content of diets resulted in a decrease in the percentage of meat in carcasses. Our results are much better than those obtained by MICHALSKA et al. (2001). In their study

Table 4

Some slaughter parameters of experimental fatteners

| Specification | Statistics | Groups | | |
|---|---|---|---|---|
| | | I | II | III |
| Lean meat percentage (%) | *x* | 58.10 | 56.51 | 57.86 |
| | *s* | 2.28 | 3.24 | 2.93 |
| Carcass dressing percentage (%) | *x* | 82.65 | 81.56 | 81.88 |
| | *s* | 1.27 | 1.02 | 1.19 |
| Loin eye area ($cm^2$) | *x* | 58.13 | 54.51 | 55.56 |
| | *s* | 6.25 | 7.22 | 7.70 |
| Length of carcass (cm) | *x* | 82.8 | 82.4 | 83.4 |
| | *s* | 2.29 | 1.89 | 1.86 |
| Backfat thickness (mm) | | | | |
| over the shoulder | *x* | 37.6 | 38.7 | 36.3 |
| | *s* | 4.51 | 4.39 | 5.31 |
| on the back | *x* | 22.2 | $24.2^a$ | $20.6^b$ |
| | *s* | 2.14 | 4.10 | 4.66 |
| over the loin I | *x* | 23.9 | 26.6 | 24.4 |
| | *s* | 4.10 | 4.23 | 4.19 |
| over the loin II | *x* | 14.2 | 15.0 | 14.6 |
| | *s* | 2.54 | 2.97 | 2.58 |
| over the loin III | *x* | 18.5 | 19.6 | 18.4 |
| | *s* | 2.42 | 3.41 | 2.5 |
| Mean from 5 measurements | *x* | 23.29 | $24.81^a$ | $22.88^b$ |
| | *s* | 1.93 | 2.80 | 2.61 |

*a, b* – $\alpha = 0.05$; *A, B* – $\alpha = 0.01$

the meat content of carcasses of crossbred pigs (Polish Landrace x Pietrain) was 49.16%.

The data concerning the proportions of some carcass elements are given in Table 5. The carcasses of pigs of the control group had the highest weights and percentages of ham, belly and shoulder. Ham weight ranged between 10.45 kg in group III to 11.04 kg in group I, whereas shoulder weight – between 6.14 in group II and 6.45 in group I. In both cases the differences between means of groups were confirmed by a statistical analysis ($\alpha = 0.05$). The weights and percentages of the other cuts were comparable in all experimental groups. Such relationships were also reported by BAIRD et al. (1975). In their experiment a diet with a high crude fiber content (approx. 8%) resulted in lower proportions of ham, loin, shoulder and belly in a carcass. The values obtained in our study were higher than those recorded by BĄK et al. (2001), concerning the slaughter quality of fatteners purchased from various producers.

Table 6 presents the fatty acid profile in *m.l.d.* samples. Polyunsaturated fatty acids (PUFA), especially linoleic and linolenic, play a fundamental role in human nutrition, mainly due to the fact that they contribute to the prevention of circulatory diseases (BANASZKIEWICZ 2001, ZIEMLAŃSKI, BUDZYŃSKA

Table 5

Share of primary elements in carcasses of experimental fatteners

| Specification | Statistics | Groups | | |
|---|---|---|---|---|
| | | I | II | III |
| Ham (kg) | *x* | $11.04^{a}$ | $10.46^{b}$ | $10.45^{b}$ |
| | *s* | 0.59 | 0.66 | 0.74 |
| (%) | *x* | 24.01 | 23.39 | 22.31 |
| | *s* | 0.68 | 1.07 | 0.88 |
| Shoulder (kg) | *x* | $6.45^{a}$ | $6.14^{b}$ | $6.44^{a}$ |
| | *s* | 0.45 | 0.29 | 0.43 |
| (%) | *x* | 14.03 | 13.73 | 13.77 |
| | *s* | 0.56 | 0.62 | 0.47 |
| Loin (kg) | *x* | 5.85 | 5.61 | 5.67 |
| | *s* | 0.45 | 0.41 | 0.41 |
| (%) | *x* | 12.72 | 12.54 | 12.13 |
| | *s* | 0.67 | 0.59 | 0.70 |
| Neck (kg) | *x* | 2.90 | 2.71 | 2.91 |
| | *s* | 0.27 | 0.25 | 0.30 |
| (%) | *x* | 6.29 | 6.05 | 6.20 |
| | *s* | 0.40 | 0.36 | 0.55 |
| Belly (kg) | *x* | $5.60^{A}$ | $5.10^{B}$ | 5.23 |
| | *s* | 0.45 | 0.43 | 0.20 |
| (%) | *x* | 12.20 | 11.40 | 11.48 |
| | *s* | 1.01 | 0.82 | 0.50 |

*a, b* – $\alpha = 0.05$; *A, B* – $\alpha = 0.01$

Table 6

Fatty acids composition in *longissimus dorsi* muscle fat (%)

| Fatty acids | Groups | | |
|---|---|---|---|
| | I | II | III |
| C-14:0 | 1.38 | 1.33 | 1.34 |
| C-16:0 | 25.30 | 25.03 | 24.43 |
| C-16:1 | 4.35 | 4.13 | 3.97 |
| C-17:0 | 0.19 | 0.19 | 0.17 |
| C-17:1 | 0.24 | 0.25 | 0.23 |
| C-18:0 | 11.57 | 11.17 | 11.31 |
| C-18:1 | 49.47 | 49.03 | 49.59 |
| C-18:2 | $5.53^{B}$ | $6.81^{A}$ | $6.62^{A}$ |
| C-18:3 | $0.40^{Bb}$ | $0.66^{a}$ | $0.81^{A}$ |
| C-20:0 | 0.15 | 0.16 | 0.23 |
| C-20:1 | 0.70 | 0.78 | 0.81 |
| C-20:4 | 0.76 | 0.89 | 0.70 |
| SFA | 38.54 | 37.82 | 37.46 |
| PUFA | $6.69^{Bd}$ | $8.36^{A}$ | $8.06^{a}$ |
| MUFA | 54.76 | 54.19 | 54.60 |

*a, b* – $\alpha = 0.05$; *A, B* – $\alpha = 0.01$

TOPOLOWSKA 1991, ZÖLLNER, TATO 1992). That is why the PUFA concentration in foodstuffs is so important, particularly in pork which until quite recently was considered to have a low dietary value. The addition of 15% or 30% of oat bran to diets for pigs caused a significant increase in the levels of linoleic acid (C18:2) and linolenic acid (C18:3), which varied from 5.53 (group I) to 6.81 (group II), and from 0.40 (group I) to 0.81 (group III) respectively, as well as in the concentrations of PUFA. The differences between the groups were statistically significant. The levels of the other fatty acids were comparable in all experimental groups.

The mean values of some indices of the serum lipid profile are shown in Table 7. The total cholesterol concentration was similar in all groups (on average 2.26) and slightly exceeded the reference values (WINNICKA 2004). Increased crude fiber levels in the experimental diets resulted in an increase in HDL cholesterol and a decrease in LDL cholesterol. The HDL concentration ranged between 1.33 in group I and 1.63 mmol · $dm^{-3}$ in group II. The differences between means were confirmed statistically. According to WINNICKA (2004), HDL cholesterol should constitute up to 40% of total cholesterol, and a decrease in the level of this fraction is undesirable. The values achieved in our experiment were considerably higher.

Table 7

Lipids profile of blood serum of experimental pigs

| Specification | Statistics | Groups | | |
|---|---|---|---|---|
| | | I | II | III |
| Total cholesterol (mmol · $dm^{-3}$) | $x$ | 2.19 | 2.37 | 2.23 |
| | $s$ | 0.262 | 0.241 | 0.507 |
| HDL (mmol · $dm^{-3}$) | $x$ | $1.33^{B}$ | $1.63^{A}$ | $1.55^{A}$ |
| | $s$ | 0.159 | 0.183 | 0.188 |
| LDL (mmol · $dm^{-3}$) | $x$ | $0.70^{A}$ | $0.55^{B}$ | $0.53^{B}$ |
| | $s$ | 0.101 | 0.116 | 0.170 |
| Triglycerides (mmol · $dm^{-3}$) | $x$ | $0.40^{B}$ | $0.46^{b}$ | $0.65^{Aa}$ |
| | $s$ | 0.218 | 0.136 | 0.245 |

*a, b* – $\alpha = 0.05$; *A, B* – $\alpha = 0.01$

The highest level of triglycerides was recorded in group III (0.65 mmol · $dm^{-3}$), and the lowest – in group I (0.40 mmol · $dm^{-3}$). These differences were found to be statistically significant, and slightly different from the reference values (WINNICKA 2004).

## Conclusions

1. Dietary supplementation with oat bran negatively affected the growth rate of pigs and feed conversion ratio, but had no significant effect on the meat content of a carcass.

2. Elevated levels of crude fiber in diets caused a considerable increase in the concentrations of polyunsaturated fatty acids (PUFAs) in *m.l.d.* samples.

3. The addition of oat bran to diets resulted in an increase in the blood levels of HDL cholesterol and a decrease in the blood levels of LDL cholesterol, but had no significant influence on the blood levels of total cholesterol.

Translated by Aleksandra Poprawska

Accepted for print 2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 217-227, Y. 2006

# EFFECT OF ADDING β-CAROTENE AND VITAMINS C AND E TO CLA-ENRICHED DIETS ON DIETETIC AND EATING QUALITY OF PORK

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K e y w o r d s: β-carotene, vitamin C, vitamin E, CLA, pigs, meat quality, eating quality.

A b s t r a c t

The study was designed to enrich meat in CLA and antioxidant vitamins, to prevent fatty acids from excessive oxidation, and to determine the effect of these factors on the dietetic and sensory traits of pork. The experiment involved 50 Polish Large White fattening pigs, which were randomly assigned to 5 groups (5 gilts and 5 barrows per group). All the rations contained 0.5% CLA preparation (Edenor 6010, Henkel). Vitamins were added to the diets in the following amounts (mg/kg): group I – 30 vitamin E; group II – 200 β-carotene; group III – 200 vitamin C; group IV – 300 vitamin E; group V – 200 β-carotene, 200 vitamin C and 300 vitamin E. There was a downward tendency for linoleic acid (C18:2) and α-linolenic acid (C18:3) ($P<0.05$) in group II. The level of PUFA in group V tended to decrease in comparison with group II ($P<0.05$). Compared to barrows, gilts were characterized by a significantly lower level of saturated acids in meat (mainly C10:0, C14:0, C16:0 and C18:0) and a significantly higher level of unsaturated acids (C18:2, C18:2 *c9c11*, C18:3, C*γ*18:3 and C20:4) ($P<0.01$). The meat of gilts had a tendency towards lower dry matter content ($P<0.05$) and significantly lower content of ether extract and $pH_{24}$ ($P<0.01$). In all the experimental groups, yellowness was significantly reduced ($P<0.01$). There was a significant increase in the vitamin E content of meat in groups III, IV and V receiving higher supplements of vitamins C and E ($P<0.01$). A lower TBARS value was found in the group supplemented with vitamin E (group IV) and in group V receiving vitamins C and E and β-carotene supplements compared to group II which received a higher dose of β-carotene ($P<0.05$). The meat of barrows had a tendency towards higher TBARS value compared to the meat of gilts ($P<0.05$). Meat tenderness and juiciness were found to improve in group V, which received a mixture of β-carotene and vitamins C and E compared to the other groups. Sex had no effect on the sensory evaluation of pork.

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**WPŁYW DODATKU β-KAROTENU, WITAMIN C i E W DAWKACH POKARMOWYCH WZBOGACONYCH W CLA NA CECHY DIETETYCZNE I KULINARNE WIEPRZOWINY**

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S ł o w a k l u c z o w e: β-karoten, witamina E, witamina C, świnie, jakość mięsa, jakość kulinarna.

A b s t r a k t

Celem badań było wzbogacenie mięsa w CLA i witaminy o działaniu antyoksydacyjnym i próba zabezpieczenia kwasów tłuszczowych przed nadmiernym utlenianiem oraz zbadanie wpływu tych czynników na cechy dietetyczne i sensoryczne mięsa wieprzowego. Doświadczenie przeprowadzono na 50 tucznikach rasy wbp podzielonych losowo na 5 grup (po 5 loszek i 5 wieprzków w każdej). Wszystkie dawki pokarmowe zawierały w swym składzie 0,5% preparatu CLA (Edenor 6010, Henkel). W mieszankach zastosowano następujące dodatki witamin ($mg \cdot kg^{-1}$): gr. I – 30 wit. E; gr. II – 200 β-karotenu; gr. III – 200 wit. C; gr. IV – 300 wit. E; gr. V- 200 β-karotenu, 200 wit. C i 300 wit. E. Stwierdzono tendencję do obniżania poziomu kwasu linolowego (C18:2) oraz kwasu α-linolenowego (C18:3) ($P<0,05$) w gr. II. Ponadto stwierdzono tendencję do obniżania poziomu PUFA w gr. V w porównaniu z gr. II ($P<0,05$). W mięsie loszek wykazano istotnie niższą zawartość kwasów nasyconych, głównie: C10:0, C14:0, C16:0 i C18:0, w porównaniu z mięsem wieprzków, oraz istotnie wyższą zawartość kwasów nienasyconych: C18:2, C18:2 *c9c11*, C18:3, Cγ18:3, C 20:4) ($P<0,01$). W mięsie loszek wykazano tendencję do niższej zawartości suchej masy ($P<0,05$) oraz istotnie niższą zawartość ekstraktu eterowego oraz $pH_{24}$ ($P<0,01$). We wszystkich grupach doświadczalnych stwierdzono istotne zmniejszenie wysycenia barwy w kierunku żółtym ($P<0,01$). Wykazano istotny wzrost zawartości wit. E w mięsie tuczników z gr. III, IV i V otrzymujących odpowiednio wyższy dodatek wit. C i E ($P<0,01$). Stwierdzono niższy poziom TBARS w grupie otrzymującej dodatek wit. E (gr. IV) i w gr. V otrzymującej mieszaninę wit. C, E i β-karotenu, w porównaniu z grupą II otrzymującą większy dodatek β-karotenu ($P<0,05$). W mięsie wieprzków stwierdzono tendencję do wyższej zawartości TBARS w porównaniu z mięsem loszek ($P<0,05$). Stwierdzono poprawę kruchości i soczystości mięsa w gr. V otrzymującej mieszaninę β-karotenu, wit. C i E, w porównaniu z innymi grupami. Nie stwierdzono wpływu płci na ocenę sensoryczną wieprzowiny.

## Introduction

Over the years, the chemical composition of fatty acids in meat production gained in importance in light of its effect on human health and meat quality traits (De Smet et al. 2004). The incidence of cardiovascular diseases, cancers, insulin-independent diabetes and obesity has increased in economically developed countries since the 1950s as a result of lifestyle and dietary changes. Conscious consumers realize that the increased consumption of polyunsaturated fatty acids (PUFA), especially *n-3* PUFA, reduces the risk of cardiovascular and cardiac diseases. Specialists from the World Health Organization (WHO) recommend that not more than 30% of the dietary energy should come from fat, including less than 10% from saturated fatty acids (SFA), and the ideal PUFA to SFA ratio is 0.7.

Animal products are usually characterized by a high content of fats, which are dominated by saturated fatty acids. The drastic increase in the consumption of fats and the decrease in the *n-3* to *n-6* PUFA ratio is accompanied by a reduced consumption of dietary fibre and natural antioxidant vitamins.

However, the composition of fatty acids in food of animal origin, including pork, can be modified through nutrition by changing the composition of total fatty acids in feed. The use of conjugated linoleic acid (CLA) is an effective way of changing the composition of lipid fatty acids in pigs (TISCHENDORF et al. 2002, MIGDAŁ et al. 2004). The effect of CLA on the increased saturation of fatty acids in porcine lipids has been confirmed by many authors (JOO et al. 2002, MIGDAŁ et al. 2004). In several experiments, the CLA preparation used caused an increase in the level of saturated fatty acids, mainly C18:0, which is positively correlated with meat taste and tenderness (WOOD et al. 2004). The use of supplemental CLA in pig diets increased the level of *n-3* acids and decreased the level of *n-6* acids (TISCHENDORF et al. 2002). In addition, the increased supply of CLA in the diet increased CLA deposition in the muscle and adipose tissue (THIEL-COOPER et al. 2001).

The use of PUFA-rich diets in pig nutrition is desirable in terms of consumer health, because these acids improve the dietetic value of meat, but on the other hand their excessive amount in animal fat has an adverse effect on sensory traits (taste and aroma) of meat and its storage life (LEKSANICH et al. 1997). Tenderness, firmness and juiciness of meat are closely related to the water and collagen content of muscle tissue and to the level of stearic (C18:0) and linoleic acids (C18:2). Stearic and linoleic fatty acids differ in melting temperature (69.6 and -5°C, respectively), which has a significant effect on the cohesiveness and firmness of meat. It was also found that stearic acid (C18:0) plays a greater role in the formation of these traits. Comprehensive studies concerning the effect of fatty acid composition on the taste of meat have shown a positive correlation between this trait and saturated and unsaturated acids, and a negative correlation for unsaturated acids, mainly linolenic (C18:3) (WOOD et al. 2004). Such meat and its products are characterized by shorter storage life resulting from the greater susceptibility of PUFA to oxidation processes, even after the freezing of meat.

Supplements that influence meat quality include vitamins C and E and β-carotene, which show antioxidant action. The choice of vitamins C and E was not accidental, because these antioxidants show synergic action as a result of mutual cooperation in the first line of antioxidant defence (CHEPTA et al. 2001). Results of many studies have confirmed the positive effect of the above antioxidants on the colour, water binding capacity and sensory traits of meat such as juiciness and tenderness (BUCKLEY et al. 1995, HANCZAKOWSKA 2004).

The aim of the study was to enrich meat in CLA and antioxidant vitamins, to prevent fatty acids from excessive oxidation, and to determine the effect of these factors on the dietetic and sensory traits of pork.

## Material and Methods

The experiment involved 50 Polish Large White fattening pigs, which were randomly assigned to 5 groups with 5 gilts and 5 barrows per group. The animals were fattened from 50 to 105 kg body weight using diets differing in the level of antioxidants. All the diets contained supplemental CLA (Edenor UKD 6010, Henkel) at 0.5% of the ration and vitamins in amounts given in Table 1. CLA contained 61.3% isomers, including 18.6% of C18:2 *c9t11,* 20.7% of C18:2 *t10c12,* 21.2% of C18:2 *c9c11*, and 0.8% of C18:2 *t9t11*. The dietary premixes used in the experiment were manufactured by the BASF Premix Plant in Kutno. Due to the instability of ascorbic acid and $\alpha$-tocopherol, both vitamins were added to the premix in the form of calcium salt of ascorbic acid monophosphate and $\alpha$-tocopherol acetate (Table 2). The level of metabolizable energy (13.0 MJ) was calculated from the diet composition, assuming tabular values for particular components (*Normy żywienia świń* 1993). The diet formula is given in Table 1. Basic and amino acid composition of the diets was determined using standard methods (AOAC 1995). The nutrient content per 1 kg of the diet was 170 g crude protein, 36.4 g crude fibre, 20.9 g crude fat, 5.19 g total phosphorus, 8.5 g calcium, 9.3 g lysine, 5.9 g methionine with cystine, and 1.9 g tryptophan. The samples of meat was analysed for chemical composition (BUDSŁAWSKI, DRABENT 1972). Meat quality traits were measured 24 h postmortem in the samples of the *longissimus* muscle taken between the $4^{th}$ and $5^{th}$ lumbar vertebrae. Sensory analysis of meat quality traits was performed on loin steaks (20 mm thick), which were stored for 24 h at 4°C and then cooked using a grill plate (Toshiba, Japan). Steaks were cooked for about 5 min until an internal temperature of 85°C was reached. The cooked samples of loin were cooled for 2 min and cut into 4 parts. They were evaluated on a 5-point scale (0=inadequate, 1=adequate, 2=satisfactory, 3=good, 4=very good, 5=excellent) in terms of tenderness, juiciness, aroma and taste, taking into account intensity and quality, in accordance with a modified method of BARYŁKO-PIKIELNA (1975). Meat colour in the CIE LAB system (CIE, 1976) was determined using a reflectance spectrophotometer (Minolta CR 310, Japan), 24 h postmortem. pH was measured using a pH meter (Matthaus, Germany) with a glass electrode standardized at pH 4.01 and 7.0 in *M. longissimus lumborum* at the level of $10^{th}$ rib. Water binding capacity was determined in accordance with the method described by GRAU and HAMM (1953). The

composition of higher fatty acids in meat was determined using gas chromatography (GC) after lipid extraction according to FOLCH et al. (1957). Chromatographic analysis of $\alpha$-tocopherol was performed using HPLC based on a modified method (UEDA, IGARASHI 1987). The samples of the *longissimus* muscle were analysed for TBARS value according to a method described by SALIH et al. (1987) after 90 days of storage at -19°C. The results were analysed statistically using two-way analysis of variance (ANOVA) and Tukey's test using Statgraphics 4.0 software.

Table 1

Complete diets and the composition of fatty acids (% of total fatty acids)

| Ingredient (%) | Composition (%) |
|---|---|
| Ground wheat | 44.5 |
| Ground triticale | 20 |
| Ground maize | 10 |
| Soybean meal | 21 |
| Limestone | 1.5 |
| Dicalcium phosphate | 0.5 |
| CLA | 0.5 |
| Vitamin/mineral premix* | 2 |
| Composition of fatty acids (%) | |
| UFA | 82.41 |
| MUFA | 26.15 |
| PUFA | 56.26 |
| PUFA *n-6* | 48.52 |
| PUFA *n-3* | 7.74 |
| PUFA *n-6/n-3* | 6.27 |

* premixes were manufactured on the basis of Lutamix complet, BASF (Kutno, Poland) containing in 1 kg: 80 g lysine, 12 g methionine, 18 g methionine and cystine, 12 g threonine, 250 g Ca, 75 g P, 80 g Na, 8 g Mg, 210 g NaCl, 2 mg Mn, 30 mg J, 3000 mg Fe, 1500 mg Cu, 4000 mg Zn, 15 mg Se, 20 mg Co and vitamins A 400.000 IU, $D_3$ 60.000, $K_3$ 75 mg, $B_1$ 60 mg, $B_2$ 150, $B_6$ 75, $B_{12}$ 1 mg, folic acid 10 mg, pantothenic acid 400 mg, nicotinic acid 600 mg, choline 600 mg

Table 2

Addition of $\beta$-carotene and vitamins C and E to the feed mixture (mg · kg$^{-1}$ of mixture)

| Item | Groups | | | | |
|---|---|---|---|---|---|
| | I standard + vit. E | II standard + $\beta$-carotene | III standard + vit. C | IV standard + vit. E | V standard + $\beta$-carotene, vit. C and E |
| $\beta$-carotene | – | 200 | – | – | 200 |
| Monophosphate L-ascorbic acid (vit. C) | – | – | 200 | – | 200 |
| $\alpha$-tocopherol acetate (vit. E) | 30 | 30 | 30 | 300 | 300 |

## Results and Discussion

Analysis of the composition of fatty acids in the *longissimus* muscle showed a significant difference ($P<0.05$) in the level of PUFA between group V, which was supplemented with vitamins C and E and $\beta$-carotene and group II, which received a diet supplemented with $\beta$-carotene only. The lowest level of PUFA in group V was due to the low levels of linoleic acid (C18:2) and $\alpha$-linolenic acid (C18:3). Significant differences in the composition of fatty acids depending on sex were found. Compared to barrows, the fatty acid profile of gilts was characterized by a significantly lower level of saturated acids ($P<0.01$) and a significantly higher level of unsaturated acids. Gilts had significantly lower levels of capric (C10:0), myristic (C14:0) and palmitic acids ($P<0.01$), with a tendency towards a lower level of stearic acid (C18:0) ($P<0.05$). The vitamin supplements used also resulted in a significantly lower level of palmitoleic acid (C16:1) in gilts. This was accompanied by a sex by diet interaction ($P<0.05$) (Table 3). These data concur with the findings of WARNANTS et al. (1999), who reported a higher level of SFA in barrows than in gilts. This fact was attributed to the higher lipogenic activity in barrows, which was reflected in a higher *de novo* synthesis of fatty acids and MUFA and a lower level of PUFA. Differences in the composition of fatty acids between gilts and barrows were also reported by HANCZAKOWSKA (2004), who used increased dietary supplements of vitamins E and C and $\alpha$-carotene, possibly indicative of the differences in the metabolism of fatty acids in both sexes. The higher level of unsaturated acids in gilts was due to linoleic (C18:2), $\gamma$-linolenic (C18:3) and arachidonic acids (C 20:4) ($P<0.01$). In gilts, a significantly higher increase in the level of CLA, mainly *c9c11* and *c9t11* isomers ($P<0.01$) was found, with an interaction between the type of diet and sex. CLA is fairly easily incorporated into adipose tissue lipids and increases the saturation of fatty acids, as confirmed by many experiments (JOO et al. 2002, MIGDAŁ et al. 2004). As a result of the increased level of unsaturated acids, gilts had a tendency towards an increased ratio between unsaturated and saturated acids ($P<0.05$).

The experimental factors did not negatively affect the physico-chemical properties of meat (Table 4). No significant changes in the content of dry matter, protein and fat under the influence of the dietary supplements were found. There were significant differences in the content of crude fat ($P<0.01$) and dry matter ($P<0.05$) depending on the sex of pigs. Compared to barrows, gilts were characterized by a lower content of crude fat and dry matter (1.96 and 2.6%; 26.1 and 26.7%, respectively). It is supposed that the differences in the ether extract content of meat can be due to factors related to the pigs' protein deposition capacity, with gilts being superior in this respect than barrows, thanks to which their carcasses are characterized by lower fatness and higher meatiness (FANDREJEWSKI 1996).

pH value measured in gilts 24 h *post-mortem* was 5.44 and was significantly lower than the pH value (5.55) of meat from barrows ($P<0.01$). The significantly lower pH values in gilts are evidence of the lower glycolytic potential compared to barrows. In a review paper, BENDALL, SWATLAND (1988) reported that ultimate pH is one of the indicators of pork quality. Average value of $pH_{24}$ in our studies suggest that pork is with defect of RSE.

Meat colour measured 24 h *post-mortem* on the L*a*b* scale shows that the high vitamin supplement reduced yellowness (b*) ($P<0.01$). The dietary vitamin supplements did not have any effect on colour lightness (L*) or redness (a*). A similar effect was reported by HASTY et al. (2002). ASGHAR et al. (1991) reported a significant increase in redness of fresh pork from pigs receiving a dietary supplement of 200 IU · $kg^{-1}$ vitamin E, although it did not have any effect on colour lightness or yellowness. This effect is attributed to the inhibitory effect of vitamin E on the oxidation of myoglobin, a pigment that gives meat its reddish hue in contrast to its oxidized forms. HOUBEN et al. (1998) did not find any effect of a vitamin E supplement on the change of colour in fresh pork.

The meat from animals receiving a supplement of vitamins, especially vitamins E and C (groups III and IV) was characterized by lower water binding capacity (29.1 and 28.6%, respectively) compared to the control group (32.4%), but the differences were not significant. Improved water binding capacity of meat as a result of supplementing dietary CLA and natural antioxidants was reported by BUCKLEY et al. (1995), JOO et al. (2002) and DAZA et al. (2005). The observed lower values of water binding capacity of meat in fatteners receiving the vitamin E and C supplement is evidence of improved pork quality as reflected in water binding capacity, which is important as a parameter of technological evaluation and organoleptic evaluation by the consumer.

In the present study, we showed a significant difference ($P<0.05$) in the content of malonaldehyde after 3 months of storing meat at -19°C between groups IV (receiving vitamin E), V (receiving vitamins C and E and β-carotene) and V (receiving β-carotene). The level of substances reacting with TBA was 0.507, 0.411 and 0.424 mg · $kg^{-1}$ meat in groups II, IV and V, respectively. The results obtained are indicative of the weaker antioxidant activity of β-carotene compared to vitamin E given at 300 mg · $kg^{-1}$ feed (group IV) and vitamins E and C and β-carotene given jointly in one ration. The effect of vitamins E and C and β-carotene on limiting lipid oxidation in pigs was reported by BUCKLEY et al. (1995), HANCZAKOWSKA (2004) and DAZA et al. (2005), among others. A significantly higher level of TBARS in barrows than in gilts was also shown ($P<0.05$). Although a higher level of unsaturated acids in meat lipids of gilts was found, which might suggest a higher susceptibility to oxidation, an almost 24% higher total fat content of barrows; meat probably caused a significant

increase in TBARS. The vitamin supplements used in the study caused changes in the vitamin E content of meat. The use of a higher dietary supplement of vitamin E alone and together with vitamin C and $\beta$-carotene caused a significant increase in vitamin E from 1.23 $\mu g \cdot mg^{-1}$ (group I) to 2.88 $\mu g \cdot mg^{-1}$ (group IV) and 3.06 $\mu g \cdot mg^{-1}$ (group V). Vitamin C, given at 200 $mg \cdot kg^{-1}$ feed together with the use of standard premix, caused a significant increase in vitamin E in meat to 1.76 $\mu g \cdot mg^{-1}$. The higher doses of $\alpha$-tocopherol acetate (groups IV and V) used in the experiment increased the vitamin E content of meat, which is in agreement with the findings of other authors (HANCZAKOWSKA 2004, DAZA et al. 2005). The results of sensory analysis of *M. longissimus dorsi* are presented in Table 5.

In the present experiment it was found that giving dietary vitamin C and a mixture of vitamins E and C and $\beta$-carotene reduced the level of some polyene acids such as C18:2 and C 20:4 PUFA (*n-6*) responsible for the sensory value,

Table 3

Fatty acid composition of *M. longissimus* in pigs receiving different amounts of vitamins and 0.5% CLA

| Fatty acids | Groups | | | | | SEM | Sex | | SEM | Diet × sex interaction[1] |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | | gilts | barrows | | |
| C 10:0 | 0.14 | 0.14 | 0.15 | 0.13 | 0.13 | 0.007 | $0.12^{A}$ | $0.15^{B}$ | 0.004 | NS |
| C 12:0 | 0.11 | 0.10 | 0.10 | 0.09 | 0.09 | 0.004 | 0.09 | 0.10 | 0.03 | NS |
| C 14:0 | 1.76 | 1.76 | 1.71 | 1.62 | 1.69 | 0.05 | $1.61^{A}$ | $1.81^{B}$ | 0.03 | NS |
| C 16:0 | 26.65 | 26.75 | 26.94 | 26.81 | 27.50 | 0.37 | $26.00^{A}$ | $27.86^{B}$ | 0.23 | NS |
| C 16:1 *n-7* | 2.46 | 2.43 | 2.41 | 2.27 | 2.64 | 0.011 | $2.30^{A}$ | $2.59^{B}$ | 0.07 | * |
| C 18:0 | 12.53 | 12.68 | 12.85 | 12.82 | 12.77 | 0.26 | $12.48^{a}$ | $12.98^{b}$ | 0.16 | NS |
| C 18:1 *n-9* | 38.77 | 37.51 | 39.47 | 38.64 | 39.84 | 0.74 | 38.39 | 39.30 | 0.46 | NS |
| C 18:2 *n-6* | $13.72^{ab}$ | $14.50^{b}$ | $12.76^{ab}$ | $13.72^{ab}$ | $11.72^{a}$ | 0.64 | $14.75^{B}$ | $11.82^{A}$ | 0.40 | NS |
| C a18:3 *n-6* | 0.10 | 0.11 | 0.10 | 0.10 | 0.10 | 0.007 | $0.12^{B}$ | $0.09^{A}$ | 0.004 | NS |
| C 18:3 *n-3* | $0.20^{ab}$ | $0.20^{ab}$ | $0.21^{b}$ | $0.20^{ab}$ | $0.18^{a}$ | 0.07 | $0.22^{A}$ | $0.19^{B}$ | 0.004 | NS |
| C 18:2 *c9t11* | 0.29 | 0.30 | 0.29 | 0.26 | 0.28 | 0.016 | $0.30^{b}$ | $0.27^{a}$ | 0.010 | NS |
| C 18:2 *t10c12* | 0.21 | 0.24 | 0.21 | 0.19 | 0.19 | 0.018 | 0.22 | 0.19 | 0.011 | NS |
| C 18:2 *c9c11* | 0.27 | 0.28 | 0.26 | 0.25 | 0.27 | 0.012 | $0.28^{B}$ | $0.25^{A}$ | 0.007 | * |
| C 18:2 *t9t11* | 0.55 | 0.59 | 0.56 | 0.56 | 0.56 | 0.019 | 0.56 | 0.57 | 0.012 | NS |
| C 20:0 | 0.14 | 0.15 | 0.15 | 0.14 | 0.13 | 0.006 | 0.14 | 0.15 | 0.003 | NS |
| C 20:4 *n-6* | 1.86 | 2.01 | 1.60 | 1.95 | 1.66 | 0.13 | $2.15^{B}$ | $1.48^{A}$ | 0.08 | NS |
| C 20:5 *n-3* | 0.09 | 0.09 | 0.09 | 0.10 | 0.09 | 0.007 | $0.10^{b}$ | $0.08^{a}$ | 0.004 | NS |
| SFA | 41.60 | 41.36 | 41.93 | 41.64 | 42.36 | 0.48 | $40.48^{A}$ | $43.07^{B}$ | 0.30 | NS |
| UFA | 58.63 | 58.39 | 58.06 | 58.35 | 57.63 | 0.48 | $59.51^{B}$ | $56.92^{A}$ | 0.30 | NS |
| MUFA | 41.24 | 39.95 | 41.89 | 40.92 | 42.49 | 0.78 | 40.49 | 41.90 | 0.49 | NS |
| PUFA | $17.38^{ab}$ | $18.43^{b}$ | $16.17^{ab}$ | $17.42^{ab}$ | $15.14^{a}$ | 0.80 | $18.81^{B}$ | $15.01^{A}$ | 0.50 | NS |
| PUFA/SFA | 0.42 | 0.44 | 0.39 | 0.42 | 0.36 | 0.02 | $0.46^{B}$ | $0.35^{A}$ | 0.01 | NS |
| PUFA *n-6*/ PUFA *n-3* | 45.5 | 46.3 | 40.9 | 44.8 | 40.4 | 1.81 | 44.4 | 42.8 | 1.14 | NS |
| Sum of CLA | 1.33 | 1.43 | 1.34 | 1.28 | 1.31 | 0.04 | $1.38^{b}$ | $1.29^{a}$ | 0.03 | NS |

*a, b* – $P<0.05$; *A, B* – $P<0.01$

[1] NS – $P>0.05$; * – $P<0.05$

which could improve meat tenderness and juiciness ($P<0.05$) (ENSER 1999). In addition, the lower content of *n-3* PUFA, mainly linolenic acid (C18:3) in group V, could affect the higher sensory score by a panel of evaluators (CAMERON, ENSER 1990). No effect of sex on the taste value of pork was found. In earlier studies, PIESZKA et al. (2004) found an improvement in pork aroma when adding 300 mg · kg$^{-1}$ α-tocopherol acetate to the diet, and the meat was characterized by a higher level of saturated acids, mainly stearic acid (C18:0) and palmitic acid (C16:0).

Table 4

Physico-chemical and sensory traits of the fatteners receiving CLA and different vitamin supplements

| Item | Groups | | | | | SEM | Sex | | SEM | Diet × sex interaction |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | | gilts | barrows | | |
| Dry matter, % | 26.2 | 26.1 | 26.6 | 26.1 | 27.0 | 0.31 | $26.1^{a}$ | $26.7^{b}$ | 0.20 | NS |
| Crude protein, % | 23.6 | 23.2 | 23.7 | 23.4 | 23.8 | 0.19 | 23.6 | 23.4 | 0.12 | NS |
| Crude fat, % | 2.07 | 2.39 | 2.35 | 2.02 | 2.73 | 0.20 | $1.96^{A}$ | $2.66^{B}$ | 0.13 | NS |
| $pH_{45}$ min. | 6.70 | 6.91 | 6.86 | 7.00 | 6.91 | 0.08 | 6.91 | 6.86 | 0.05 | NS |
| $pH_{24h}$ | 5.45 | 5.43 | 5.49 | 5.57 | 5.55 | 0.04 | $5.44^{A}$ | $5.55^{B}$ | 0.02 | NS |
| Water binding capacity, % | 32.4 | 31.8 | 28.6 | 29.1 | 31.9 | 1.19 | 29.8 | 31.7 | 0.75 | NS |
| Thermal losses, % | 32.2 | 33.4 | 32.0 | 32.0 | 32.9 | 0.98 | 32.8 | 32.2 | 0.62 | NS |
| Colour of meat: | | | | | | | | | | |
| L* | 54.2 | 53.9 | 54.8 | 54.5 | 54.5 | 0.56 | 54.3 | 54.5 | 0.35 | NS |
| a* | 15.3 | 15.2 | 14.8 | 14.6 | 15.2 | 0.29 | 14.9 | 15.2 | 0.18 | NS |
| b* | $6.8^{B}$ | $5.0^{A}$ | $5.2^{A}$ | $5.2^{A}$ | $5.3^{A}$ | 0.27 | 5.4 | 5.5 | 0.17 | NS |

For explanations see Table 3

Table 5

Effect of different vitamin supplements to the CLA-enriched diet on eating quality and dietetic value of pork meat

| Item | Groups | | | | | SEM | Sex | | SEM | Diet × sex interaction |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | | gilts | barrows | | |
| Tenderness, points | $4.73^{a}$ | $4.69^{a}$ | $4.91^{b}$ | $4.74^{a}$ | $4.88^{b}$ | 0.07 | 4.76 | 4.78 | 0.05 | NS |
| Juiciness, points | $4.71^{a}$ | $4.66^{a}$ | $4.84^{b}$ | $4.69^{a}$ | $4.79^{b}$ | 0.06 | 4.72 | 4.74 | 0.04 | NS |
| Flavour: | | | | | | | | | | |
| intensity, points | 4.79 | 4.77 | 4.87 | 4.79 | 4.87 | 0.07 | 4.77 | 4.82 | 0.04 | NS |
| quality, points | 4.81 | 4.78 | 4.88 | 4.86 | 4.88 | 0.06 | 4.82 | 4.86 | 0.03 | NS |
| Palatability: | | | | | | | | | | |
| intensity, points | 4.73 | 4.71 | 4.83 | 4.82 | 4.79 | 0.06 | 4.79 | 4.76 | 0.04 | NS |
| quality, points | 4.67 | 4.69 | 4.79 | 4.77 | 4.73 | 0.06 | 4.73 | 4.73 | 0.04 | NS |
| TBARS, mg · kg$^{-1}$ | $0.449^{ab}$ | $0.507^{b}$ | $0.471^{ab}$ | $0.411^{a}$ | $0.424^{a}$ | 0.029 | $0.428^{a}$ | $0.476^{b}$ | 0.018 | NS |
| α-tocopherol, μg · g$^{-1}$ | $1.23^{A}$ | $1.13^{A}$ | $1.76^{B}$ | $2.88^{C}$ | $3.06^{C}$ | 0.06 | 2.03 | 1.99 | 0.04 | NS |

For explanations see Table 3

## Conclusions

1. It is concluded that the use of $\beta$-carotene, vitamin E and vitamin C supplements in complete diets enriched in conjugated linoleic acid in the second period of pig fattening altered the composition of meat fatty acids by reducing its PUFA level.

2. Differences were found in the composition of fatty acids between gilts and barrows, possibly indicative of differences in fatty acid metabolism depending on sex.

3. The supplement of vitamin E and a mixture of $\beta$-carotene, vitamins E and C limited the lipid oxidation processes by increasing the oxidative stability of meat lipids.

4. There were improvements in the sensory traits of meat, including meat tenderness and juiciness, especially when a mixture of vitamins (gr. V) and a higher level of vitamin C (gr. III) were used.

5. Higher doses of vitamin E in the diet resulted in a higher deposition of vitamin E in meat in groups IV and V.

Translated by Jerzy Pilawski

Accepted for print 04.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 229-237, Y. 2006

# ILEAL DIGESTIBILITY OF ASH AND MINERALS IN DIETS USED IN POLAR FOX NUTRITION DURING THE REPRODUCTION PERIOD*

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K e y w o r d s: polar fox, digestibility, ash, minerals.

A b s t r a c t

The aim of the present research was to determine the apparent ileal digestibility of ash and minerals in diets used in feeding of polar foxes during the reproduction preparation period, reproduction and pregnancy (A1 and B1 diets) and lactation (A2 and B2 diets) on two domestic farms (A and B), differing in reproduction results. The diets were based on offal of animal origin and cereals. The diets on farm B showed a deficit of the content of calcium, phosphorus and magnesium. Diets from farm A demonstrated a 2.5 to 3.4-fold higher crude ash content and a much greater content of calcium, phosphorus and magnesium, as compared with farm B diets. The digestibility experiments carried out on foxes with "end-to-end" ileorectal anastomoses showed a higher apparent ileal digestibility of ash, calcium, phosphorus, magnesium, sodium and potassium in diets B. Too high content of crude ash and calcium in diets used in feeding polar foxes on farm A decreased significantly the ileal digestibility of the macroelements.

## STRAWNOŚĆ JELITOWA POPIOŁU I SKŁADNIKÓW MINERALNYCH W DIETACH STOSOWANYCH W ŻYWIENIU LISÓW POLARNYCH W OKRESIE REPRODUKCYJNYM

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* Supported by the Polish State Committee for Scientific Research (KBN), Project No. 5PO6E02618

Abstrakt

Celem badań było określenie pozornej strawności jelitowej popiołu i składników mineralnych w dietach stosowanych w żywieniu lisów polarnych w okresie przygotowania do rozrodu, rozrodu i ciąży (diety A1 i B1) oraz laktacji (diety A2 i B2) na dwóch, różniących się wynikami w rozrodzie, fermach krajowych (A i B). Diety bazowały na odpadach pochodzenia zwierzęcego i zbożach. Diety stosowane w żywieniu lisów na fermie B były niedoborowe pod względem zawartości wapnia, fosforu i magnezu. Diety z fermy A zawierały 2,5 – 3,4 krotnie więcej popiołu surowego oraz miały znacznie wyższą koncentrację wapnia, fosforu i magnezu w porównaniu z dietami z fermy B. Doświadczenia strawnościowe przeprowadzone na lisach z zespoleniami jelitowo-rektalnymi „end to end" wykazały wyższą pozorną strawność jelitową popiołu, wapnia, fosforu, magnezu, sodu i potasu w dietach pochodzących z fermy B. Zbyt wysoka koncentracja popiołu surowego i wapnia w dietach stosowanych w żywieniu lisów polarnych na fermie A w stopniu istotnym obniżyła pozorną strawność jelitową badanych makroelementów.

## Introduction

The main component of the diets used in feeding carnivorous fur animals are feeds of animal origin containing a rich composition of mineral salts. The composition of ingredients and an adequate balancing of the ration play an essential role in the availability of bioelements. Their absorption depends on their quantitative ratios between respective minerals in the diet, the degree of dissociation, deficit or surplus in the feed and the presence of factors stimulating or inhibiting absorption (DELZENNE et al. 1995, BRONNER, PANSU 1998, HOWARD et al. 1998, LIU et al. 2000, DOBENECKER 2002).

Traditionally the digestibility of nutrients in carnivorous animals is determined along the gastrointestinal tract. This method, however, does not cover fermentation processes in large intestine caused by bacterial enzymes (WOLF et al. 1998, HILL et al. 2001) and endogenous secretion of minerals (LARSEN, SANDSTROM 1993, DINTZIS et al. 1995). Experiments on mink demonstrated that, despite a short digesta passage time and a considerably lower bacterial activity, as compared with other species of monogastric animals (WILLIAMS et al. 1998), in carnivorous animals the large intestine shows processes of microbiological degradation of carbohydrates (BØRSTING et al. 1995) and endogenous secretion (LAERKE et al. 2004). The intestinal method of estimating the apparent digestibility, as compared with the fecal analysis method, is more precise while evaluating the absorption of minerals ingested with feed.

The aim of the present research was to determine the apparent ileal digestibility of ash and minerals in diets used in feeding of reproductive polar foxes during the reproduction and lactation periods on two domestic farms A and B.

# Material and Methods

## Experimental animals

The digestibility experiments included five, from the same litter, male polar foxes of a similar body weight (6.18 ± 0.15 kg). In those animals "end-to-end" ileorectal anastomoses were made surgically following the method developed in foxes by SZYMECZKO (2001). Foxes were premedicated with tranquilizer (0.08 ml · $kg^{-1}$) and atropine (0.03 mg · $kg^{-1}$). General anaesthesia was induced with ketamine (10 mg · $kg^{-1}$) and foxes were placed on back side for the surgical procedure. The abdominal cavity was opened and the marked segment of large intestine for resection was located. In the mesentery anastomosed to it – successive blood vessels were ligated and then the large intestine was resected. Free sections of ileum and rectum were connected with two continuous sutures with the "end-to-end" technique. Having completed the anastomoses, the ends of the free mesentery and body layers were sutured, the area around the wound was washed and disinfected. Over five days following the operation, antibiotic and tranquilizers, antihaemorrhage and antiswelling medications were administered to the foxes with dosages recommended by the producers. Two days after surgery, liquids were supplemented, administering subcutaneous physiological saline solution and glucose. During the following days the foxes were fed with semi-liquid diet with an increasing concentration of nutrients and from the tenth day, the animals were given an entire daily feed ration, with higher, as compared with the requirements, metabolizable energy (ME) concentration (NRC 1982) . After the veterinary examination in the third week after the operation, the foxes were considered clinically healthy. They were housed separately in metabolic cages, in a controlled temperature of the room. The use and handling of animals for this experiment were approved by the Local Ethical Committee in Bydgoszcz.

## Experimental diets

The research involved two reproduction polar fox farms. Farm A demonstrated the best (8.1 kits per female), and on farm B – the worst (1 kit per female) reproductive results. There were prepared two batches of complete diets (Table 1) used in females nutrition of parents stock on each of the farms, in two feeding periods, which covered the period of reproduction and pregnancy (01.12.-15.05. : A1 and B1 diets) and lactation (15.05.-15.07. : A2 and B2 diets). The diets used in digestibility experiments were homogenized and mixed with 5 g · $kg^{-1}$ of $Cr_2O_3$ (SZYMECZKO, BURLIKOWSKA 1996).

Table 1
Composition of diets fed to polar foxes on farm A (diet A1 and A2) and B (diet B1 and B2) during the reproduction period, $g \cdot kg^{-1}$ fresh matter

| Feeding period | 01.12. – 15.05. | | 15.05. – 15.07. | |
|---|---|---|---|---|
| | diets | | | |
| | A1 | B1 | A2 | B2 |
| Beef offal | 165 | 600 | – | 600 |
| Poultry offal | 165 | – | 214 | – |
| Fish offal | 496 | – | 428 | – |
| Fish meal | 33 | – | 43 | – |
| Beef meat | – | – | 143 | – |
| Beef liver and spleen | – | 100 | – | 50 |
| Milk powder | 17 | – | 29 | – |
| Excruded cereals | 124 | – | 143 | – |
| Precooked barley | – | 300 | – | 350 |
| Vit.–min. mixture (Polfamix LN)* | – | 1 | – | 1 |
| Vit.–min. mixture (Guyofox Plus)** | 1 | – | 1 | – |
| Iron preparation (Aniron)*** | 0.5 | – | 0.5 | – |
| Iron preparation (Taiga Fur)**** | – | 0.5 | – | 0.5 |

* Concentration per 1 g: vit. A 3500 j.m; D3 500 j.m; E 28 mg; $K_3$ 0.2 mg; $B_1$ 1.5 mg; $B_2$ 2.8 mg; $B_6$ 2.8 mg; $B_{12}$ 0.02 mg; H 0.2 mg; folic acid 0.2 mg; PP 10 mg; calcium panthotenate 7 mg; methonine 200 mg; choline chloride 50 mg; Fe 17 mg; Zn 2 mg; Cu 1 mg; Mn 1 mg; Co 1 mg; J 0.1 mg; Se 0.6 mg;

** Concentration per 1 g: vit. A 3000 j.m.; $D_3$ 300 j.m.; E 50 mg; K 0.5 mg; $B_1$ 22 mg; $B_2$ 3 mg; $B_6$ 3 mg; $B_{12}$ 0.02 mg; H 0.03 mg; folic acid 0.3 mg; PP 5.0 mg; calcium panthotenate 3.15 mg; choline chloride 50 mg; Mn 7.5 mg; Zn 10.0 mg; organic Fe 20.0 mg; non-organic Fe 4.8 mg; Se 0.058 mg; Cu 1.25 mg; Co 0.01 mg;

*** Concentration in 1 ml of preparation: liver extract 543 mg; ferrous sulphate 75 mg; managnese sulphate 3.5 mg; cupric sulphate 3.5 mg; cobalt chloride 1.5 mg;

**** Concentration in 1 ml of preparation: ferrous gluconate 205 mg; ferric sulphate 97.5 mg; cupric sulphate 5.9 mg; cobalt sulphate 1.76 mg.

## Digestibility experiments

The digestibility experiments were carried out on foxes with ileorectal anastomoses to determine the apparent ileal digestibility of crude ash and minerals in each of the 4 farm diets. Foxes were fed once a day at 8 a.m., at the approximate maintenance level (90 kcal ME $kg^{-1}$ body weight; NRC 1982) and had free access to water. Each diet was fed for 8 days. Testing each diet was divided into two 4-day periods; preliminary, constituting a period of animal adaptation to the diets investigated and the period of a continuous 96-hour ileal digesta collection over the last four days. Having completed the collections, the digesta was stored at -25°C, freeze-dried, and grinded before being analysed.

## Chemical analysis

The content of nutrients in diet and digesta samples was determined with standard methods. The content of minerals in diets and ileal digesta were determined after a prior samples mineralization (20 min) in the nitric and perchloric acids medium at 230°C using the Milestone Ethos Plus Microwave Labstation. In the samples the contents of calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and chromium (Cr) were determined using the atomic absorption spectrophotometer 969 by Unicam, applying adequate wavelengths: Ca – 422.7 nm, Mg – 285.2 nm, Na – 589 nm, K – 766.5 nm and Cr – 357.9 nm. The content of phosphorus (P) was determined with the colorimetric method at the wavelength of 420 nm (SAPEK, SAPEK 1997). The apparent digestibility of crude ash and minerals was defined with the indicator method (BURACZEWSKI, ZIOŁECKA 1991).

## Statistical analysis

The results obtained were verified with statistical analysis with Student's *t*-test for dependent samples using Statistica software (STANISZ 1998). The level of significance was set at $P<0.05$.

# Results and Discussion

The chemical composition and the content of minerals in the dry matter of diets and the results of digestibility experiments are given in Table 2. Diets A, with a varied ingredient composition, demonstrated a higher content of protein, carbohydrates and crude ash. Diets used in polar fox nutrition on farm B, based on beef offal and ground barley only, however, contained a two-fold greater amount of crude fat in dry matter. The greatest differences concerned the concentration of crude ash whose content in farm A diets was 2.5 to 3.4-fold higher than in B farm diets and over the lactation (15.05. – 15.07.) exceeded the nutrient requirements recommended for this animal species (NRC 1982). A high content of ash in the diets fed on farm A corresponds to a higher content of calcium, phosphorus and magnesium, accompanied by a relatively balanced amount of sodium and potassium. As compared with the polar fox feeding recommendations for the reproduction period, diets B showed calcium, phosphorus and magnesium deficits, while rations used in the nutrition of females on farm A showed an excessive amount of calcium, 4 – 8-fold exceeding the permissible values,

accompanied by an adequate content of phosphorus and magnesium in the dry matter of the diet (NRC 1982, SŁAWOŃ 1987, JAROSZ 1994).

Table 2
Chemical content ($g \cdot kg^{-1}$) and apparent ileal digestibility of crude ash and minerals (%) in diets fed to polar foxes on farm A (diet A1 and A2) and B (diet B1 and B2) during the reproduction period

| Feeding period | 01.12. – 15.05. | | | 15.05. – 15.07. | | |
|---|---|---|---|---|---|---|
| | diets | | | | | |
| | A1 | B1 | P< | A2 | B2 | P< |
| Dry matter, $g \cdot kg^{-1}$ | 967.1 | 969.4 | | 976.8 | 972.1 | |
| | $g \cdot kg^{-1}$ dry matter | | | | | |
| Crude protein | 416.9 | 390.6 | | 436.3 | 374.4 | |
| Crude fat | 147.5 | 315.4 | | 164.6 | 361.0 | |
| Carbohydrates* | 266.3 | 191.1 | | 161.2 | 148.5 | |
| Crude ash | 119.5 | 48.3 | | 201.1 | 59.3 | |
| Calcium | 27.3 | 2.0 | | 46.6 | 2.9 | |
| Phosphorus | 7.5 | 2.0 | | 9.6 | 2.6 | |
| Magnesium | 1.6 | 0.9 | | 1.9 | 1.0 | |
| Sodium | 4.2 | 4.7 | | 5.2 | 4.3 | |
| Potassium | 6.2 | 6.0 | | 6.4 | 6.0 | |
| | Digestibility, %** | | | | | |
| Ash | 14.2±3.3 | 35.3±1.1 | 0.05 | 11.6±4.0 | 22.5±4.2 | 0.05 |
| Calcium | 22.5±1.2 | 58.8±7.2 | 0.05 | 22.5±2.3 | 77.0±1.4 | 0.05 |
| Phosphorus | 47.5±3.7 | 77.5±2.9 | 0.05 | 40.6±3.2 | 45.2±8.3 | NS*** |
| Magnesium | -12.9±8.1 | 42.1±3.1 | 0.05 | -7.0±3.5 | 37.2±4.8 | 0.05 |
| Sodium | 62.2±3.9 | 72.4±1.4 | 0.05 | 53.5±5.8 | 78.9±5.3 | 0.05 |
| Potassium | 78.9±6.2 | 86.2±1.9 | 0.05 | 82.8±1.8 | 90.8±1.1 | 0.05 |

* by difference
** digestibility results expressed as means ± SD
*** non significant

The digestibility results showed a higher apparent ileal digestibility of ash and the macroelements investigated from diets used in polar fox nutrition on farm B in both feeding periods. As for most minerals studied (except for the digestibility of phosphorus in the second feeding period), the differences were statistically significant ($P<0.05$).

A high content of ash in feed decreases the digestibility of organic substance, protein and fat, deteriorating the concentration of metabolizable energy in the diets used in feeding carnivorous fur animals (SŁAWOŃ 1987, ROUVINEN, KIISKINEN 1991). The results of the present digestibility experiments demonstrate that an excessively high content of crude ash decrease the ileal absorption of minerals. Apparent ileal digestibility of ash and the macroelements studied from diets A in which the amount of ash fall within the maximum permissible values (A1 diet) or exceeded them (A2 diet), was significantly lower

($P<0.05$) than in farm B diets, which coincides with the results of earlier reports of the present authors into the ileal digestibility of minerals in rations for polar foxes (SZYMECZKO et al. 2005). Experiments with mink (ROUVINEN, KIISKINEN 1991) and blue foxes (VALAJA et al 2000) also demonstrated that an increase in the amount of ash in diets decreases the apparent digestibility of this nutrient and the absorption of calcium and phosphorus in the whole digestive tract of these animals. It shall be noted that the results of research on the crude ash digestibility reported in literature in foxes shows a high variation. AHLSTRØM and SKREDE (1998) and AHLSTRØM et al. (2003), investigating the total digestibility of nutrients in diets for carnivorous animals, recorded apparent ash digestibility coefficient values ranging from 34 to 58%, in which the optimal concentration of this nutrient in the feed ranged from 6 to 7%.

The degree of utilization of calcium and phosphorus depends also on the ratio of these macroelements. An optimal Ca : P ratio in fox diets ranges from 1:1 to 1.7:1, and increasing this range may deteriorate the growth of bone, even when the diet contains large amounts of vitamin D (NRC 1982). DOBENECKER (2002), SCHOENMAKERS et al. (1999), in the experiments with dogs, demonstrated that an excessive amount of calcium at an increased ratio of Ca : P leads to a significant decrease in the absorption of these elements and disturbances in their metabolism. In dogs which were fed deficit diet, covering only 15% of the calcium requirements, the absorption of both calcium and phosphorus was significantly better than in dogs fed with the feed 3-fold exceeding the permissible values (DOBENECKER 2002). The highest apparent digestibility of calcium (19.9%) and phosphorus (50.9%) was identified in the alimentary canal of growing foxes fed low-ash-content diets (47 $g \cdot kg^{-1}$ of dry matter) and at the optimal ratio of Ca : P (1.4:1) (VALAJA et al. 2000). This relationship coincides with the results of the present research in which there was noted a significantly higher ($P<0.05$) apparent digestibility of calcium and phosphorus from farm B diets of a low concentration of calcium and phosphorus, however with a balanced Ca : P ratio (B1-1:1; B2-1:1.1).

The present research recorded negative values of coefficients of apparent ileal digestibility of magnesium in foxes fed diets A, which can be caused by a few factors. It is commonly known that the absorption of magnesium is lowered by the excess of calcium. At the Ca : Mg ratio in feed more than 5, there is a greater risk of magnesium deficit in the body, partially due to a lowered absorption but also as a result of an increased amount of magnesium excreted by kidneys (VASKONEN 2003). Cat experiments demonstrated that an increased content of calcium in the feed decreased the absorption of magnesium significantly, inducing symptoms of an intensified hypomagnesemia (PASTOOR et al. 1994, HOWARD et al. 1998). According to BRINK et al. (1992), a negative effect of

calcium on the absorption of magnesium in rats could have been due to a formation of insoluble calcium-magnesium-phosphate complexes in the lumen of the intestine, decreasing the solubility of minerals, and thus deteriorating their bioavailability. Additionally magnesium, similarly as calcium, can form, with free fatty acids, soaps insoluble and non-absorbable from digesta (SEELIG 1989, ROUVINEN, KIISKINEN 1991, VASKONEN 2003).

Besides the results of the present investigations, there seem to be no reports on ileal digestibility of minerals in polar foxes. In the experiment which involved cannulated dogs fed 100% beef-protein diet, HILL et al. (2001) observed a definitely lower apparent ileal digestibility of phosphorus (25.9%), magnesium (12.4%) and sodium (36.8%) and higher – of potassium (92.9%), as compared with the digestibility of these minerals from diets B1 and B2 based on beef offal.

## Conclusions

The present results demonstrate that an excessively high content of crude ash and calcium in diets applied in polar fox feeding decreases the apparent ileal digestibility of ash, calcium, phosphorus, magnesium, sodium and potassium significantly.

Translated by authors

Accepted for print 12.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 239-248, Y. 2006

# CORRELATIONS BETWEEN SOME BLOOD CHEMICAL INDICES AND MEAT QUALITY IN DUCKS

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Key words: ducks, blood chemical indices, meat quality, coefficients of phenotypic correlation.

Abstract

The experiment was performed on 100 (50 ♂ and 50 ♀) sexed ducklings of the A-44 strain. The birds were kept indoor, in pens (males and females separately), on rye straw litter, and fed a starter diet (from one day to three weeks of age) containing 19.70% of protein and 12.40 MJ of metabolizable energy, followed by a grower diet (from four weeks of age to the end of the experimental period – seven weeks) containing 16.01% of protein and 12.23 MJ of metabolizable energy. At the completion of the rearing period 20 males and 20 females were selected, by stratified sampling, for slaughter and slaughter analysis. The right breast muscles were dissected from carcasses chilled at 4°C for 18 hours, to determine the physicochemical and sensory properties of meat. Blood was taken from the jugular vein during slaughter. The serum concentrations of glucose, total protein, calcium, total cholesterol, total lipids and cholinoesterase (ChE) were determined using a biochemical analyzer, EPOLL-20 BIO.

In the majority of cases no significant correlations were found between the serum levels of the above chemical indices and meat quality parameters. A significant correlation was observed between total lipids and the content of fat and dry matter in breast muscles of both males and females (r from 0.487 to 0.745). Calcium content was highly correlated with meat pH ($r$ ♂ = – 0.818; $r$ ♀ = – 0.714), and glucose content – with the aroma, tenderness and taste of meat in males ($r$ from 0.664 to 0.902). The activity of cholinoesterase was closely correlated ($r$ from 0.455 to 0.940) with the levels of fat, dry matter and ash in breast muscles of both males and females.

## ZWIĄZKI MIĘDZY NIEKTÓRYMI WSKAŹNIKAMI CHEMICZNYMI KRWI A JAKOŚCIĄ MIĘSA KACZEK

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Abstrakt

Materiał do badań stanowiło 100 (50 ♂ i 50 ♀) seksowanych piskląt kaczek rodu A-44. Ptaki odchowywano w pomieszczeniu zamkniętym, w kojcach (oddzielnie samce i samice), na ściółce ze słomy żytniej. Kaczki do 3. tyg. życia żywiono mieszanką zawierającą 19,70% białka i 12,40 MJ energii metabolicznej, a od 4. tyg. do końca odchowu (7 tyg.) – mieszanką o zawartości 16,01% białka i 12,23 MJ energii metabolicznej. Po zakończeniu odchowu wybrano po 20 kaczorów i kaczek do uboju i oceny rzeźnej, stosując schemat losowania warstwowego. Z ich tuszek, po 18 h chłodzenia w temp. 4°C, wydzielano prawy mięsień piersiowy do oceny fizyczno-chemicznej i sensorycznej. Krew do badań pobierano z żyły jarzmowej podczas uboju ptaków. W surowicy krwi oznaczono zawartość: glukozy, białka ogólnego, wapnia, cholesterolu całkowitego, lipidów całkowitych, glukozy oraz wapnia i poziom cholinoesterazy (ChE). Oznaczenia wykonano za pomocą analizatora biochemicznego EPOLL – 20 BIO.

Poziom badanych wskaźników chemicznych w surowicy krwi nie wykazywał – w większości przypadków – istotnej współzależności z analizowanymi cechami jakości mięsa kaczek. Znaczną korelację stwierdzono między poziomem lipidów całkowitych a zawartością tłuszczu i suchej masy w mięśniach piersiowych ptaków obojga płci ($r$ 0,487 – 0,745). Zawartość wapnia okazała się wysoko skorelowana z pH mięśni ($r$ ♂ = – 0,818; $r$ ♀ =– 0,714), a poziom glukozy z zapachem, kruchością i smakowitością mięsa kaczorów ($r$ 0,664 – 0,902). Aktywność cholinoesterazy wykazywała znaczną współzależność ($r$ 0,455 – 0,940) z zawartością tłuszczu, suchej masy i popiołu w mięśniach piersiowych zarówno kaczorów, jak i kaczek.

## Introduction

The properties of particular chemical components of poultry meat, as well as the interdependences between them, decide about its nutritive and dietary value, palatability and technological quality (NIEWIAROWICZ 1993). In fowl the weight of muscles and – to a high degree – their quality can be shaped by the breeder (FARHAT, CHAVEZ 2000, NIEWIAROWICZ 1993). However, selection towards a fast growth rate and an increase in breast muscle weight may have a negative effect on the technological properties of poultry meat (BAEZA et al. 1997a, 2002). That is why specialists constantly search for reliable meat quality indicators that could be used for e.g. duck selection (KSIĄŻKIEWICZ et al. 1994, WAWRO et al. 1999b, 2001a,b). According to KSIĄŻKIEWICZ et al. (1995), testing the possibility of using blood biochemical indices as markers of performance traits during duck selection is an important research problem.

WAWRO et al. (2000a) demonstrated that the activity of alanine aminotransferase (ALAT) was closely correlated with the protein content of breast muscles in Pekin ducks. A significant correlation, both in males and females, was also observed between aspartate aminotransferase (AST) and the pH of muscles. The activity of alkaline phosphatase (AP) was found to be significantly correlated with the sensory properties of breast muscles in males. In Muscovy females the serum levels of ALAT and AP may be indicators of the fat content of muscles (WAWRO et al. 2001a).

FARHAT and CHAVEZ (2001) analyzed the relationships between the serum levels of glucose, triglycerides, cholesterol and uric acid and meatiness in ducks, and reported that the values of these blood indices were affected by the diet and breast muscle thickness. The interdependences between the activity of some enzymes in the blood and the proportions of carcass tissue components may be also related to the sex of birds (KSIĄŻKIEWICZ et al. 1995).

The aim of the present study was to analyze the correlations between some blood chemical indices and meat quality in Pekin ducks.

## Materials and Methods

The experiment was performed on 100 (50 ♂ and 50 ♀) sexed ducklings of the A-44 strain, purchased on the Waterfowl Breeding Farm Dworzyska. The birds were kept indoor, in pens (males and females separately), on rye straw litter, and fed a starter diet (from one day to three weeks of age) containing 19.70% of protein and 12.40 MJ of metabolizable energy, followed by a grower diet (from four weeks of age to the end of the experimental period – seven weeks) containing 16.01% of protein and 12.23 MJ of metabolizable energy.

At the completion of the rearing period the birds were placed in ascending order by body weight, and then 20 males and 20 females were selected for slaughter and slaughter analysis, using stratified sampling to ensure sample representativeness and randomness. The ducks were weighed at two-week intervals. The birds were fasted for about 12 hours, weighed, sacrificed, plucked and eviscerated, in accordance with the Polish Standards PN-84, A-66520 and PN-A-86520 of 1998, "Poultry Carcasses". The right breast muscles were dissected from carcasses chilled at 4°C for 18 hours, to determine the physicochemical and sensory properties of meat. The basic chemical composition of samples was determined by universally accepted methods, and meat acidity, color lightness and water-holding capacity – by the methods described by SOBINA et al. (1989). The sensory properties of breast muscles, i.e. aroma, tenderness, juiciness and taste, were evaluated as described by BARYŁKO-PIKIELNA (1975).

Blood was taken from the jugular vein during slaughter. The serum concentrations of glucose, total protein, calcium, total cholesterol, total lipids and cholinoesterase (ChE) were determined using a biochemical analyzer, EPOLL-20 BIO and sets provided by Bio Systems Reagents Instruments, as recommended by the manufacturer.

The statistical analysis included:

– calculation of arithmetic means ($\bar{x}$) and coefficients of variation;

– determination of the significance of differences between means of traits in males and females (analysis of variance; test $F$);
– calculation of the coefficients of phenotypic correlations (simple) between blood chemical indices and the physicochemical and sensory properties of breast muscles.

## Results and Discussion

The serum levels of the chemical indices examined were comparable in males and females (Table 1). The only exception was total cholesterol ($\bar{x}$ ♂ = 5.47, $\bar{x}$ ♀ = 4.58 mmol · dm$^{-3}$). The serum concentrations of glucose, protein and calcium were 109 mg · dl$^{-1}$, 36.0 g · dl$^{-1}$ and 2.30 mmol · dm$^{-3}$ respectively. The activity of the enzyme cholinoesterase (ChE) was also similar in males and females – $\bar{x}$ = 1.45 JU · dm$^{-3}$ and $\bar{x}$ = 1.31 JU · dm$^{-3}$ respectively.

Table 1

Arithmetic means ($\bar{x}$) and coefficients of variation ($v$) of the blood indices in ducks

| Specification | | Statistical measures | Sex | |
|---|---|---|---|---|
| | | | ♂ | ♀ |
| Glucose | (mg · dl$^{-1}$) | $\bar{x}$ | 109.80 | 107.50 |
| | | $v$ | 16.20 | 15.11 |
| Protein | (g · dl$^{-1}$) | $\bar{x}$ | 35.11 | 36.87 |
| | | $v$ | 7.89 | 9.17 |
| Cholesterol | (mmol · dm$^{-3}$) | $\bar{x}$ | 5.47$^{xx}$ | 4.61 |
| | | $v$ | 15.01 | 11.43 |
| Total lipids | (mg · dl$^{-1}$) | $\bar{x}$ | 596.40 | 596.50 |
| | | $v$ | 29.52 | 20.42 |
| Calcium | (mmol · dm$^{-3}$) | $\bar{x}$ | 2.37 | 2.24 |
| | | $v$ | 14.29 | 9.72 |
| Cholinoesterase | (IU · dm$^{-3}$) | $\bar{x}$ | 1.45 | 1.31 |
| | | $v$ | 23.72 | 23.17 |

$^{xx}$ – significant differences at $\alpha$ = 0.01

The concentrations of glucose, protein, cholesterol, total lipids and calcium, observed in our study, are slightly different than reported by other authors (ARTONI et al. 1989, CAIN et al. 1993, KRASNODĘBSKA-DEPTA et al. 1997a, KSIĄŻKIEWICZ et al. 1993, PASCALOM-PEKELNICZKY et al. 1994, PRUSZYŃSKA et al. 2000, WAWRO et al. 2000b). For instance, ARTONI et al. (1989) demonstrated that blood glucose levels may range from 114.5 mg · dl$^{-1}$ in ducks aged 23 weeks to 164.8 mg · dl$^{-1}$ in ducks aged 20 weeks. CAIN et al. (1993) and PASCALOM-PEKELNICZKY et al. (1994) pointed out to slight, age-related changes in the serum level of total protein, from about 3.8 g · dl$^{-1}$ in ducks aged four weeks to

4.5 g · dl$^{-1}$ in ducks aged twelve weeks. PRUSZYŃSKA et al. (2000) found that the serum level of cholesterol in ducks of the A-44 strain decreased with age, from 19.22 mmol · dm$^{-3}$ in day-old ducklings to 5.18 and 4.76 mmol · dm$^{-3}$ in birds aged three and seven weeks respectively. According to the above authors, the serum concentrations of cholesterol, triglycerides and phospholipids in Pekin ducks are dependent not only on sex, but also on strain. KRASNODĘBSKA-DEPTA et al. (1997 a) observed an age-related increase in the serum level of calcium in interspecific hybrids (Muscovy ♂ x A-44 ♀ ), from 1.77 ♂ and 1.65 ♀ mmol · dm$^{-3}$ at nine weeks of age to 2.35 ♂ and 2.50 ♀ mmol · dm$^{-3}$ at twelve weeks of age. Skeleton growth usually ends at this age (WILKIEWICZ-WAWRO et al. 2005), which may be reflected by lower demand for blood-transported calcium in ducks.

The serum levels of the chemical indices analyzed are considerably affected by the feeding system (KRASNODĘBSKA-DEPTA et al. 1997b, STURKIE 1970) as well as by age, sex, genus or even strain of ducks (ARTONI et al. 1989, CAIN et al. 1993, KRASNODĘBSKA-DEPTA et al. 1997a, b, PRUSZYŃSKA et al. 2000, WAWRO et al. 2000b). A low-protein diet may reduce the serum levels of total protein and – indirectly – of glucose, whereas as fat-rich diet can increase the serum concentrations of cholesterol and lipids, and indirectly decrease calcium content (KRASNODĘBSKA-DEPTA et al. 1997b).

The chemical composition as well as physicochemical and sensory properties of poultry meat have a significant effect on its technological suitability and eating quality. A particular role is played by muscle proteins, which are usually divided into three groups: sarcoplasmic proteins, myofibrillar proteins and connective tissue proteins. In the analysis of the chemical composition of meat these three groups of proteins are collectively referred to as total protein.

The breast muscles of ducks (Table 2) contained average amounts of protein ($\bar{x}$ approx. 19%), fat ($\bar{x}$ approx. 1.0%) and ash ($\bar{x}$ = 1.2%), as compared with reference data (SOBINA et al. 1987, 1989, SZEREMETA et al. 2002, WILKIEWICZ-WAWRO 1994). Particular attention should be paid to considerable free water drip from the muscles ($\bar{x}$ > 7.4 cm$^2$). This is consistent with the results obtained by SOBINA et al. (1987) and WILKIEWICZ-WAWRO et al. (2000), who recorded a lower water-holding capacity in meat from young ducks (to seven weeks of age), in comparison with older ones (eight weeks of age and above). Older ducks had a lower water content and a higher total protein content of muscles, as well as lower free water drip and better hydrophilic properties of muscular tissue.

The eating quality of meat depends also on aroma, tenderness, juiciness and palatability. The scores for these attributes of breast muscles (scale from 1 to 5 points) were high – above 4.7 points. This indicates good eating quality of duck meat, and confirms the results of previous studies conducted on Mullards (WILKIEWICZ-WAWRO 1994), Pekin and Muscovy ducks (WAWRO et al. 2004).

Table 2

Chemical composition and physicochemical and sensory properties of breast muscles

| Specification | Unit | Sex | | | |
|---|---|---|---|---|---|
| | | ♂ | | ♀ | |
| | | $\bar{x}$ | $v$ | $\bar{x}$ | $v$ |
| Protein | % | 18.90 | 4.05 | 19.08 | 3.50 |
| Fat | % | 0.90 | 21.02 | 1.10 | 27.84 |
| Dry matter | % | 22.23 | 2.33 | 23.11$^{x}$ | 1.96 |
| Crude ash | % | 1.20 | 5.60 | 1.22 | 3.31 |
| Water-holding capacity | $cm^2$ | 8.22$^{x}$ | 13.67 | 7.42 | 17.04 |
| pH | | 5.80 | 1.05 | 5.82 | 1.24 |
| Color lightness | % | 13.58 | 17.32 | 13.75 | 15.85 |
| Aroma: | | | | | |
| intensity | pnt | 4.75 | 5.52 | 4.83 | 5.13 |
| desirability | pnt | 4.74 | 5.52 | 4.82 | 5.14 |
| Tenderness | pnt | 4.71 | 7.10 | 4.79 | 6.98 |
| Juiciness | pnt | 4.88 | 6.93 | 4.78 | 6.78 |
| Taste: | | | | | |
| intensity | pnt | 4.75 | 7.10 | 4.83 | 6.74 |
| desirability | pnt | 4.88 | 6.38 | 4.83 | 6.74 |

$^{x}$ – significant differences at $\alpha = 0.05$

In the majority of cases no significant correlations were found between the serum levels of the above chemical indices and meat quality parameters (Tables 3-5). It should be noted that the relationships between the traits examined are often observed within one sex group only.

Table 3

Coefficients of correlation ($r$) between the blood indices and chemical properties of meat

| Indices | | Sex | Content in breast muscles | | | |
|---|---|---|---|---|---|---|
| | | | protein | fat | dry matter | crude ash |
| Glucose | $(mg \cdot dl^{-1})$ | ♂ | 0.592 | 0,336 | 0.431 | 0.228 |
| | | ♀ | 0.013 | -0.182 | -0.411 | -0.516 |
| Protein | $(g \cdot dl^{-1})$ | ♂ | 0.238 | 0.488 | 0.222 | -0.312 |
| | | ♀ | 0.223 | 0.099 | 0.019 | 0.063 |
| Cholesterol | $(mmol \cdot dm^{-3})$ | ♂ | 0.754 | 0.560 | 0.449 | 0.447 |
| | | ♀ | 0.396 | -0.105 | 0.113 | 0.445 |
| Lipids | $(mmol \cdot dm^{-3})$ | ♂ | 0.551 | 0.312 | 0.612 | 0.367 |
| | | ♀ | -0.214 | 0.354 | 0.51 | 0.140 |
| Calcium | $(mmol \cdot dm^{-3})$ | ♂ | 0.353 | 0.487 | 0.667 | 0.095 |
| | | ♀ | -0.678 | -0.072 | -0.001 | -0.700 |
| Cholinoesterase | $(IU \cdot dm^{-3})$ | ♂ | 0.377 | 0.537 | 0.940 | 0.714 |
| | | ♀ | 0.351 | 0.625 | 0.547 | 0.455 |

Critical values of ($r$) for: $\alpha \geq 0.05 - 0.444$; $\alpha \geq 0.01 - 0.561$

Table 4

Coefficients of correlation ($r$) between the blood indices, water-holding capacity, pH and color of meat

| Blood indices | | Sex | Properties of breast muscle | | |
|---|---|---|---|---|---|
| | | | water-holding capacity | pH | color lightness |
| Glucose | ($mg \cdot dl^{-1}$) | ♂ | -0.150 | -0.501 | -0.475 |
| | | ♀ | 0.385 | -0.748 | 0.276 |
| Protein | ($g \cdot dl^{-1}$) | ♂ | -0.204 | -0.502 | -0.056 |
| | | ♀ | 0.154 | -0.065 | 0.314 |
| Cholesterol | ($mmol \cdot dm^{-3}$) | ♂ | -0.362 | -0.023 | -0.448 |
| | | ♀ | -0.415 | -0.333 | -0.803 |
| Lipids | ($mmol \cdot dm^{-3}$) | ♂ | -0.455 | -0.103 | -0.271 |
| | | ♀ | -0.856 | 0.408 | -0.477 |
| Calcium | ($mmol \cdot dm^{-3}$) | ♂ | -0.180 | -0.818 | -0.554 |
| | | ♀ | 0.049 | -0.714 | -0.629 |
| Cholinoesterase | ($IU \cdot dm^{-3}$) | ♂ | -0.435 | -0.146 | -0.732 |
| | | ♀ | 0.021 | 0.136 | -0.547 |

Critical values of $r$ for: $\alpha \geq 0.05 - 0.444$; $\alpha \geq 0.01 - 0.561$

Table 5

Coefficients of correlation ($r$) between the blood indices and sensory properties of meat

| Indices | Sex | Sensory properties of breast muscles | | | | | |
|---|---|---|---|---|---|---|---|
| | | aroma | | tenderness | juiciness | taste | |
| | | intensity | desirability | | | intensity | desirability |
| Glucose | ♂ | 0.901 | 0.902 | 0.781 | 0.438 | 0.777 | 0.664 |
| ($mg \cdot dk^{-3}$) | ♀ | -0.357 | -0.357 | 0.904 | 0.212 | 0.350 | 0.351 |
| Protein ($g \cdot dl^{-1}$) | ♂ | -0.212 | -0.212 | -0.299 | -0.599 | -0.330 | -0.228 |
| | ♀ | 0.876 | 0.876 | -0.131 | -0.421 | -0.112 | -0.110 |
| Cholesterol | ♂ | 0.746 | 0.745 | 0.403 | 0.404 | 0.776 | 0.582 |
| ($mmol \cdot dm^{-3}$) | ♀ | 0.334 | 0.333 | -0.120 | 0.108 | 0.298 | 0.300 |
| Lipids | ♂ | 0.530 | 0.530 | -0.061 | -0.585 | -0.160 | -0.398 |
| ($mmol \cdot dm^{-3}$) | ♀ | 0.411 | 0.412 | -0.800 | 0.236 | -0.301 | -0.277 |
| Calcium | ♂ | 0.425 | 0.426 | 0.236 | -0.273 | -0.069 | 0.138 |
| ($mmol \cdot dm^{-3}$) | ♀ | 0.440 | 0.440 | -0.245 | 0.093 | 0.021 | 0.094 |
| Cholinoesterase | ♂ | 0.423 | 0.424 | -0.188 | -0.356 | -0.049 | -0.140 |
| ($IU \cdot dm^{-3}$) | ♀ | 0.367 | 0.367 | 0.101 | -0.201 | -0.072 | -0.118 |

Critical values of $r$ for: $\alpha \geq 0.05 - 0.444$; $\alpha \geq 0.01 - 0.561$

Glucose, being the main energy source for the organism, permits efficient protein utilization and control over fat metabolism, due to its anti-ketone activity. The blood concentration of glucose was positively correlated with the protein content of breast muscles in males ($r = 0.592$), and negatively with the ash content of breast muscles in females ($r = -0.516$, Table 3).

Cholesterol is a steroid found in all animal cells. The serum level of cholesterol was significantly correlated (Table 3) with the content of protein,

fat, dry matter and ash in breast muscles in males ($r$ from 0.44 to 0.754), and with the ash content of breast muscles in females ($r = 0.445$).

A significant correlation was observed between the serum concentration of total lipids and the dry matter content of breast muscles in both males and females ($r$ ♂= 0.617, $r$ ♀ = 0.578 – Table 3). A strong correlation was also found between this chemical trait and ChE activity ($r$ from 0.547 in females to 0.940 in males). In humans, and probably also in birds, cholinoesterase is the enzyme characterized by the highest esterolytic activity. Reduced activity of this enzyme is observed in neoplastic and liver diseases as well in numerous infectious and devastating diseases. Increased activity of cholinoesterase accompanies nephrosis and diabetes.

The sensory properties of meat (tenderness, juiciness) are greatly affected by the water-holding capacity. The serum level of lipids was significantly negatively correlated with the water-holding capacity of breast muscles, both in males and females ($r$ ♂ = – 0.455, $r$ ♀ = – 0.856 – Table 4). WAWRO et al. (1999b, 2001b) also observed a close correlation between the water-holding capacity of breast muscles and blood hemoglobin levels in Pekin and Mullard ducks of both sexes.

Apart from the water-holding capacity, also pH has a substantial effect on the technological quality of meat. Both in males and females a high negative correlation was recorded between breast muscle acidity and the serum concentrations of glucose ($r < – 0.500$) and calcium ($r < – 0.713$ – Table 4). There was also a significant correlation, in both sex groups, between color lightness of breast muscles and the serum levels of cholesterol and calcium, and cholinoesterase activity ($r$ from – 0.448 to – 0.803).

The sensory quality of meat, usually reflected by aroma, tenderness and juiciness (BARYŁKO-PIKIELNA 1975), is a function of a variety of factors. No significant relationships were found in the present experiment between the blood chemical indices and the sensory properties of breast muscles (Table 5). Only glucose content was highly correlated with meat tenderness, both in males ($r = 0.781$) and females ($r = 0.904$). The results of our study are partly consistent with the findings of WAWRO et al. (2001a). According to these authors, the activity of such enzymes as alanine aminotransferase (ALAT), aspartate aminotransferase (AST), alkaline phosphatase (AP) and acid phosphatase (ACP) generally do not show significant correlations with the sensory properties of breast muscles in Muscovy ducks sacrificed at a different age.

The blood levels of chemical and biochemical indices depend on the species, strain, sex, age and physiological condition of ducks, but primarily on the diet (FARHAT, CHAVEZ 2001, KRASNODĘBSKA-DEPTA et al. 1997b, STURKIE 1970). The same factors decide also about the quality of poultry meat (NIEWIAROWICZ 1993). BAEZA et al. (1998) reported that in Muscovy ducks meat tenderness and

juiciness decrease with age, whereas pH increases and the meat becomes darker due to a larger amount of hemoglobin. The ambiguity of our results is most probably a consequence of sex and young age (seven weeks) of experimental ducks, since the levels of blood chemical and biochemical indices stabilize in fully mature birds.

## Conclusions

In the majority of cases no significant correlations were found between the serum levels of the blood chemical indices and meat quality parameters. A significant correlation was observed between total lipids and the content of fat and dry matter in the breast muscles of both males and females ($r$ from 0.487 to 0.745). Calcium content was highly correlated with meat pH ($r$ ♂ = – 0.818; $r$ ♀ = – 0.714), and glucose content – with the aroma, tenderness and taste of meat from males ($r$ from 0.664 to 0.902). The activity of cholinoesterase was closely correlated ($r$ from 0.455 to 0.940) with the levels of fat, dry matter and ash in the breast muscles of both males and females.

Translated by JOANNA JENSEN

Accepted for print 30.03.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 249-257, Y. 2006

# MORPHOLOGICAL CHARACTERISTICS OF MUSCLE FIBRES OF *M. LONGISSIMUS LUMBORUM* IN CROSSBREED FATTENERS WITH DIFFERENT PROPORTION OF HAMPSHIRE BREED

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K e y w o r d s: muscle fibres, *m. longissimus lumborum*, fatteners.

A b s t r a c t

On 46 fatteners from three different genotypes group as follows: group I – Hampshire and crossbreeds with different proportion of Hampshire breed: group II [♀ Duroc x Hampshire ♂)], and group III [♀ Polish Landrace x (♀ Duroc x Hampshire ♂) ♂)] muscle fibres traits of *m. longissimus lumborum* (LL) were examined. Samples of muscle were taken to categorize muscle fibres according to their mATP-ase activity (I, IIA and IIB). Muscle fibre percentage, cross-sectional area (CSA), relative area (RA) and phenotypic correlation between traits of muscle fibres were estimated. Moreover, percentage of giant muscle fibres was examined. The obtained results indicated that genotype had effect on muscle fibre composition. The greatest CSA of all examined muscle fibre types was found in fatteners with 25% Hampshire bloodlines (group III), whereas in group I the smallest ones were observed. Moreover, crossbreeds with the lowest percentage of Hampshire breeds (group III) had a higher percentage and RA of type IIB fibres and a lower percentage and RA of type I fibres compared to other group. The frequency of giant fibres was the highest in group III than the others, as well. Additionally, phenotypic correlation between fibre types percentages were negative, in contrast to positive correlation between fibre type CSA. Fibre type RA was much more closely related to fibre percentages than to CSA. Moreover, percentage of giant muscle fibres positively low correlated with frequency and RA of type IIB fibres.

## CHARAKTERYSTYKA MORFOLOGICZNA WŁÓKIEN MIĘŚNIOWYCH *M. LONGISSIMUS LUMBORUM* TUCZNIKÓW MIESZAŃCÓW Z RÓŻNYM UDZIAŁEM RASY HAMPSHIRE

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S ł o w a k l u c z o w e: włókna mięśniowe, *m. longissimus lumborum*, tuczniki.

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Abstrakt

Na 46 tucznikach trzech grup genotypowych: tuczniki rasy Hampshire – grupa I, mieszańcach z różnym udziałem rasy Hampshire w schemacie krzyżowania: [♀ Duroc x Hampshire ♂)] – grupa II oraz [♀ Polish Landrace x (♀ Duroc x Hampshire ♂) ♂)] – grupa III analizowano cechy włókien mięśniowych *m. longissimus lumborum*. Na podstawie aktywności ATP-azy miozynowej określono 3 typy włókien mięśniowych (I, IIA oraz IIB). Badano udział procentowy, powierzchnię przekroju poprzecznego (CSA), powierzchnię względną (RA) włókien mięśniowych, a także obliczono współczynnik korelacji *r* dla zależności między poszczególnymi cechami włókien mięśniowych. Dodatkowo oszacowano udział procentowy włókien mięśniowych olbrzymich. Stwierdzono, że genotyp wpływa istotnie na kompozycję włókien mięśniowych. Największy udział procentowy oraz RA włókien mięśniowych typu IIB, a równocześnie najmniejszy udział procentowy oraz RA włókien mięśniowych typu I, a także największy udział procentowy włókien olbrzymich wykazano u tuczników z grupy o najmniejszym udziale rasy Hampshire (grupa III). Ponadto największą CSA wszystkich analizowanych typów włókien mięśniowych stwierdzono u świń z grupy III (25% dolewu rasy Hampshire), a najmniejszą w grupie I. Współczynniki korelacji *r* między udziałem procentowym włókien mięśniowych okazały się ujemne, natomiast zależności dodatnie oszacowano między CSA włókien mięśniowych. Stwierdzono także silniejsze zależności między RA włókien mięśniowych a ich udziałem procentowym, w porównaniu z CSA włókien mięśniowych. Dodatnie współczynniki korelacji oszacowano także dla zależności między udziałem procentowym włókien olbrzymich a udziałem procentowym i RA włókien mięśniowych typu IIB.

## Introduction

Histological and histochemical investigations on pig muscles have revealed that muscle fibre type composition and fibre areas of specific muscles are important factors influencing many of the peri- and post-mortal biochemical processes and thereby meat quality (CAMERON et al. 1998, OKSBJERG et al. 2000). Fibres of mammalian skeletal muscle have been classified on the basis of various physiological, morphological and biochemical parameters, namely slow-twitch oxidative, fast-twitch oxidative glycolytic and fast-twitch glycolitic fibres (PETER et al. 1972). Using histochemical methods these fibre types are designated as types I, IIA and IIB by BROOKE and KAISER (1970), types $\beta R$ (red), $\alpha R$ and $\alpha W$ (white) by ASHMORE et al. (1972), respectively. This classification of the muscle fibres can be applied to all mammalian muscle. Muscle metabolism is the summation of the activities of the individual muscle fibres, which comprise the muscle. Some fibre characteristics are mostly determined genetically, while others can be affected by external factors, such as animal's age and sex (LARZUL et al. 1997, WOJTYSIAK et al. 2003, BEE et al. 2004), its physical activity, nutrition or intensive selection (BROCKS et al. 1998, KŁOSOWSKA, FIEDLER 2003, GENTRY et al. 2004). The *m. longissimus* is the most frequently used indicator muscle in meat quality studies in pigs. Few studies suggest that muscle fibre composition is on one hand affected by growth rate and, on the others, itself affected the carcass lean content. Moreover histochemical profile of muscle fibres is specific for different pig breeds or lines (LARZUL et al. 1997, RUUSUNEN, PUOLANNE 1997).

Therefore the aim of this study was to determine muscle fibres traits of *m. longissimus lumborum* between fatteners with different proportion of the Hampshire breed.

## Material and Methods

The research was carried out on 46 fatteners from three different genotypes group as follows: group I – Hampshire ($n=12$) and crossbreeds with different proportion of Hampshire breed: group II [♀ Duroc x Hampshire ♂)] (50% Hampshire bloodlines, $n=18$), and group III [♀ Polish Landrace x (♀ Duroc x Hampshire ♂) ♂)] (25% Hampshire bloodlines, $n=16$). Hampshire fatteners were slaughtered on average at 105 kg body weight and both groups of crossbreeds fatteners at 107 kg body weight. Animals were feed *ad libitum* and slaughtered at commercial slaughterhouses according to routine procedure. Muscle samples for histochemical analyses were taken from the right carcass-side from the *m. longissimus lumborums* (LL) 15-min post mortem at the level of 5$^{th}$ lumbar vertebra and deep within the muscle. Samples were immediately cut into 1-cm$^3$ pieces (parallel to the muscle fibres) and frozen in isopentane that was cooled using liquid nitrogen and stored at -80°C until hitochemical analyses were performed. For determination of muscle fibre type percentage and cross-section area (CSA), the frozen samples were sectioned at 10-μm thickness at -20°C in cryostat (Slee MEV, Germany) and stained for mATPase activity according to the method of BROOKE and KAISER (1970). After both acid (pH 4.6) and alkaline (pH 10.3) preincubation the method identified type I (slow twitch oxidative, red), type IIA (fast twitch oxidative and glycolytic, intermediate) and type IIB (fast twitch glycolytic, white) fibres. Additionally, the relative area (RA) occupied by each fibre type was calculated from the corresponding numerical percentages and mean CSA. Moreover, percentage of giant muscle fibres (GF) was examined. The Multi Scan v. 14.02 computer image analysis software was used to evaluate percentage and muscle fibre cross-section area (CSA). A minimum of 300 fibres was counted in each section. Additionally, correlation ($r$) of fibre traits were also estimated.

Differences between examined group for histochemical characteristics of the muscle fibres were tested using analysis of variance by Statgraphics 5.0 (STSC Inc., Rockville, MD) and tested for differences by Tuckey test. Body weight at slaughter was included as a covariant. Differences were considered significant at $P < 0.05$.

## Results and Discussion

Morphological study (ATP-ase activity) of LL muscle from three examined groups of fatteners showed three types of muscle fibres (Figure 1). Type I fibres were usually distributed side by side with each other and they grouped together in the characteristic "nests" formed from 4 to 8 fibres. Many of type IIA fibres (intermediate) were observed at lower frequency spread separately or in very small groups close to the red fibres. On the other hand, the most numerous muscle fibres IIB that surrounded other fibre types were observed.

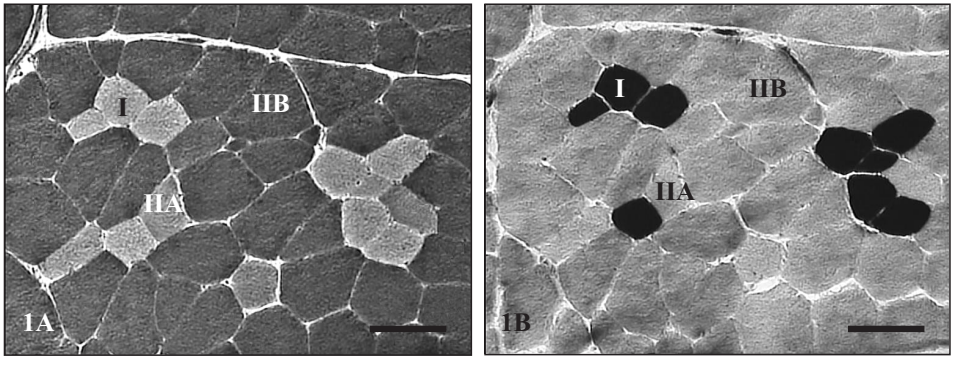


Fig. 1. Serial cross-sections of *m. longissimus lumborum* of Duroc x Hampshire pigs stained by reactions for alkaline-ATPase – pH 10.3 (1A) and acid – pH 4.6 (1B) activities: I – red fibres, IIA – intermediate fibres, IIB – white fibres. Scale bar 100 μm

The morphological characteristics of fibre types from LL muscle are presented in Table 1. There were significant differences in both the percentage of fibre types, their CSA, RA and additionally in proportion of giant muscle fibre between the examined groups of fatteners. The highest frequency of giant fibres characterised fatteners from group III compared to group I and II. Moreover, the fatteners with the smallest percentage of Hampshire breeds (group III) had a higher percentage and RA of type IIB fibres and a lower percentage and RA of type I fibres, than muscles from others group, between that no significant differences were observed. However, in group II compared to group I, a tendency of declining number of type I fibres and increasing number of type IIB was observed. Moreover, present study showed that genotype had effect on muscle fibre size. Fatteners with 25% Hampshire bloodlines (group III) characterised the largest CSA of all examined muscle fibre types, whereas in group I the smallest ones were observed. Similarly, LEFAUCHEUR et al. (2004) showed a strong effect of breed on mean CSA of muscle fibres, and more interestingly, that this effect was highly dependent on

fibre and muscle types. Moreover, differences in fibre types composition observed in present study confirms the earlier results of KARLSSON et al. (1993) and LARZUL et al. (1997), who demonstrated that muscle fibre composition and size is specific for different pig breeds or lines. BROCKS et al. (1998) and KŁOSOWSKA and FIEDLER (2003) suggest that muscle fibre characteristics are mainly determined genetically. In pigs, the number of muscle fibres is determined before birth (STICKLAND and GOLDSPINK 1973), whereas the differentiation of muscle fibres continues in early postnatal life. The postnatal growth of muscle tissue is dependent on the total number of fibres and muscle fibre CSA and length. Significant lower percentage of type IIB fibres and higher percentage of type I in fatteners from group I and II observed in present study are in agreement with ESSEN-GUSTAVSSON and FJELKNER-MODIG (1985), who reported that the muscles of Hampshire are more oxidative than those of Landrace or Yorkshire pigs. Pig muscles with many oxidative fibres tolerate stressful conditions better during transport, resulting in a slower fell in pH after slaughter. Muscle fibres affect postmortem changes in muscle due to the differences in their glycolytic or oxidative capacity. After slaughter, the pH value of light muscle decreases more rapidly and to a lower ultimate value than that of dark muscle because light muscle contains more glycogen and more glycolytic enzymes. WEILER et al. (1995) and LEFAUCHEUR et al. (2004) suggest that selection in modern pigs induced a shift in muscle metabolism toward a more glycolytic and less oxidative fibre type.

Table 1

Percentages, cross-sectional area (CSA) and relative areas (RA) of red (I), intermediate (IIA) and white (IIB) muscle fibres, and percentage of giant fibres (GF) in *m. longissimus lumborum* of three examined genotypes group of fatteners

| Traits | Group I $\bar{x} \pm SE$ | Group II $\bar{x} \pm SE$ | Group III $\bar{x} \pm SE$ |
|---|---|---|---|
| Percentages of muscle fibres (%) | | | |
| IIB | $58.54 \pm 1.18^a$ | $60.25 \pm 1.06^a$ | $65.80 \pm 1.13^b$ |
| IIA | $12.59 \pm 0.92^a$ | $12.64 \pm 1.36^a$ | $14.02 \pm 1.64^a$ |
| I | $28.87 \pm 0.96^a$ | $27.11 \pm 0.87^a$ | $20.18 \pm 0.82^b$ |
| Cross-section area (CSA) ($\mu m^2$) | | | |
| IIB | $4568.31 \pm 98.54^a$ | $5184.61 \pm 92.86^b$ | $5864.53 \pm 99.87^c$ |
| IIA | $3082.53 \pm 61.12^a$ | $3342.36 \pm 71.63^b$ | $3826.97 \pm 68.56^c$ |
| I | $2894.78 \pm 51.36^a$ | $3147.69 \pm 91.82^b$ | $3708.26 \pm 78.19^c$ |
| Relative area (%) | | | |
| IIB | $68.60 \pm 1.08^a$ | $71.00 \pm 0.93^a$ | $75.02 \pm 1.12^b$ |
| IIA | $9.96 \pm 0.47^a$ | $9.60 \pm 0.62^a$ | $10.43 \pm 0.56^a$ |
| I | $21.44 \pm 1.12^a$ | $19.40 \pm 0.96^a$ | $14.55 \pm 0.81^b$ |
| Percentages of giant muscle fibres (%) | | | |
| GM | $0.96 \pm 0.04^a$ | $1.03 \pm 0.05^a$ | $1.48 \pm 0.07^b$ |

*a, b, c* – means (in rows) with different superscripts differ significantly at $P < 0.05$

According to ESSEN-GUSTAVSSON and FJELKNER-MODIG (1985) muscle oxidative capacity affects meat quality by improving the sensory properties of meat. Moreover, muscle characteristics, including fibre type frequency may be a source of variation in eating quality (KARLSSON et al. 1993, BEE et al. 2004, GENTRY et al. 2004,). Thus, muscle with a high frequency of type I fibres may be expected to be more tender due to higher rates of protein turn-over (GARLICK et al. 1985) and higher levels of m calpain (STEVENS et al. 1997). On the other hand, these results still remain open to criticism. SAZILI et al. (2005) suggest that exactly how muscle fibre composition and meat tenderness are related is unclear and a number of inconsistent reports exist. Studies in cattle (O'HALLORAN et al. 1997) and in pigs (KARLSSON et al. 1993) have shown that the frequency of type IIB fibres is negatively correlated with toughness. On the other hand, RENAND et al. (2001), in study on bulls, found a positive correlation between the proportion of type I fibres and toughness. In contrast, MALTIN et al. (1998) supported a positive relationship between the frequency or percentage area of type I fibres and sensory tenderness and negative relationship between the frequency of type IIB fibres and tenderness. Instead CAMERON et al. (1998) suggested that proportion of type IIB fibres, contrary to that of type I, is negatively correlated with meat tenderness. Other studies showed that differences between the same muscles may only be evident in specific breeds of any one species. It has been shown that the shear force of *longissimus* muscle differ significantly in Duroc, but not in Berkshire and Large White pigs (CHANG et al. 2003). RUUSUNEN and PUOLANE (1997) found that the variation in muscle fibre composition in pigs within the breeds is larger than the average variation between the breeds. Moreover, GENTRY et al. (2004) suggest that presence or lack of differences in fibre type composition observed in literature may be affected by a lack of uniformity in the classification of muscle fibre types, especially type II, and additionally, by different histochemical methods used for the identification of muscle fibre types.

The variations of fibre type size and frequency shown here may explain some of the variability in their mechanical properties and eating quality characteristics as juiciness, drip loss, intramuscular fat and colour (WARRISS et al. 1990, LARZUL et al. 1997, OKSBJERG et al. 2000). Meat colour is a major factor limiting the quality and acceptability of meat and meat products. The right colour of meat can be conditioned by the presence of ferrous oxymioglobin – oxyMb (PHILIPS et al. 2001) which is directly connected with the percentage and size of the muscle fibre types (WARRISS et al. 1990). Therefore, changes in percentage and CSA of the muscle fibre between examined group of fatteners observed in the present study can have also influence for meat colour and eating quality.

Correlations between muscle fibre traits are presented in Table 2. All phenotypic correlation between fibre type percentages were significantly nega-

tive. LARZUL et al. (1997) and WOJTYSIAK et al. (2003) also reported negative correlations between percentage of all fibre types. Additionally, phenotypic correlations between fibre type CSA were all positive and highly significant as previously shown by LARZUL et al. (1997). On the other hand, correlation between fibre percentages and CSA were not significant. In contrast, study by LARZUL et al. (1997) showed low correlations between fibre percentages and CSA. Moreover, fibre type RA was much more closely related to fibre percentages than to CSA. These results confirm the earlier results of LARZUL et al. (1997) and WOJTYSIAK et al. (2003). Furthermore, percentage of giant muscle fibres positively low correlated only with frequency and RA of type IIB fibres. DIETL at al. (1993) founded positive phenotypic and genetic correlation coefficients between the frequency of giant fibres and the rate of glycolysis post mortem. On the other hand, BERGMANN (1979) described this process as the consequence of the exposure to stress, whereas SOŚNICKI (1987) considered their development to be a result of a degenerative process.

Table 2

Phenotypic correlation between *longissimus lumborum* muscle fibre characteristics: I – red fibres, IIA – intermadiate fibres, IIB – white fibres, GF – giant fibres

| Specification | Percentage (%) | | Cross-sectional area (CSA) | | | Relative area (RA) | | | Percentage (%) |
|---|---|---|---|---|---|---|---|---|---|
| | IIA | IIB | I | IIA | IIB | I | IIA | IIB | GF |
| % I | -0.12* | -0.46** | ns | ns | ns | 0.31* | -0.21* | -0.27* | ns |
| % IIA | | -0.42** | ns | ns | ns | -0.19* | 0.49* | -0.21* | ns |
| % IIB | | | ns | ns | ns | -0.20* | -0.28* | 0.53* | 0.09* |
| CSA I | | | | 0.73** | 0.65** | 0.11* | 0.06* | -0.12* | ns |
| CSA IIA | | | | | 0.79** | 0.08* | 0.05* | -0.09* | ns |
| CSA IIB | | | | | | -0.07* | -0.05* | 0.06* | ns |
| RA I | | | | | | | ns | -0.07 | ns |
| RA IIA | | | | | | | | ns | ns |
| RA IIB | | | | | | | | | 0.05* |

ns – differences not significant, * differences significant at $P < 0.05$, ** $P < 0.01$

## Conclusions

1. Genotype had effect on muscle fibre size. The greatest CSA of all examined muscle fibre types was found in fatteners with 25% Hampshire bloodlines (group III), whereas in group I the smallest ones were observed.

2. Muscle from group of fatteners with the lowest percentage of Hampshire breed (group III) had a higher percentage and RA of type IIB fibres and a lower percentage and RA of type I fibres, and the highest frequency of giant fibres compared to other group.

3. Phenotypic correlation between fibre types percentages were negative, in contrast to positive correlation between fibre type CSA. Moreover, percentage of giant muscle fibres positively low correlated with frequency and RA of type IIB fibres.

Translated by Dorota Wojtysiak

Accepted for print 26.04.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 259-276, Y. 2006

# REGIONALIZATION OF RED DEER (*Cervus elaphus* L.) BREEDING – THE WEIGHT OF ANTLERS AS A PARAMETER DIFFERENTIATING THE RED DEER POPULATION IN WARMIA AND MAZURY

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K e y w o r d s: red deer (*Cervus elaphus* L.), regionalization, habitat, individual quality, antlers, quality classes of hunting grounds.

A b s t r a c t

The aim of the study was to analyse the gross weight of antlers, the types of forest habitats and selected features of antlers of Mazurian red deer with the aim of distinguishing the regions presenting a qualitative diversity of the red deer population in Warmia and Mazury. The research made it possible to state that regionalization, distinguished on the basis of the weight of antlers, is the most useful one for game management as regards the population of Mazurian red deer. As follows from this regionalization, the best red deer in the vicinity of Olsztyn, in their full individual development, between their $9^{th}$ and $14^{th}$ year of life, live in the following areas: Gierdawy, Kętrzyn, Borki, Sadłowo W., Sadłowo M., Gązwa, Spychowo, Szczytno, Grunwald, Miłomłyn and Tarda, Koniuszyn, Danielewo, whereas the poorest stags as regards quality are found in areas: Bartniki, Bukowiec, Ponarzyny, Mrągowo, Racibór, Wesołowo, Dłużek and Drwęca.In the areas of the poorest individual quality of red deer stags, the average weight of 16 analysed red deer antlers was 5.73 kg, the total CIC score, i.e. without taking into consideration additional points or deductions, was 166.15 pts. For the sake of comparison: in the above areas where the strongest stags were harvested, they were characterized by the following features: the weight of antlers – 7.76 kg, the total CIC score – 182.20 pts. The analysis of features in regionalization 1 facilitated a better demonstration of the differences between the analysed regions, thus offering the possibility to introduce breeding regions into hunting practice. To sum up, it can be stated that in the Province of Olsztyn, in the hunting season, a smaller percentage share of fresh coniferous forest and fresh mixed coniferous forest accompanied by a larger percentage share of fresh forest produces better quality Mazurian red deer.

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**REJONIZACJA HODOWLI JELENIA SZLACHETNEGO (*Cervus elaphus* L.) – MASA POROŻA JAKO PARAMETR RÓŻNICUJĄCY POPULACJĘ JELENI NA WARMII I MAZURACH**

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Słowa kluczowe: jeleń (*Cervus elaphus* L.), rejonizacja, siedlisko, jakość osobnicza, poroże, bonitacja łowisk.

Abstrakt

Celem pracy była analiza masy wieńców brutto, typów siedliskowych lasu oraz wybranych cech poroża jelenia mazurskiego, co miało posłużyć do wyróżnienia rejonów o zróżnicowanych jakościowo populacjach jeleni na Warmii i Mazurach. Wykazano, że do prowadzenia gospodarki łowieckiej w populacji jelenia mazurskiego najbardziej przydatna jest rejonizacja wyróżniona na podstawie masy wieńca. Wynika z niej, że w Olsztyńskiem najlepsze, w pełni rozwoju osobniczego jelenie, od 9. do 14. roku życia, żyją w obrębach: Gierdawy, Kętrzyn, Borki, Sadłowo W., Sadłowo M., Gązwa, Spychowo, Szczytno, Grunwald, Miłomłyn, Tarda, Koniuszyn, Danielewo, a jakościowo najsłabsze byki w obrębach: Bartniki, Bukowiec, Ponarzyny, Mrągowo, Racibór, Wesołowo, Dłużek i Drwęca. W obrębach o najsłabszej jakości osobniczej jeleni byków średnia masa 16 analizowanych wieńców jeleni wynosiła 5,73 kg, suma punktów pomiarowych CIC, czyli bez uwzględnienia punktów dodatkowych i potrąceń, 166,15 pkt. Dla porównania, w ww. obrębach, gdzie pozyskano najmocniejsze byki, masa wieńca wynosiła 7,76 kg, suma punktów pomiarowych CIC – 182,20 pkt. Analizując cechy w ramach rejonizacji 1, wyraźniej dostrzeżono różnice między analizowanymi rejonami, co daje możliwość wprowadzania rejonów hodowlanych do praktyki łowieckiej. Stwierdzono, że w woj. olsztyńskim, w okresie sezonu polowań, im niższy był udział procentowy boru świeżego i boru mieszanego świeżego, a większy udział lasu świeżego, tym jakość jelenia mazurskiego była lepsza.

## Introduction

In 1977, the Mazurian Provincial Hunting Board developed a programme for regionalization of Mazurian red deer breeding in former administrative boundaries of the Province of Olsztyn. However, the program was never implemented and today few people realize that such document exists. Research supports the view that regionalization is not only a necessity, but also a requirement of rational hunting management in red deer breeding. For this reason, the present study undertakes the subject of breeding regions for Mazurian red deer in the Province of Olsztyn, and particularly of the assessment of selected phenotypical qualities of red deer males (*Cervus elaphus hippelaphus* Erxleben 1977) in the aspect of natural environment conditions.

The red deer in Poland feeds on about 265 various species of plants (DZIĘCIOŁOWSKI 1969c). These animals can consume a significant part of their forage, even up to 50% of the demand in some hunting grounds, by feeding on crops (GĘBCZYŃSKA 1980, PICARD et al. 1991). In each habitat where the red

deer can found, 70-80% of the forage is composed only of about a dozen species of plants (GĘBCZYŃSKA 1980). Due to their various densities in the flora of local habitats, the forage significantly differs. Consequently, in each of the local habitats, the deer makes a choice of a specific set of plants (KOSSAK 1976, HOMOLKA 1990). The choice depends on the availability of plants in a given habitat as well as on deer preferences (KOSSAK 1976, WICKSTROM et al. 1984, EDGE et al. 1988). According to HRABE and KOUBEK (1990), the linear dimensions of the body, and therefore the weight and branching of antlers, are mainly influenced by feeding factors and not by a geographical location.

The aim of the study was to analyse the gross weight of antlers, the types of forest habitats and selected features of antlers of Mazurian red deer with the aim of distinguishing those regions with a qualitative diversity in the red deer population in Warmia and Mazury.

## Environmental conditions of the Province of Olsztyn

The Province of Olsztyn is situated in the north-eastern part of Poland and occupies an area of 233 138 ha. It is 147 km long and 130 km wide, which results in a climatic differentiation between the north-eastern and the north-western parts of the province. The forest complexes in the Province of Olsztyn, despite their advanced anthropomorphization, belong to the least distorted environments that best resemble the original plant complexes occurring in the area of the province. The surface area of forests in the province has increased in comparison to the pre-war period and currently amounts to 33%. The area of the Province of Olsztyn includes 393,128 ha of grounds owned by the State Forestry and 13.770 ha of forests managed by the Regional Directorates of the State Forests (RDLP) in Olsztyn (*Information...* 1994).

The route connecting the towns of Górowo Iławeckie, Dobre Miasto, Olsztynek and Działdowo basically corresponds with the division of the province into forest and natural lands (CHODNIK 1988), of which the western part of the province is located in land I, the so-called Baltic land, and the eastern in land II – Mazovia-Podlasie. Additionally, the first part is composed of the following districts: Elbląg-Warmia, Mazurian Lakeland and North Masovia.

## Elbląg-Warmia District

The border of the district approximately corresponds to the eastern range of beech trees. It is also the north border of spruce, sycamore and great sessile

oak. A pure stand of beech trees with the addition of larch grow in the Małdyty area. The district provides optimum conditions for pine (Taborskie Forests in the vicinity of Ostróda). Forests in this district are much diversified as regards habitats. Mixed coniferous forests (BM) and mixed forests (LM) prevail in the area, with a large percentage share of forest types. The undergrowth is lush and differentiated as regards the choice of species (MROCZKIEWICZ 1964).

Mazurian Lakeland District

The district is characterized by scattered forest areas, with rich undergrowth and underbrush. The most common type of habitat is fresh coniferous forests, mixed coniferous forest (BM), humid forest (Lw) and wet deciduous forest.

### North Masovia District

It partly covers the south-eastern part of the province, and its vast areas are occupied with forests. Coniferous forest and forest habitats, dominated by pine trees, with the contribution of oak and hornbeam, as well as small amounts of spruce, are found in the northern part of the district.

The climate is characterized by a significant variability of weather conditions. The annual mean temperature for the province is 6.6°C. The coldest area is the north-eastern part of the province, as well as Górowskie Elevation and central part of Dylewskie Hills. The course of January isotherm indicates a spatial decline in temperatures, reaching 4°C towards the east. Significant differences occur between the north-eastern part of the province and its south-western part. There are visible differences between the number of cold and freezing days and the date of the last frost. The length of vegetation period is about 125 days. The precipitation in the prevailing part of the province area amounts to about 550-660 mm. Snow cover is kept, on average, for more than 60 days in the west of the province and up to 80 days in its east part; whereas in the area of Dylewskie Hills and Górowskie Elevation this period can be even longer (JARUBAS 1979).

## Materials and Methods

### Quality assessment of antlers of culled red deer stags

Experimental material for the study was composed of the antlers of stags, harvested in the Province of Olsztyn in the seasons 1988/89-1990/91. Research material was collected in 170-173 of hunting grounds out of 184 situated in the Province of Olsztyn. In total, 3.192 stags were culled in these grounds, which

constituted 89.04% of all specimens harvested in this period and the conducted analysis involved the examination of 1,704 pieces (53.38%), of which 274 pieces between 9 and 14 years of age were selected..

The following information was collected with regard to the culled animals: age of harvested specimens, place of harvesting – the hunting ground, the weight of carcass and the form of antlers. Elements of antler CIC scoring were also collected, taking into account only those parameters that are used for calculating CIC points, i.e. the length of tines, the length of brow tines and tray tines, the circumference of coronets as well as the upper and lower circumferences of tine, the weight of antlers, the number of tines and the inside spread) (STACHOWIAK 1994, VARIĆAK 2001), considering them to be most objective in assessing the quality of deer antlers.

In order to establish more reliable measurements of gross weight of antlers in a trophy harvested by the so-called hunters paying in hard currency, 10% was deducted from its original weight to compensate for water evaporation from bone tissue (VARIĆAK 2001, JACZEWSKI 1981).

All analyses were conducted exclusively in relation to animals belonging to the class of strong stags, i.e. game and junior individuals harvested using so-called improper or wrongful culling (*Information*. 1983 ) in their $9^{th}$-$14^{th}$ year of life, i.e. those that took the stag antlers with the crown on both sides and had the weight of antlers of a minimum of 5 kg.

While determining the total CIC score in order to calculate the net weight, deductions of 0.50-0.70 kg were applied for the analysed group, on the basis of preliminary research for individual period of life.

## Determination of the percentage share of forest habitat types in the Province of Olsztyn

In order to characterize the habitats and possibly differentiated regions of red deer breeding, it was necessary to prepare, on the basis of surveys of forest management for forest divisions, a list of habitat types according to quality classes of hunting areas for the cervid family as well as to determine the structure of species and ages of tree stands, as per areas. They are the smallest administrative units, in relation to which the performance of such divisions can be carried out.

The data for this part of experiment was prepared according to the studies for forest management. In total, 45 areas situated in the area of 20 forest districts were analysed (Table 1) and characterized according to 16 habitat types (Table 2). In these habitat styles, the age structure of tree stands, of 10 commonly used classes and subclasses, was determined (*Forest Breeding...* 1988).

Table 1
Schema presenting administrative division of forest divisions of the Province of Olsztyn into areas, with the assignment of proper hunting grounds

| Forest division | | Area | | Ground |
|---|---|---|---|---|
| 1 | | 2 | | 3 |
| Srokowo | 1 | Gierdawy | 1 | 1, 2, 3, 12, 13, 14, 15, 16, 26, 27 |
| | | Kętrzyn | 2 | 24, 25, 33, 34, 35, 36 |
| Bartoszyce | 2 | Sępopol | 3 | 4, 5, 6, 7, 17, 18, 19 |
| | | Bartniki | 4 | 28, 29, 30, 37, 38, 46, 47, 48, 49 |
| Górowo Ił. | 3 | Borki | 5 | 8, 9, 10, 20 |
| | | Bukowiec | 6 | 11, 21, 22, 23, 31 |
| | | Pieniężno | 7 | – |
| Wichrowo | 4 | Orneta | 8 | – |
| | | Łaniewo | 9 | 32, 41 |
| | | Wichrowo | 10 | 39, 40, 50, 51, 52, 65 |
| Dobrocin | 5 | Dobrocin | 11 | 83, 84, 96, 97 |
| | | Ponarzyny | 12 | 68, 69, 81, 82, 95 |
| Kudypy | 6 | Kudypy | 13 | 93, 94, 103, 104 |
| | | Łyna | 14 | 53, 66, 67, 79, 80, 91, 92 |
| Wipsowo | 7 | Purda Leś. | 15 | 75, 89, 90, 101, 114 |
| | | Sadłowo W. | 16 | 59, 61, 62, 63, 64, 76 |
| | | Wipsowo | 17 | 77, 78 |
| Mrągowo | 8 | Mrągowo | 18 | 72, 73, 86 |
| | | Sadłowo M. | 19 | 45, 58, 60 |
| | | Gązwa | 20 | 42, 43, 44, 55, 56, 57, 74 |
| Strzałowo | 9 | Strzałowo | 21 | 71 |
| | | Krutyń | 22 | 85, 98 |
| | | Babięta | 23 | 99, 100 |
| Spychowo | 10 | Spychowo | 24 | 122 |
| | | Racibór | 25 | 111, 123 |
| | | Chochół | 26 | – |
| Wielbark | 11 | Wielbark | 27 | 159, 170 |
| | | Wesołowo | 28 | 149, 160 |
| | | Chorzele | 29 | – |
| Szczytno | 12 | Korpele | 30 | 87, 88, 112, 113, 124, 138, 139 |
| | | Małdaniec | 31 | 136, 137, 148, 158 |
| | | Szczytno | 32 | 150 |
| Jedwabno | 13 | Dłużek | 33 | 140, 151 |
| | | Zimna Woda | 34 | 161, 162, 171 |
| Nowe Ramuki | 14 | Nowe Ramuki | 35 | 102, 115, 116, 125, 126 |
| | | Stawiguda | 36 | 127 |
| Olsztynek | 15 | Olsztynek | 37 | 141, 142, 153, 165 |
| | | Grunwald | 38 | 131, 143, 144, 154, 155, 166 |
| Stare Jabłonki | 16 | St. Jabłonki | 39 | 118, 130 |
| | | Jagiełek | 40 | 117, 128, 129 |
| Miłomłyn | 17 | Miłomłyn | 41 | 108, 109, 119, 132, 183 |
| | | Tabórz | 42 | 105 |
| | | Tarda | 43 | 106, 107 |

cont. Table 1

| 1 | | 2 | | 3 |
|---|---|---|---|---|
| Iława | 18 | Iława | 44 | 135, 146, 147 |
| | | Smolniki | 45 | 157, 167, 168, 169 |
| | | Drwęca | 46 | 120, 133, 134, 145, 156 |
| Nidzica | 19 | Nidzica | 47 | 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182 |
| | | Koniuszyn | 48 | 152, 163, 164 |
| Młynary | 20 | Młynary | 49 | – |
| | | Markowo | 50 | – |
| | | Danielewo | 51 | 70 |

Table 2

Suitability of individual habitats for deer breeding

| Habitat No. | Symbol | Name | Division of habitats acc. to Bobek et al. | Division of habitats acc. to IBL |
|---|---|---|---|---|
| 1 | Bs | dry coniferous forest | I (coniferous forests) | III (Bs + Bb) |
| 2 | Bb | marshy coniferous forest | | |
| 3 | Bśw | fresh coniferous forest | | I (coniferous forest habitats) |
| 4 | Bw | humid coniferous forest | | |
| 5 | BM | mixed coniferous forest | II (mixed habitats) | |
| 6 | BMśw | fresh mixed coniferous forest | | |
| 7 | BMw | humid mixed coniferous forest | | |
| 8 | BMb | marshy mixed coniferous forest | | |
| 9 | LMśw | fresh mixed forest | | II (forest habitats) |
| 10 | LMw | humid mixed forest | | |
| 11 | LMb | marshy mixed forest | | |
| 12 | Lśw | fresh forest | III (deciduous forests) | |
| 13 | Lw | humid forest | | |
| 14 | Ll | riparian forest | | |
| 15 | OL | wet deciduous forest | | |
| 16 | OLS | ash wet deciduous forest | | |

Variants of regionalization for the red deed breeding were prepared based on the antler weights of shot deer in the analysed district of animal.

In order to provide characteristics of individual qualities of the red deer, a list was prepared of habitats covering the forests of the former Province of Olsztyn, taking into consideration their suitability for deer game breeding, as established by the Research Institute of Forestry (IBL) (DZIĘCIOŁOWSKI 1977) – Table 2 and taking into consideration the research by BOBEK et al. (1991),

allowing for the considerable stock of individual food nutrients in lowland deciduous forest, mixed coniferous, and coniferous forest habitats (Table 2).

In order to adapt and optimize the distinguished regions for red deer breeding in the Province of Olsztyn from the point of view of practical hunting and, taking into consideration statistical analysis results conducted on the basis of diagram presenting the assignment of forest areas, with the weight of antlers as a factor differentiating the population of this species in the Province of Olsztyn (Table 3), the regionalization 1 and 2 was prepared, according to the presented study experiments (Table 4 and 5).

Table 3

Schema presenting the assignment of forest areas with regard to the weight of antlers as a factor differentiating the population of this species in the Province of Olsztyn

| Group | Weight of antlers (kg) | Numbers of forest areas included into each group |
|---|---|---|
| 1 | $x \geq 7.7$ | 2, 5, 19, 24, 32, 38, 41, 43, 48, 51 |
| 2 | $7.3 \leq x < 7.7$ | 1, 16, 20 |
| 3 | $7.0 \leq x < 7.3$ | 13, 17, 31, 37, 42 |
| 4 | $6.8 \leq x < 7.0$ | 10, 14, 22, 40, 45 |
| 5 | $6.3 \leq x < 6.8$ | 3, 9, 15, 21, 23, 27, 30, 35, 44 |
| 6 | $x < 6.3$ | 4, 6, 12, 18, 25, 28, 33, 46 |
| Areas, in which no deer was recorded in the strong stag class, shot between its $9^{th}$ and $14^{th}$ year of life | | 7, 8, 11, 26, 29, 34, 36, 39, 47, 49, 50 |

Table 4

Regionalization 1 singled out according to the Schema presenting the assignment of forest areas with regard to the weight of red deer antlers as a factor differentiating the population of this species in the Province of Olsztyn, providing their regions and corresponding groups from Table 3

| Regionalization | Groups (1-6) and corresponding regions (I-IV) in regionalization 1 | | | |
|---|---|---|---|---|
| Group (acc. to Table 3) | 1 | 2-4 | 5 | 6 |
| Regionalization 1 | I | II | III | IV |

Table 5

Regionalization 2 singled out according to the Schema presenting the assignment of forest areas with regard to the weight of red deer antlers as a factor differentiating the population of this species in the Province of Olsztyn, providing their regions and corresponding groups from Table 3

| Regionalization | Groups (1-6) and corresponding regions (I-III) in regionalization 2 | | |
|---|---|---|---|
| Group (acc. to Table 3) | 1-2 | 3-5 | 6 |
| Regionalization 2 | I | II | III |

While characterizing the population of deer stags in specified regionalization, statistical calculations were used to calculate the variance analysis. The whole of statistical material was collected and analysed using a spreadsheet in Excel 2003, and the calculation was performed with the use of SPSS 6.0.

# Results and Discussion

## The assignment of forest areas according to the weight of antlers as a factor differentiating population in the Province of Olsztyn

Groups of individuals included in this study were differentiated on the basis of antlers of culled deer (Table 3).

This is a parameter, which unambiguously, as reported by many authors, characterizes individual quality of a stag, and indirectly – the whole population (ZALEWSKI, SZCZEPAŃSKI 2004).

The average antler weight ranged between 5.73 kg (group 6) and 7.87 kg (group 1) – Table 6. The average weight of antlers in group 1 is worth emphasising, as its value statistically differs at the level of $\alpha \leq 0.05$ or $\alpha \leq 0.01$

Table 6

Analysis taking into consideration the Schema presenting the assignment of forest areas, according to own conception, with regard to the weight of antlers as a factor differentiating the population of this species in the Province of Olsztyn

| Features | Statistical measurements | Regions | | | | | | Significance of differences |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| Weight of antlers (kg) | *n* | 65 | 21 | 42 | 34 | 96 | 16 | 1>3*; 1-5>6**<br>1.2>5**; 1>4** |
| | *x* | **7.87** | **7.39** | **7.12** | **6.91** | **6.56** | **5.73** | |
| | *s* | 1.51 | 1.24 | 1.18 | 1.03 | 1.02 | 0.62 | |
| | *v* | 19.19 | 16.78 | 16.57 | 14.91 | 15.55 | 10.82 | |
| Total CIC score (pts) | *n* | 65 | 21 | 42 | 34 | 96 | 16 | 1-5>6** |
| | *x* | **182.37** | **181.66** | **177.95** | **176.83** | **174.60** | **166.15** | |
| | *s* | 15.12 | 11.57 | 11.97 | 10.62 | 10.44 | 8.95 | |
| | *v* | 8.29 | 6.37 | 7.73 | 6.01 | 5.98 | 5.39 | |
| Number of tines (pcs) | *n* | 65 | 21 | 42 | 34 | 96 | 16 | 1,3,4>6*<br>2.5>6** |
| | *x* | **13.80** | **13.71** | **13.12** | **13.09** | **13.36** | **11.94** | |
| | *s* | 2.15 | 1.38 | 2.06 | 1.88 | 1.77 | 1.53 | |
| | *v* | 15.58 | 10.07 | 15.70 | 14.36 | 13.25 | 12.81 | |
| Inside spread (%) | *n* | 64 | 21 | 42 | 34 | 96 | 16 | 1.2>5*<br>4.6>1-3* |
| | *x* | **72.48** | **72.01** | **72.58** | **77.64** | **75.07** | **78.16** | |
| | *s* | 10.26 | 9.85 | 10.83 | 10.33 | 8.31 | 8.30 | |
| | *v* | 14.16 | 13.68 | 14.92 | 13.30 | 11.07 | 10.62 | |

* $P \leq 0.05$
** $P \leq 0.01$

from all remaining parameters, except individuals from group 2. The range of medium values of total CIC score in groups 1-6 was clearly differentiated and ranged from 166.15 pts CIC (group 6) to 182.37 pts. CIC (group 1) was maintained at levels of 8.29% and 5.39%, respectively.

## Analysis in regionalization 1 and 2

The statistical analysis conducted according to the scheme presented in Table 3 made it possible to distinguish four regions in the regionalization diagram 1, as described in Methods (Table 4), in relation to the gross weight of antlers. This statement, in comparison to the presented above diagram presenting the assignment of forest areas taking into consideration the weight of antlers, makes it possible to demonstrate even more clearly the differences between the analysed regions, and at the same time allows the introduction of breeding regions to hunting practice. Excessive fragmentation of hunting grounds, on the other hand, is not a favourable condition for this process.

The division of the province following regionalization 1 – into four regions I-IV (Table 7) allows us to state that there are statistically significant differences between all distinguished regions in relation to the weight of the antlers. This diversity is much less clear in relation to the total CIC score. The analysis

Table 7

Analysis of features in regionalization 1

| Features | Statistical measurements | Regions | | | | Significance of differences |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | |
| Weight of antlers (kg) | *n* | 65 | 97 | 96 | 16 | |
| | *x* | **7.87** | **7.10** | **6.56** | **5.73** | II>III* |
| | *s* | 1.51 | 1.15 | 1.02 | 0.62 | I>II, III, IV** |
| | *v* | 19.12 | 16.15 | 15.63 | 10.88 | II, III>IV** |
| Total CIC score (pts) | *n* | 65 | 97 | 96 | 16 | |
| | *x* | **182.37** | **178.36** | **174.60** | **166.15** | I, II, III>IV** |
| | *s* | 15.12 | 11.45 | 10.44 | 8.95 | I>III** |
| | *v* | 8.29 | 6.42 | 5.98 | 5.39 | |
| Number of tines (pcs) | *n* | 65 | 97 | 96 | 16 | |
| | *x* | **13.08** | **13.24** | **13.36** | **11.94** | I, II, III>IV** |
| | *s* | 2.15 | 1.87 | 1.77 | 1.53 | |
| | *v* | 16.41 | 14.12 | 13.21 | 12.78 | |
| Inside spread (%) | *n* | 64 | 97 | 96 | 16 | |
| | *x* | **72.48** | **74.23** | **75.07** | **78.16** | IV>I* |
| | *s* | 10.26 | 10.65 | 8.31 | 8.30 | |
| | *v* | 14.15 | 14.34 | 11.07 | 10.62 | |

* $P \leq 0.05$
** $P \leq 0.01$

of this parameter shows that only region IV is statistically different from all other regions. A similar tendency can be noted in relation to the number of tines. However, in relation to the inside spread expressed in the percentage, diversification is practically non-existent, except for statistically significant differences at the level of $\alpha \leq 0.05$ between regions IV and I.

Statistically significant differences shown within the diagram presenting the assignment of forest areas (Table 6), do not allow for the clear creation of only one variant of regionalization, therefore, along with regionalization 1, regionalization 2 was created according to the scheme presented in Methods (Table 5).

This regionalization resulted in three regions (I-III) – Table 8, which were created based on the gross weight of antlers. These two features in regionalization 2 demonstrate the highly significant differences in all differentiated regions, except for the analysis of the total CIC score between regions I and II, where statistical differences were found only at the level of $\alpha \leq 0.05$.

Table 8

Analysis of features in regionalization 2

| Features | Statistical measurements | Regions | | | Significance of differences |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| Total CIC score (PTS) | *n* | 86 | 172 | 16 | I>II* I>III** |
| | *x* | **182.20** | **175.86** | **166.15** | |
| | *s* | 14.28 | 10.90 | 8.95 | |
| | *v* | 7.84 | 6.20 | 5.39 | |
| Weight of antlers (kg) | *n* | 86 | 172 | 16 | I>II, III** II>III** |
| | *x* | **7.76** | **6.76** | **5.73** | |
| | *s* | 1.45 | 1.09 | 0.62 | |
| | *v* | 18.69 | 16.12 | 10.82 | |
| Number of tines (pcs) | *n* | 86 | 172 | 16 | I, II>III** |
| | *x* | **13.23** | **13.25** | **11.94** | |
| | *s* | 2.00 | 1.86 | 1.53 | |
| | *v* | 15.12 | 14.04 | 12.81 | |
| Inside spread (%) | *n* | 85 | 172 | 16 | III>I* |
| | *x* | **72.37** | **74.97** | **78.16** | |
| | *s* | 10.10 | 9.48 | 8.30 | |
| | *v* | 13.96 | 12.65 | 10.62 | |

* $P \leq 0.05$
** $P \leq 0.01$

As regards the number of tines, regions I and II statistically highly significantly differ from region III, and in relation to the inside spread expressed in percentage value, differences at the confidence level $\alpha \leq 0.05$ occur between regions III and I.

From a practical point of view, this regionalization seems to be more useful than the one described previously and can be used for conducting breeding and hunting procedures, realized in the population of the Mazurian deer (Table 9, Figure 1).

Table 9

Weight of antlers and total CIC score as parameters deciding on the assignment of areas to regions of individual quality of the Mazurian deer population singled out in regionalization 2

| Region | Weight of antlers (kg) | Total CIC score (pts) | Numbers of forest areas included into regions (acc. to Fig. 1) |
|---|---|---|---|
| I | 7.76 | 182.20 | 1, 2, 5, 16, 19, 20, 24, 32, 38, 41, 43, 48, 51 |
| II | 6.76 | 175.86 | 3, 9, 10, 13, 14, 15, 17, 21, 22, 23, 27, 30, 31, 35, 37, 40, 42, 44, 45 |
| III | 5.76 | 166.15 | 4, 6, 12, 18, 25, 28, 33, 46 |

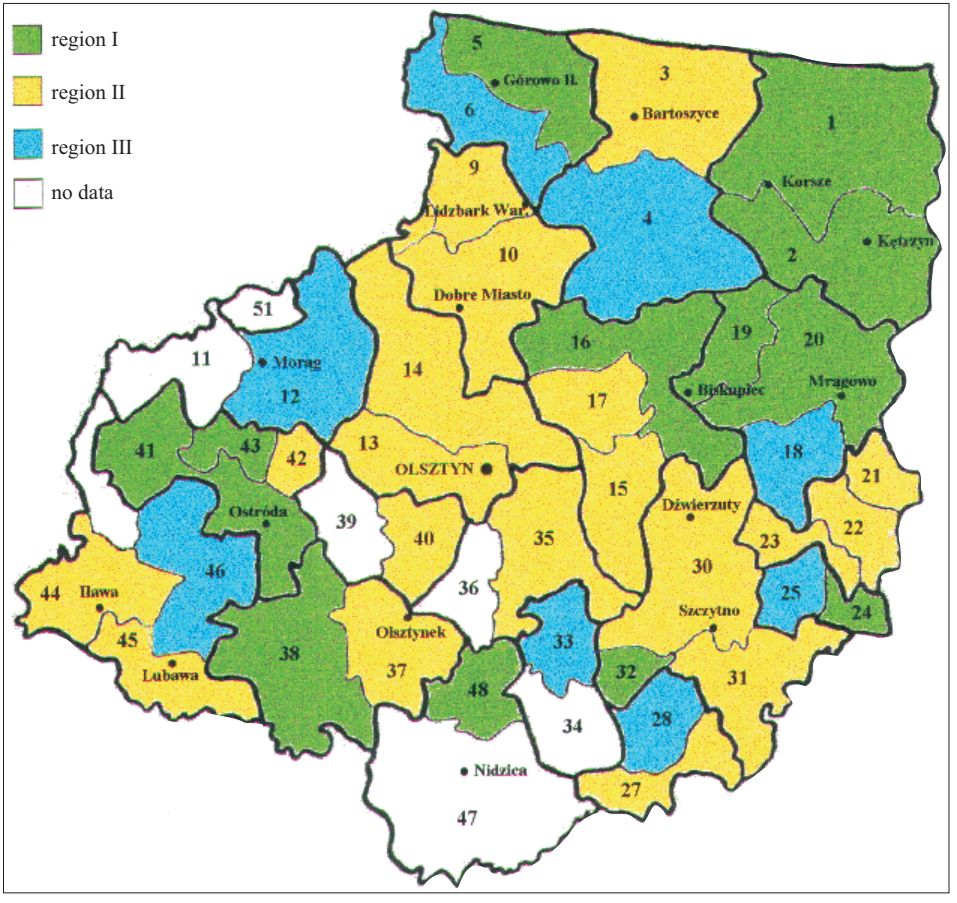


Fig. 1. Map of the province presenting the regions of individual quality of the red deer population in regionalization 2

## Characteristics of habitats in the regions singled out in regionalization 2

While characterizing this regionalization, it can be claimed that forests of the Province of Olsztyn, as regards the age structure of stands in the distinguished regions, have a very similar percentage share (Table 10) of classes Ia and Ib, which include tree stands with up to 20-year-old cover, for each of the regions, respectively: I – 18.73%; II – 16.72% and III – 22.48% (BOBEK et al. 1991). In age classes II-VI, i.e. stands 20-120 years old, equalization of age structure is exceptional. One can clearly observe the percentage share of old stands in VII age class in the regions (3.04%) and II (2.96%) in comparison to region III (1.0%). This is clear not only in this class, but also in relation to stands that are over 120 years old, which occupy in individual regions:

I – 5.44% / 4645.89 ha,
II – 4.59% / 6876.05 ha,
III – 2.68% / 1663.50 ha.

In regionalization 2, one can observe a certain regularity as regards the total CIC score and the gross weight of antlers (Table 8), since the data concerning age structure of stands presented above suggests their possible influence on the quality of deer (BOBEK et al. 1991).

Among the analysed regions, special attention should be paid to the lowest percentage share of habitat 3 (fresh coniferous forest – Bśw) and the highest percentage share of habitat 12 (fresh forest – Lśw) in region I (Table 11). In region III, one can find 34.20% of fresh coniferous forest, which is the largest share of this habitat type among all regions under analysis.

Confirmation of the performed division in regionalization 2 is seen while performing an analysis of habitats according to BOBEK et al. classification (1991) – Table 11. As follows from the analysis, the percentage share of coniferous forest habitats is inversely proportional to the individual quality of red deer stags. In the province, the percentage share of fresh coniferous forests (habitat 3) is very important for 4 coniferous forest habitats, since damp and wet coniferous forest occupy only about 1.27% of all habitats and dry coniferous forest is practically missing (1.06 ha – 0%).

The percentage of other quality classes according to BOBEK et al. (1991) does not confirm the division suggested in regionalization 2. However, the confirmation is accomplished in the summary covering the structure of habitats in I and II quality class according to IBL (Table 11), determining their proportions within the regions. This concerns fresh mixed coniferous forest (BMśw) and fresh forests (Lśw), the percentage of which in individual regions amounts to:

Table 10

Characteristics of habitats according to age classes of tree stands in regionalization 2

| Region | | Surface area of the forest unforested | Age class of the stand (years) | | | | | | | | | | | | | | | Total region area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ia (1-10) | Ib (11-20) | IIa (21-30) | IIb (31-40 | IIIa (41-50) | IIIb (51-60) | IVa (61-70) | IVb (71-80) | Va (81-90) | Vb (91-100) | VI (101-120) | VII (120-140) | VIII over 140 | K.O. | KDO | |
| I | (ha) | 1449.2 | 7515.08 | 8479.92 | 12466.10 | 10782.40 | 6886.04 | 7550.22 | 7667.54 | 5927.57 | 4365.87 | 3543.70 | 4086.00 | 2594.53 | 727.66 | 957.30 | 366.40 | 85365.5 |
| | (%) | 1.70 | 8.80 | 9.93 | 14.60 | 12.63 | 8.07 | 8.84 | 8.98 | 6.94 | 5.11 | 4.15 | 4.79 | 3.04 | 0.85 | 1.12 | 0.43 | 100.00 |
| Total | (%) | 1.70 | 18.73 | | 74.11 | | | | | | | | | 5.44 | | | | 100.00 |
| II | (ha) | 2015.62 | 10116.22 | 14912.50 | 21822.21 | 22639.34 | 11417.58 | 12634.40 | 11788.06 | 10594.77 | 9243.29 | 7818.19 | 7833.18 | 4432.41 | 1269.80 | 659.19 | 514.65 | 149711.41 |
| | (%) | 1.35 | 6.76 | 9.96 | 14.58 | 15.12 | 7.63 | 8.44 | 7.87 | 7.08 | 6.17 | 5.22 | 5.23 | 2.96 | 0.85 | 0.44 | 0.34 | 100.00 |
| Total | (%) | 1.35 | 16.72 | | 77.34 | | | | | | | | | 4.59 | | | | 100.00 |
| III | (ha) | 850.18 | 9382.40 | 4604.29 | 8670.01 | 8206.12 | 4654.06 | 4251.72 | 5697.81 | 4532.74 | 4042.81 | 3467.84 | 2202.54 | 621.07 | 485.93 | 476.26 | 80.24 | 62226.02 |
| | (%) | 1.37 | 15.08 | 7.40 | 13.93 | 13.19 | 7.48 | 6.83 | 9.16 | 7.28 | 6.50 | 5.57 | 3.54 | 1.00 | 0.78 | 0.77 | 0.13 | 100.00 |
| Total | (%) | 1.37 | 22.48 | | 73.48 | | | | | | | | | 2.68 | | | | 100.00 |

Table 11

Characteristics of habitats in regionalization 2 according to quality classed proposed by IBL and Bobek et al.

| Quality class according to IBL | III | | I | | | | | II | | | | | | | Total area of the region |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Quality class according to Bobek et al. | I | | | | II | | | | | | III | | | | |
| Habitat / Region | 1 (Bs) | 2 (Bb) | 3 (Bśw) | 4 (Bw) | 6 (BMśw) | 7 (BMw) | 8 (BMb) | 9 (LMśw) | 10 (LMw) | 11 (LMb) | 12 (Lśw) | 13 (Lw) | 15 (OL) | 16 (OLS) | |
| I (ha) | 1.06 | 464.71 | 12372.75 | 603.40 | 19093.92 | 3026.24 | 608.27 | 13106.33 | 628.62 | 745.80 | 27518.59 | 1247.23 | 4042.38 | 700.53 | 84159.83 |
| (%) | 0.00 | 0.55 | 14.70 | 0.72 | 22.69 | 3.60 | 0.72 | 15.57 | 0.75 | 0.89 | 32.70 | 1.48 | 4.80 | 0.83 | 100.00 |
| Total for the quality class (ha) | 0.55 | | 42.43 | | | | | 57.02 | | | | | | | 100.00 |
| (%) | 15.97 | | | | 44.22 | | | | | | 39.81 | | | | 100.00 |
| II (ha) | 849.10 | 579.25 | 43991.26 | 1702.00 | 56023.88 | 4622.17 | 150.59 | 24793.99 | 1136.07 | 268.30 | 9179.17 | 1044.67 | 4049.03 | 506.72 | 148896.20 |
| (%) | 0.57 | 0.39 | 29.54 | 1.14 | 37.63 | 3.10 | 0.10 | 16.65 | 0.76 | 0.18 | 6.16 | 0.70 | 2.72 | 0.34 | 100.00 |
| Total for the quality class (ha) | 0.96 | | 72.08 | | | | | 27.51 | | | | | | | 100.00 |
| (%) | 31.64 | | | | 58.42 | | | | | | 9.92 | | | | 100.00 |
| III (ha) | 20.65 | 104.79 | 19861.26 | 534.07 | 14758.74 | 1273.83 | 280.76 | 9546.71 | 523.91 | 324.35 | 8283.01 | 447.65 | 1872.78 | 233.69 | 58066.20 |
| (%) | 0.04 | 0.18 | 34.20 | 0.92 | 25.42 | 2.19 | 0.48 | 16.44 | 0.90 | 0.56 | 14.26 | 0.77 | 3.23 | 0.40 | 100.00 |
| Total for the quality class (ha) | 0.22 | | 63.25 | | | | | 36.56 | | | | | | | 100.00 |
| (%) | 35.34 | | | | 45.99 | | | | | | 18.66 | | | | 100.00 |

I – 55.39%,
II – 43.79%,
III – 39.68% all habitat types within the region.

According to ŁABUDZKI (1993), in lowland hunting areas, optimum conditions for the growth of antlers are found in habitats in which 60-80% of the surface area is covered by forests and 20-40% by mixed coniferous forests. The most attractive type of habitats for deer in the summer period, according to BOBEK et al. (1991), consists of deciduous forests, due to a large percentage of herbs and leaved forage. The next in order of attractiveness are mixed forests, which, in turn, are abundant with potential food, and finally coniferous forests. However, in winter, reserves of current growth of shrubs and green parts of herbs, grasses and sedges are found in the following habitats: Bśw, Bw, BM, with the exception of Bs. At the same time, those are habitats most preferred by red deer, where they can find the largest amounts of good food and proper protective conditions.

To sum up, it can be stated that in the Province of Olsztyn, in the hunting season, the smaller percentage share of fresh coniferous forest with a larger percentage of fresh forest produce better quality Mazurian red deer.

## Conclusions

1. The conducted research made it possible to state that the most useful type of regionalization for conducting game management in the population of Mazurian red deer in the Province of Olsztyn is regionalization 2, the one that has been singled out on the basis of the weight of antlers. As follows from the regionalization, the best deer quality in the province can be found in the following areas: 1 – Gierdawy, 2 – Kętrzyn, 5 – Borki, 16 – Sadłowo W., 19 – Sadłowo M., 20 – Gązwa, 24 – Spychowo, 32 – Szczytno, 41 – Miłomłyn, 43 – Tarda, 48 – Koniuszyn and 51 – Danielewo (Figure 1).

2. The analysis performed demonstrates that definitely the weakest deer were culled in the areas of: 4 – Bartniki, 6 – Bukowiec, 12 – Ponarzyny, 18 – Mrągowo, 25 – Racibór, 28 – Wesołowo, 33 – Dłużek and 46 – Drwęca (Figure 1).

3. In the areas of the poorer individual quality of red deer stags, the average gross weight of 16 analysed red deer antlers was 5.73 kg, the total CIC score without taking into consideration additional points or deductions was on average, 166.15 pts. A statistically culled stag in these areas had regular twelve-pointed antlers with the crown on both sides, and was characterized by the largest inside spread of antlers among all analysed regions. For comparison: in areas 1 – Gierdawy, 2 – Kętrzyn, 5 – Borki,

16 – Sadłowo W., 19 – Sadłowo M., 20 – Gązwa, 24 – Spychowo, 32 – Szczytno, 38 – Grunwald, 41 – Miłomłyn, 43 – Tarda, 48 – Koniuszyn and 51 – Danielewo, where the strongest stags were harvested, were characterized by, respectively: the gross weight of antlers – 7.76 kg, the total CIC score – 182.20 pts, the form of antlers – regular fourteen-pointed antlers with both crowns (13.23 tines) and the inside spread – 72.37%.

4. The analysis of features within regionalization 1 and 2 helped to demonstrate the differences between the analysed regions more clearly. This proves the need to conduct further research on the possibilities of introducing breeding regions into hunting practice. On the other hand, excessive fragmentation of hunting grounds does not favour this process, which is confirmed in Table 6.

5. The analysis of habitats according to the classification suggested by BOBEK et al. (1991) in regionalization 2 indicates that the increase in the percentage of coniferous forest habitats results in the decrease in the individual quality of red deer stags. The percentage of other quality classes according to BOBEK et al. (1991) does not confirm the division suggested in regionalization 2. However, the confirmation is accomplished in the summary covering the structure of habitats in I and II quality class, according to IBL.

6. A positive dependence was found between the quality of stag antlers harvested in the hunting season and the percentage share of fresh forests in the area of the Province of Olsztyn.

7. The effects of research, resulting from a detailed analysis of selected environmental features and deer antlers, should become source material to determine the principles of managing the population of Mazurian deer and to establish regional criteria of culling red deer stags in the area of the former Province of Olsztyn.

Translated by JOANNA JENSEN

Accepted for print 2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 277-289, Y. 2006

# THE QUALITY OF ROE DEER (*Capreolus c. capreolus* L.) AND AN ASSESSMENT OF THE MANAGEMENT OF ITS POPULATIONS IN THE OLSZTYN DISTRICT OF THE POLISH HUNTING ASSOCIATION

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K e y w o r d s: roe deer (*Capreolus capreolus* L.), antlers, game management.

A b s t r a c t

The aim of the paper was to present the ontogenetic quality of the roe deer and the assessment of breeding and hunting procedures realized in its population in hunting breeding regions located in the Olsztyn district of the Polish Hunting Association.

Research material analysed in this paper was the roe deer population in the area of the Olsztyn district of the Polish Hunting Association. Statistical material was gathered from 203 hunting grounds, i.e. all grounds located in the area of the Olsztyn district of the Polish Hunting Association. Hunting grounds are grouped in hunting breeding regions for which long-term breeding plans are developed.

Data collected in the paper originates from annual hunting plans for the season 2002/2003 and from the sheets of compliance for culling deer males shot in the hunting season 2001/2002. The performed analysis allowed to formulate the following conclusions and generalizations: – in the analysed regions, the indicator of the realization of plans of the roe deer harvesting in the hunting season 2001/2002 was between 89-99%, and the indicators: of planned harvesting (B) and of population exploitation (C) ranged from 20-34% to 87-100%, respectively; – in most hunting breeding regions, as follows from the performed analysis, reduction culling was planned; – the structure of roe deer harvesting in sex and age groups indicates too large percentage of shot kids (14.69-23.04%) in relation to established breeding norms; – what is alarming is the fact that in as many as in three regions out of regions No. 1 – 4, the culling of age class I of roebucks exceeds 50% of shot males; – in the 2nd year the average carcass weight of a male roe deer ranges from 14.0 to 15.7 kg, the weight of antlers: 145.6-164.8 g, and a roebuck at this age was a statistical a spike-antlered buck or an irregularly fork-antlered buck; – the weight of carcass of males in their 6th year and older, was 17.0-18.9 kg, the weight of antlers: 257.3-343.4 g. A statistical male at this age was an irregularly six-point-antlered buck, and every 4th-5th animal was a regularly six-point-antlered buck; – users of hunting areas and persons responsible for approving annual hunting plans disregard basic rules of game management, which is demonstrated by the errors made in game planning and in its documentation; – within the next years there should be organized cycles of training for users of hunting areas, concerning game planning and keeping proper breeding documentation.

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**JAKOŚĆ SARNY (*Capreolus c. capreolus* L.) ORAZ OCENA GOSPODAROWANIA JEJ POPULACJAMI W OKRĘGU OLSZTYŃSKIM POLSKIEGO ZWIĄZKU ŁOWIECKIEGO**

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S ł o w a k l u c z o w e: sarna (*Capreolus capreolus* L.), poroże, gospodarka łowiecka.

A b s t r a k t

Zaprezentowano jakość osobniczą sarny europejskiej oraz oceniono zabiegi hodowlano-łowieckie realizowane w jej populacji w rejonach hodowlanych. Materiałem badawczym była populacja sarny występującej na terenie okręgu olsztyńskiego PZŁ. Materiał statystyczny zebrano w odniesieniu do 203 obwodów łowieckich, tj. wszystkich położonych na terytorium okręgu olsztyńskiego PZŁ. Na podstawie analizy wyciągnięto następujące wnioski i uogólnienia: wskaźnik realizacji planów pozyskania sarny w sezonie łowieckim 2001/2002 wyniósł w analizowanych rejonach 89-99%, wskaźniki planowanego pozyskania (B) i eksploatacji populacji (C) wynosiły odpowiednio 20-34% i 87-100%; w 2. roku życia przeciętna masa tuszy sarny rogacza oscylowała w przedziale 14,0-15,7 kg, masa poroża 145,6-164,8 g, natomiast rogacz w tym wieku był przeciętnie szpicakiem lub widłakiem nieregularnym; masa tuszy rogaczy w 6. roku życia i starszych wynosiła 17,0-18,9 kg, a masa poroża 257,3-343,4 g. Stwierdzono, że użytkownicy obwodów łowieckich i osoby odpowiedzialne za zatwierdzanie rocznych planów łowieckich lekceważą podstawowe zasady hodowli zwierzyny, o czym świadczą błędy w planowaniu łowieckim i prowadzonej dokumentacji.

## Introduction

The density of the roe deer in many hunting areas in Poland seems to be far below the level that is encouraged by the current guidelines with regard to this issue. These guidelines, until quite recently, explicitly pointed out that the density of roe deer in Polish hunting areas should be 60-150 animals per 1.000 ha of hunting ground. The anticipated realized growth, taken into consideration in annual hunting plans, should not exceed 30% of the spring population. Additionally, taking into account the excessive density of the fox population in Polish hunting areas (BRESIŃSKI, PANEK 2000), it should be stated that this is the level which considerably exceeds the reality of growth realized in the population of this species. The roe deer management principles establish the shooting of males in age classes I and II in the analysed period at a 1:1 ratio. To verify how game management was conducted in the populations in the Warmia and Mazury region, it has been and it is still necessary is to monitor the actions of game management (ZALEWSKI et al. 2001).

The aim of the paper is to present the ontogenetic quality of the European roe deer and the assessment of breeding and hunting procedures conducted in its population in hunting breeding regions in the area of the Olsztyn district of the Polish Hunting Association.

## Materials and Methods

The research material analysed in this paper was the roe deer population in the area of the Olsztyn district of the Polish Hunting Association. Statistical material was gathered with reference to 203 hunting grounds, i.e. all grounds that are located within the Olsztyn district of the Polish Hunting Association. Hunting grounds are grouped in hunting breeding regions (Figure 1) – Regional Board of the State Forests in Olsztyn and include:
„North Mazury / Warmia and Mazury" – region No. 1;
„Wipsun" – region No. 2;
„The Napiwodzko-Ramucki Region" – region No. 3;
„The Taborskie Forests" – region No. 4;
„The Piska Primeval Forest" – region No. 6 – a part of its area belongs to the Commune of Mikołajki (Regional Board of the State Forests in Białystok).

182 hunting grounds are used by hunting clubs, and the management over the remaining 21 grounds is held by Game Breeding Centres.

Data collected in the paper originates from annual hunting plans for the season 2002/2003 and from the sheets of compliance for culling deer males shot in the hunting season 2001/2002. While analysing the data contained in the assessment sheets of the compliance of roebuck culling, the following factors were taken into account: the age of the animal, the weight of its carcass, the gross weight of spikes and the form of antlers according to the CIC system (VARIĆAK 2001, STACHOWIAK 1994). While determining the number of tins in the antlers, the sum of the tins on both spikes were included. Moreover, the roebucks were divided into two age classes: class I – roebucks in their 2nd and 3rd years (first and second antlers) and class II – roebucks from their 4$^{th}$ year on – third and further antlers (*Resolution...* 2005).

Additionally, male deer were divided into four age groups:
– group 1 – in their 2nd year,
– group 2 – in their 3rd year,
– group 3 – in their 4-5th year,
– group 4 – in their 6th year and older.

The statistical data originating from the annual hunting plans, was used for calculating the following breeding and hunting indicators for hunting breeding regions:

Realization of planned harvest of the roe deer for a hunting season (A):

$$A = \frac{\text{culling in the season 2001/2002}}{\text{a culling plan in the season 2001/2002}} \cdot 100\%$$

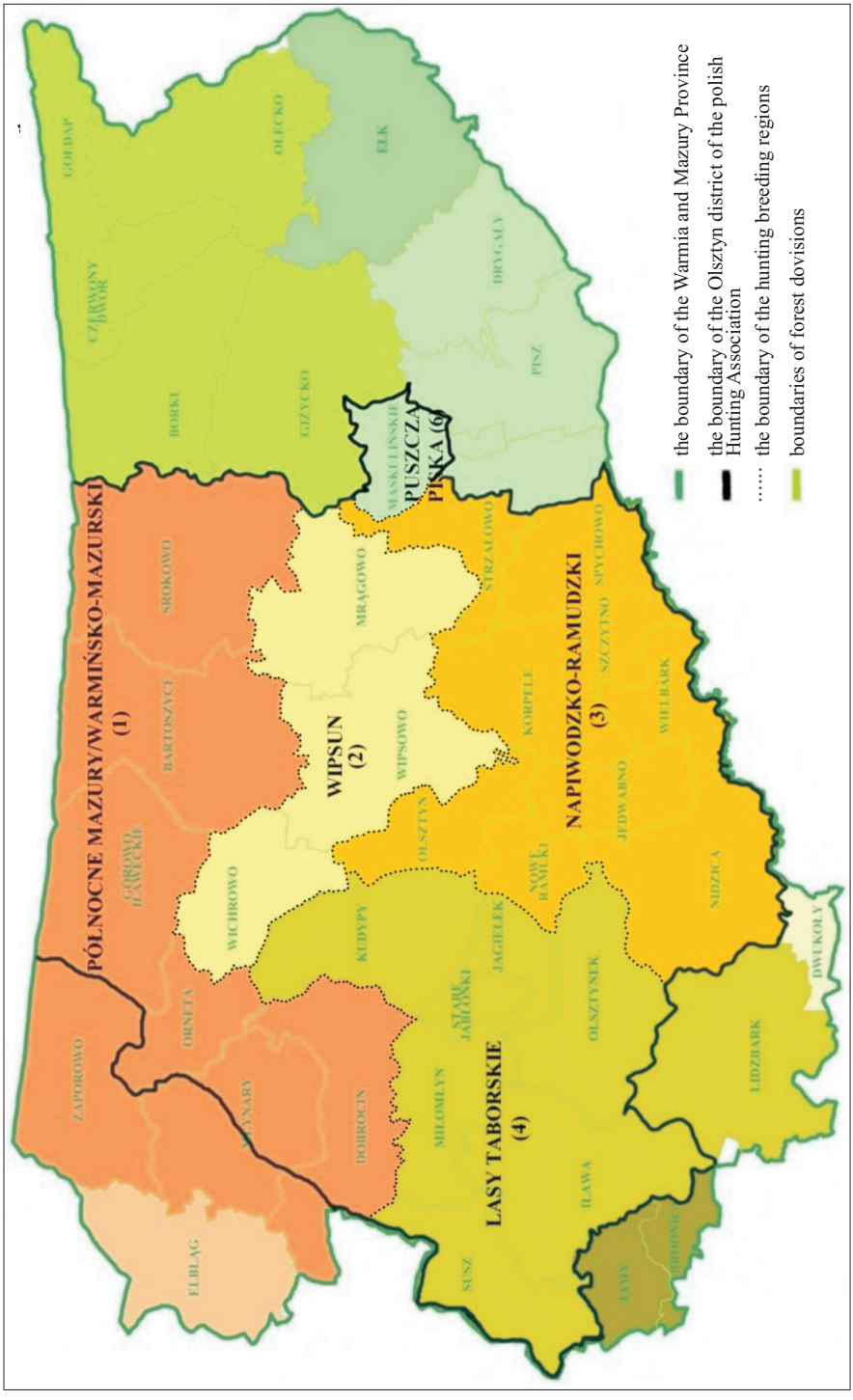


Fig. 1. Hunting breeding regions in the Olsztyn district of the Polish Hunting Association

Planned harvest of the roe deer in relation to their spring population (B)

$$B = \frac{\text{a culling plan for the hunting season 2002/2003}}{\text{state as of 31.03.2002}} \cdot 100\%$$

Exploitation of the roe deer population (C)

$$C = \frac{\text{(state before the hunting season 2002/2003)} - \text{(state as of 31.03.2002)}}{\text{a culling plan for the hunting season 2002/2003}} \cdot 100\%$$

The analysis of the data gathered in the sheets for assessing the compliance of roebuck culling consisted in providing the number of animals ($n$) and calculating the statistics: the arithmetic mean ($x$) and the standard deviation ($s$). The calculations were made in an Excel spread sheet, and the obtained results were presented in the form of tables and charts.

## Results

### 1. Breeding and hunting indicators concerning game management conducted in the roe deer population

In discussing the results, it must be remarked that hunting breeding region No. 6 only partially belongs to the Olsztyn district (Figure 1). Therefore, the results obtained in this region differ from the others. The analysis of the collected data (Table 1) concerning the roe deer population in the area of the Olsztyn district of the Polish Hunting Association showed that the culling of the roe deer in the season 2001/2002 involved 9.665 animals.

According to the performed stock taking, the roe deer population as of 31 March 2002 amounted to 34.418 animals, and the estimated population before the hunting season was 44.145 animals.

The breeding and hunting indicators for the discussed populations reached the following values: indicator (A) for the Olsztyn district – 92%, indicator (B) – 31%, and the exploitation of population (C) in the season under analysis – 91%.

On the scale of the Olsztyn district, the planned harvesting was slightly above the maximum level of the realized growth. However, after obtaining the realization of the plan of game harvesting in the recent years (ZALEWSKI et al.

Table 1
Game management realized in the roe deer population in the season 2001/2002 in the area of hunting and breeding regions established in the Olsztyn district of the Polish Hunting Association

| Hunting breeding region | Roe deer (no. of animals) | | | | | Breeding indicators (%) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | A | B | C |
| | culling plan 2001/2002 | realization of culling 2001/2002 | state as of 31.03.2002 | state before the season 2002/2003 | culling plan 2002/2003 | realization of harvesting plan for the season 2001/2002 | planned harvesting in relation to spring population | exploitation of population |
| | 1 | 2 | 3 | 4 | 5 | 2/1 | 5/3 | 4-3/5 |
| 1 | 2298 | 2105 | 8579 | 11 050 | 2546 | 92 | 30 | 97 |
| 2 | 2063 | 1973 | 6201 | 8054 | 2121 | 96 | 34 | 87 |
| 3 | 2707 | 2403 | 8223 | 10 559 | 2592 | 89 | 32 | 90 |
| 4 | 3265 | 3020 | 10595 | 13 497 | 3258 | 92 | 31 | 89 |
| 6 | 165 | 164 | 820 | 985 | 165 | 99 | 20 | 100 |
| Total | 10 498 | 9665 | 34 418 | 44 145 | 10 682 | 92 | 31 | 91 |

2001) it should be stated that indicator B, which takes into account the real harvesting, runs at the level of the standard established for this population. The rule itself, which establishes a low realized growth in the annual hunting plan, and at the same time a higher level of harvesting (indicator C), despite the fact that it can fit within current standards, seems to be completely unjustified.

The highest population occurred in breeding regions No. 4 – 10.595, and No. 1 – 8.579 animals. These are the regions where the most intensive culling was carried out in the season 2001/2002. In hunting breeding region No. 4, the number of shot animals was 3.020, and in region No. 1 – 2.105. In region No. 6 only 820 animals were recorded. And it was there where their smaller number was harvested – 164 animals. This is related, as it has been mentioned above, to the small number of grounds that belong to region No. 6 and form a part of the Olsztyn district of the Polish Hunting Association (Figure 1).

Upon an analysis of breeding and hunting indicators, it can be stated that indicator (A) in all regions reached a high level: the smaller value was obtained in breeding region No. 3 – 89%, and the highest value in region No. 6 – 99%. Indicator (B) reached its lowest value in breeding region No. 6-20% and in region No. 1 – 30%. However, in these regions the highest value of indicator (C) was recorded, which in region No. 6 reached the level of 100%, and in region No. 1 – 97%. The highest value of indicator (B) was observed in breeding region No. 2 – 34%, where the exploitation of the population (C) reached the smallest

value – 87%. While analysing the values of indicators (B) and (C), it can be stated that the higher is value of indicator (B), the lower is the value of indicator (C) and the other way round. This means that increasing the harvesting above the realized growth indicates reduction culling in many hunting breeding regions of the Olsztyn district. What can be a consolation is the fact that, undoubtedly, the stocktaking of the roe deer population does not reflect its real number in the Olsztyn district, and the underestimation of the population compensates for the indicated disadvantageous phenomena caused by game management.

The analysis performed for the hunting and breeding regions supports the previous findings that the plans for roe deer harvesting in the regions are, generally, not met. Culling plans for the season were often above the real growth realized for a given hunting area. As follows from the annual hunting plans, a reduction of the roe deer population was planned in these regions. More animals were planned for culling in a given season than could result from the established realized growth (indicator C). An ideal situation is found in four mentioned hunting grounds belonging to hunting and breeding region No. 6, where breeding and hunting indicators run at the following levels: A = 99%, C = 100%, and B = 20%, and this is, on all accounts, the correct situation, taking into consideration the increasing density of fox in our hunting areas, which has a considerable influence on the survival rate of kids (DZIĘCIOŁOWSKI 2000, KAMIENIARZ, BRESIŃSKI 2000).

## 2. Sex structure of conducted culls in the roe deer population

While analysing the data related to the structure of roe deer harvesting in sex groups for the hunting season 2001/2002 over the area of hunting breeding regions established in the Olsztyn district (Table 2), it should be concluded that culling of roebucks was close to the value set in the then in force standards of structural culling (harvesting), and amounted at 40%. In the analysed hunting breeding regions it was between 36.29% (region No. 2) and 42.07% (region No. 3). Upon the analysis of the remaining two sex groups, it can be claimed that the culling of does ran at a low level in relation to the value of harvesting set by standards at the level of 50%. It was between 37.96% (region No. 1) and 43.99% (region No. 2). In total, 4.069 does were shot in the whole of Olsztyn district, which was 42.10% in relation to the total number of culled deer.

A certain inaccuracy can be noticed in the culling of kids. It was then assumed that the harvesting of kids in stabilized populations should be at the level of 10%. However, in the season 2001/2002, 1.722 animals were shot,

Table 2
Structure of roe deer harvesting in sex and age groups in the hunting season 2001/2002 in the area of hunting and breeding regions established in the Olsztyn district of the Polish Hunting Association

| Hunting breeding region | Roe deer (total) | | Roebucks | | Does | | Kids | |
|---|---|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % | *n* | % |
| 1 | 2105 | 100 | 821 | 39.00 | 799 | 37.96 | 485 | 23.04 |
| 2 | 1973 | 100 | 716 | 36.29 | 868 | 43.99 | 389 | 19.72 |
| 3 | 2403 | 100 | 1011 | 42.07 | 1039 | 43.24 | 353 | 14.69 |
| 4 | 3020 | 100 | 1258 | 41.66 | 1293 | 42.81 | 469 | 15.53 |
| 6 | 164 | 100 | 68 | 41.46 | 70 | 42.68 | 26 | 15.85 |
| Total | 9665 | 100 | 3874 | 40.08 | 4069 | 42.10 | 1722 | 17.82 |

which made 17.82% in relation to the culled deer. Improper harvesting of kids was recorded particularly in region No. 1 – 23.04%. According to current breeding standards, the aim should be to harvest the largest possible amount of does without kids, as well as weak and sick animals. In the case of kids, all harvested animals should be females. Excessive culling of kids in the population could result in upsetting its age structure.

In the analysed hunting breeding regions, the general rule in performing structural culling according to sex and age groups is shooting roebucks and does in similar proportions, with slightly more females and with too many shot kids. The worst situation is in hunting and breeding region No. 1, where it definitely departs from the current standards. Additionally, with large natural losses of kids (KAMIENIARZ, BRESIŃSKI 2000) this seems to be unjustified.

## 3. Age structure of realized culls in the roebuck population

The structure of roebuck harvesting in age classes in the hunting season 2001/2002 was at the following level: in age class I (2nd – 3rd year) – 1,074 roebucks were shot, which accounted for 51.09% of the total number of culled roebucks. Roebuck harvesting in class II (from the 4th year on) was at the level of 1.028 animals, which constituted 48.91% of all roebucks shot in this season (Table 3). According to general breeding rules binding for the analysed period, the culling of roebucks in age class I should be between 40 and 50% of the total number of harvested males (KAMIENIARZ, BRESIŃSKI 2000). In breeding region No. 4, the level of harvested roebucks was the closest to current breeding rules. In this hunting breeding region, 289 roebucks in age class I were shot, which accounts for 50.61%, and 282 animals of age class II, i.e. 49.39% of the total number of harvested roebucks. On the basis of the data (Table 3), it can be

claimed that the culling of roebucks in age class II in hunting breeding regions was understated. The harvesting of roe deer males in breeding regions in age class II ranged from 45.72% in region No. 2 to 54.34% in breeding region No. 3.

Table 3
Structure of roebuck harvesting in age classes in the hunting season 2001/2002 in the area of hunting and breeding regions established in the Olsztyn district of the Polish Hunting Association

| Hunting breeding region | Roebucks (total) | | Class I | | Class II | |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % |
| 1 | 457 | 100 | 242 | 52.95 | 215 | 47.05 |
| 2 | 573 | 100 | 311 | 54.28 | 262 | 45.72 |
| 3 | 438 | 100 | 203 | 46.35 | 238 | 54.34 |
| 4 | 571 | 100 | 289 | 50.61 | 282 | 49.39 |
| 6 | 63 | 100 | 29 | 46.03 | 34 | 53.97 |
| Total | 2102 | 100 | 1074 | 51.09 | 1028 | 48.91 |

What can be alarming is the fact that, while taking into consideration hunting and breeding regions No. 1 – 4, in as many as 75% of regions, culling of age class I at a level exceeding 50% of shot males was recorded. This can be even more alarming, as commissions responsible for assessment of the compliance with roebuck culling make the most frequent mistakes by classifying animals in their 3rd year as 4th year. In this context, we are far from meeting the goal of culling the males of roe deer in the ratio: 40% – class I, and 60% – class II (ZALEWSKI 2002).

## 4. Characteristics of the carcass weight, the gross weight of spikes and the form of antlers of roebucks shot in the hunting season 2001/2002

In order to characterize the quality of the roebuck population in hunting and breeding regions of the Olsztyn district of the Polish Hunting Association, the parameters of antlers and weight of their carcass were analysed (Table 4, Figures 2-4). Statistical material was gathered in relation to five breeding regions. In the season 2001/200, the weight of carcass of males in the first age group (in their 2nd year) was on average 15.2 kg (Figure 2). The weight of carcasses in this age group ranged from 14.0 kg in region No. 6 to 15.7 kg in region No. 1 (Table 4).

Table 4

Characteristics of the carcass weight, the gross weight of spikes and the form of antlers of roebucks shot in the season 2001/2002 in the Olsztyn district of the Polish Hunting Association

| Age group | Hunting breeding region | Carcass weight (kg) | | Spikes gross weight (g) | | Number of tines | |
|---|---|---|---|---|---|---|---|
| | | *n* | *x* | *n* | *x* | *n* | *x* |
| 2nd year (I) | 1 | 185 | 15.7 | 185 | 163.4 | 185 | 0.8/2.8* |
| | 2 | 197 | 15.4 | 197 | 155.6 | 197 | 0.6/2.6* |
| | 3 | 111 | 14.8 | 111 | 145.6 | 111 | 0.5/2.5* |
| | 4 | 183 | 14.8 | 183 | 164.4 | 183 | 0.6/2.6* |
| | 6 | 17 | 14.0 | 17 | 164.8 | 17 | 0.6/2.6* |
| Total | *n* | 693 | | 693 | | 693 | |
| | *x* | 15.2 | | 158.6 | | 0.6/2.6* | |
| | s | 2.0 | | 47.8 | | 0.9 | |
| 3rd year (II) | 1 | 57 | 16.6 | 57 | 225.1 | 57 | 2.0/4.0* |
| | 2 | 114 | 16.9 | 114 | 212.0 | 114 | 1.8/3.8* |
| | 3 | 92 | 15.3 | 92 | 182.9 | 92 | 1.3/3.3* |
| | 4 | 106 | 15.6 | 106 | 207.8 | 106 | 1.3/3.3* |
| | 6 | 12 | 15.9 | 12 | 235.4 | 12 | 1.8/3.8* |
| Total | *n* | 381 | | 381 | | 381 | |
| | *x* | 16.1 | | 206.5 | | 1.6/3.6* | |
| | s | 2.2 | | 52.9 | | 1.3 | |
| 4th – 5th year (III) | 1 | 143 | 18.1 | 143 | 286.6 | 143 | 2.7/4.7* |
| | 2 | 189 | 17.7 | 189 | 263.2 | 189 | 3.0/5.0* |
| | 3 | 176 | 16.9 | 176 | 244.1 | 176 | 2.5/4.5* |
| | 4 | 170 | 17.4 | 170 | 279.0 | 170 | 2.9/4.9* |
| | 6 | 18 | 17.2 | 18 | 276.3 | 18 | 3.3/5.3* |
| Total | *n* | 696 | | 696 | | 696 | |
| | *x* | 17.5 | | 267.4 | | 2.8/4.8* | |
| | s | 2.7 | | 66.0 | | 1.2 | |
| 6th year and older (IV) | 1 | 72 | 18.9 | 72 | 343.4 | 72 | 3.3/5.3* |
| | 2 | 73 | 18.5 | 73 | 318.9 | 73 | 3.1/5.1* |
| | 3 | 59 | 17.1 | 59 | 257.3 | 59 | 2.9/4.9* |
| | 4 | 112 | 17.6 | 112 | 340.2 | 112 | 3.2/5.2* |
| | 6 | 16 | 17.0 | 16 | 321.2 | 16 | 3.4/5.4* |
| Total | *n* | 332 | | 332 | | 332 | |
| | *x* | 18.0 | | 320.6 | | 3.2/5.2* | |
| | s | 2.1 | | 82.5 | | 1.2 | |

* While determining the form of antlers in this paper, the sum of the number of tins from both spikes was included. A tin was defined as both the anterior and posterior tins as well as prickets.

The average gross weight of spikes in the first age group was 158.6 g (Figure 3). The maximum weight of spikes was noted in hunting breeding region No. 6 – 164.8 g, and the smallest – in region No. 3 – 145.6 g (Table 4). A statistical harvested male in this age group was a spike-antlered buck or an irregularly fork-antlered buck. While determining the form of antlers in this paper, the sum of the number of tins from both spikes was included. A tin was defined as both the anterior and posterior tins as well as prickets. In the

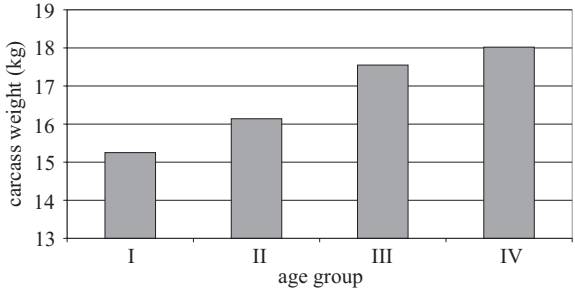


Fig. 2. Characteristics of the carcass weight of roebucks in the season 2001/2002

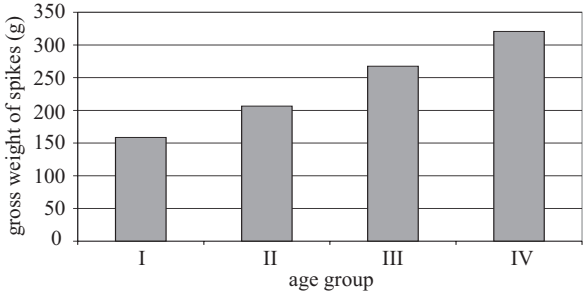


Fig. 3. Characteristics of the gross weight of spikes of roebucks shot in the season 2001/2002

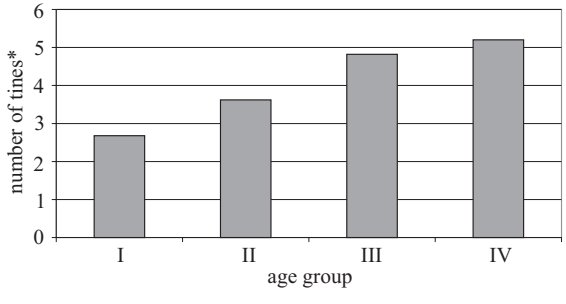


Fig. 4. Characteristics of the form of antlers of roebucks shot in the season 2001/2002
* While determining the from of antlers in this paper, the sum of the number of tins from both spikes was included. A tin was defined as both the anterior and posterior tins as well as prickets.

above-mentioned case, the average number of tins including two prickets was 2.6 (Figure 4).

The gross weight of spikes in the second age group averaged 206.5 g (Figure 3). In individual regions, the weight of spikes ranged from 182.9 g (region No. 3) to 235.4 g (region No. 6) – Table 4. The weight of carcass

averaged 16.1 kg. A statistical male in its third year was an irregularly or regularly fork-antlered buck ($x$ = 3.6 tines). In the third age group, the average weight of carcass was 17.5 kg. It reached the lowest value of 16.9 kg in region No. 3 and the largest value of 18.1 kg in breeding region No. 1. The average gross weight of spikes in this age group was 267.4 g. Its maximum value amounted to 286.6 g in breeding region No. 1 and the smallest value – 244.1 g in region No. 3. Statistically, a harvested roebuck was in its $4^{th}$ – $5^{th}$ year, a regularly fork-antlered or irregularly six-point-antlered buck, with x = 4.8 tines. In the fourth age group among the 6th year and older specimens, the average carcass weight was 18.0 kg and ranged from 17 kg (region No. 6) to 18.9 kg (region No. 1). The average gross weight of spikes of roebucks in their 6th year and older was 320.6 g (Figure 3). The lowest value of 257.3 g was noted in region No. 3, and the highest – 343.4 g in hunting breeding region No. 1 (Table 1). The average number of tines in the fourth age group was 5.2. This indicates that, statistically, a culled male deer in this age group was an irregularly six-point-antlered buck and every fifth male deer was a regularly six-point-antlered buck.

## Conclusions

On the basis of the performed analysis of annual hunting plans and assessments of compliance with roebuck culling for the season 2001/2002, the following conclusions and generalizations concerning the realization of game management in the roe deer population can be drawn, and an assessment of its quality in the Olsztyn district of the Polish Hunting Association can be presented:

- in the analysed regions, the indicator of the realization of plans for the roe deer harvesting in the hunting season 2001/2002 was between 89 and 99%, and indicators: of the planned harvesting (B) and the exploitation of population (C) ranged from 20-34% to 87-100%;
- in most hunting and breeding regions, the reduction culling was planned;
- the structure of harvesting deer by sex and age groups indicates an excessively large percentage of culled kids (14.69-23.04%) in relation to the established breeding standards;
- what is alarming is the fact that in as many as three out of four regions, culling of age class I of roebucks exceeds the level of 50% of shot males;
- in the $2^{nd}$ year, the average weight of roebuck carcass is between 14.0 and 15.7 kg, the gross weight of its antlers: 145.6-164.8 g, and a statistically harvested roebuck at this age was a spike-antlered buck or an irregularly fork-antlered buck;

– the weight of carcass of males in their 6th year and older is 17.0-18.9 kg, the spikes weight is 257.3-343.4 g, and a statistical buck at this age was an irregularly six-point-antlered buck, and every 4th-5th animal is a regularly six-point-antlered buck;
– users of hunting areas and those responsible for approving annual hunting plans disregard basic rules of game breeding, as demonstrated by the errors made in game planning and the documentation;
– in the future there should be organized cycles of training for users of hunting areas, which should concern game planning and keeping proper breeding documentation.

Translated by JOANNA JENSEN

Accepted for print 21.04.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 291-305, Y. 2006

# TEMPERATURE AND DISSOLVED OXYGEN CHARACTERISTICS OF THE LAKES IN THE UPPER PASŁĘKA RIVER CATCHMENT

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K e y  w o r d s: eutrophication, temperature and oxygen characteristics, water mass dynamics.

A b s t r a c t

The studies were carried out on Lakes Wymój, Sarąg, Łęguty and Isąg, through which flows the Pasłęka River. The lakes differ by morphometric properties. The setting of the lakes in the young glacial and undulating area restricts wind activity on the water table. According to the criteria by OLSZEWSKI (1959), Wymój and Łęguty can be classified as bradymictic while Sarąg and Isąg are eumictic.

In the surface water of all the examined lakes, oxygen saturation periodically exceeded 100%, evidence for intensive production processes. On the other hand, in the deeper water, especially near the bottom, oxygen was rapidly consumed or eventually completely deficient. The clinograde oxygen curves observed in the lakes in the summer point out their highly eutrophic condition.

Undoubtedly, one of the main reasons for the poor water quality in the discussed lakes is the contaminating effect of the Pasłęka River

## UKŁADY TERMICZNE I TLENOWE W JEZIORACH GÓRNEJ PASŁĘKI

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S ł o w a  k l u c z o w e: eutrofizacja, układy termiczne i tlenowe, dynamika mas wodnych.

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Abtsrakt

Badaniami objęto jeziora Wymój, Sarąg, Łęguty oraz Isąg, przez które przepływa rzeka Pasłęka. Zbiorniki te są bardzo interesującymi obiektami badań, gdyż w 1970 r. w dorzeczu Pasłęki utworzono rezerwat bobrów „Ostoja bobrów na rzece Pasłęce". W skład rezerwatu wchodzą rzeka Pasłęka od źródeł oraz południowa część jez. Isąg. Ze względu na niepowtarzalne walory krajobrazowe i przyrodnicze jeziora te powinny podlegać określonym zasadom użytkowania i ochrony.

Obecnie są prowadzone badania zmierzające do określenia stanu żyzności powyższych zbiorników oraz oddziaływania zlewni na tempo zachodzących w nich przemian. Celem pracy było rozpoznanie układów termiczno-tlenowych oraz dynamiki mas wodnych jezior górnej Pasłęki.

W jeziorach Wymój i Łęguty stwierdzono występowanie krótkiej cyrkulacji wiosennej, wyraźnej i długotrwałej stratyfikacji termicznej latem oraz niskich temperatur hypolimnionu. Takie cechy pozwalają zakwalifikować je do jezior o V stopniu statyczności wg PATALASA (1960) lub do zbiorników bradymiktycznych zgodnie z podziałem OLSZEWSKIEGO (1959) i stratyfikowanych stabilnych (ABERG, RHODE 1942).

Jeziora Sarąg i Isąg zaliczono do jezior o IV stopniu statyczności (PATALAS 1960) lub zbiorników eumiktycznych, pośrednich między łatwo- i trudnocyrkulującymi (OLSZEWSKI 1959).

W powierzchniowych warstwach wody wszystkich badanych jezior nasycenie tlenem, w niektórych okresach przekraczało znacznie 100%, co wskazywało na intensywne procesy produkcyjne. W głębszych warstwach wody, zwłaszcza przy dnie, obserwowano dość szybkie wyczerpywanie tlenu prowadzące do jego całkowitego zaniku.

Występujące latem w badanych zbiornikach krzywe tlenowe klinogradowe wskazują na eutroficzny charakter tych jezior.

Jedną z głównych przyczyn złej jakości wód w ww. jeziorach jest niewątpliwie przepływająca przez nie rzeka Pasłęka wnosząca znaczny ładunek zanieczyszczeń.

## Introduction

Lakes experience the natural process of ageing, called eutrophication, which is an increase of fertility stimulated by continuous input of matter from the land. In nature such changes occur rather slowly, but man's activities in the drainage basin accelerate this process as much as thousand times (KAJAK 1998, LOSSOW 1996, SELING and SCHLUNGBAUM 2002). The effects of the super-fertilization of a lake are, among others, oxygen deficits in the deep water and the ensuing release of nutrients from the bottom sediments to water, accumulation of hydrogen sulphide in hypolimnion, excessive growth of aquatic plants (especially plankton) responsible for unpleasant odour and taste of the water, unnatural colour, and introduction to water of unwanted organic matter with toxic properties. Overall, super-fertilization of a lake causes unfavourable changes to water quality and reduces its commercial and recreational value.

Recognition of the level of the lake's fertility and of the degradation rate is possible after a detailed analysis of its morphometric properties, water mass dynamics, physical and chemical settings, and biological communities.

The lakes of the Mazury Lakeland should be regarded as a precious and very important element of the young glacial landscape. Most of the lakes are

still relatively little degraded, yet their vulnerability to degradation should signal an alarm for care and complex protection.

Lakes Wymój, Sarąg, Łęguty and Isąg, crossed through by the Pasłęka River, comprise a very interesting field for studies. In 1970, the Regulation of the Minister of Environmental Protection, Natural Resources and Forestry (Polish Monitor 1970, 1982) established a reserve in the catchment of the Pasłęka River called "Beaver sanctuary on the Pasłęka River". The reserve then covered an area of 4.116.18 ha in the former olsztyńskie and elbląskie voivodships. At present, the reserve's surface area is 1,903 ha (ENDLER, ZIELIŃSKA 1994) and includes the Pasłęka River from its mouth to the southern section of Lake Isąg.

The lakes of the Pasłęka River catchment have been studied sporadically. The first mention of Lake Isąg appeared in OLSZEWSKI'S paper (1951) which presented conclusions from the field studies of this lake carried out by Willer in the 1930s. OLSZEWSKI et al. (1978) provide information regarding the temperature and oxygen structures in Lakes Wymój, Sarąg and Łęguty, originating from the 1950/1960s.

Now, the studies are aimed at determining the fertility of the lakes and the effect of the drainage basin on the rate of the transformations in the lakes. This paper is the first step towards recognition of the current aquatic conditions in the lakes of the Pasłęka River catchment and includes description of the water masses' dynamics and thermal and oxygen structures.

## Methods

The survey of thermal and oxygen structures in Lakes Wymój, Sarąg, Łęguty and Isąg was conducted in spring, summer and autumn, in April through November 1997. The very thin ice cover prevented the winter explorations.

On each lake, a sampling post was set up over the deepest spot in the lake; i.e,:
– on Lake Wymój at 16 m,
– on Lake Sarąg at 16.5 m,
– on Lake Łęguty at 22.7 m,
– on Lake Isąg at 54.5 m.

On the sampling post, the thermal and oxygen structure was examined with measurements at every meter of the depth with a Cole Palmer oxygen probe.

Oxygen saturation was read from the tables of saturation by Fox, with the use of the data on water temperature and DO (mg $O_2 \cdot dm^{-3}$).

## Description of the examined lakes

The mouth of the Pasłęka River can be found in the boggy meadows near the Gryźliny Village, at 188 m above the sea level. First, the river flows through small Pasłęk Lake and next through Lakes Wymój, Sarąg, Łęguty, Isąg and an artificial lake called Pierzchalski. The river discharges to the Vistula River Estuary. The Pasłęka River valley within the borders of the Warmińsko-Mazurskie Voivodship comprises a young glacial and undulating area. Its geologic and geomorphologic properties were formed at the end of the Pomeranian Phase of the Baltic Glaciation (PANFIL 1978). The lakes crossed by the Pasłęka River are quite variable morphometrically (Table 1).

Table 1
Detailed morphometric characteristics and parameters of the examined lakes (acc. IRŚ w Olsztynie)

| Parameter | Name of the lake | | | |
|---|---|---|---|---|
| | Wymój | Sarąg | Łęguty | Isąg |
| Water table surface area (ha) | 47.3 | 183.0 | 60.9 | 395.7 |
| Max. depth (m) | 16 | 16.5 | 22.7 | 54.5 |
| Mean depth (m) | 5.1 | 6.9 | 8.5 | 14.2 |
| Relative depth (m) | 0.02 | 0.012 | 0.03 | 0.027 |
| Depth index | 0.3 | 0.38 | 0.3 | 0.26 |
| Volume (thousand $m^3$) | 2413.8 | 12627.0 | 5234.0 | 56189.4 |
| Max. length (km) | 1.30 | 3.20 | 1.29 | 4.94 |
| Max. width (km) | 0.5 | 1.1 | 0.8 | 1.1 |
| Elongation | 2.6 | 2.9 | 1.5 | 4.49 |
| Shoreline of the lake bowl (km) | 3.15 | 9.35 | 3.70 | 17.53 |
| Development of the shoreline | 1.3 | 1.9 | 2.5 | 2.5 |

Lake Wymój is surrounded by forest and meadows. The eastern and western shores are mostly steep, with spots which are extremely steep and covered by pine forest. The southern and northern ends are low and wet. Along the northern shore lies Wymój Village and along the eastern shore, an estate of summer cottages.

On the southern shore of Lake Sarąg, sprawls the village, Makruty. The northern, southern and western shores of the lake are steep and afforested while the eastern is low and wet. The slopes of the lake bowl descend gently towards the deepest spot located in the centre of the lake.

Lake Łęguty has two basins, western and eastern, distinctly parted by a shallow table. On the eastern and western sides, the lake is surrounded by

forests. The other shores are adjacent to fields and meadows. The shores are flat, except for the eastern part where they rise and are sometimes steep. The southern end of the lake is surrounded by the settlements of the Łęguty and Grazymy villages. The northern shore neighbours with a recreational centre, Łęgucki Młyn.

Lake Isąg has mostly high or sporadically steep shores. Only the eastern side is flat. The northern and southern edges are grown with forest, the other with groves, tree stands or occupied by fields and meadows. Pelnik Village sits at the middle section of the eastern shore and Worliny Village on the western side of the lake. There are three islands on the lake. The largest, with the surface area of 3.5 ha, is steep and grown with forest.

## Results

Examination of the thermal and oxygen structures was initiated in the spring. In April, in Wymój and Isąg, temperature variability in the water column was rather low (Figures 1, 4). The temperature of the surface waters was 5.7 and 6.4°C and near the bottom 5.4 and 5.3°C, respectively. In Sarąg and Łęguty, in the spring, a complete homothermy was observed at 5.8°C (Figures 2, 3).

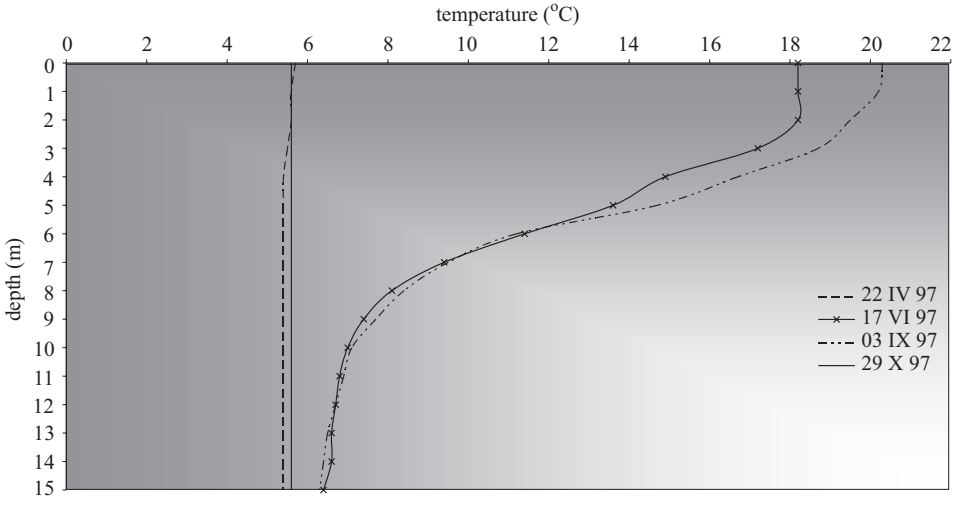


Fig. 1. Seasonal variation of water temperature in lake Wymój

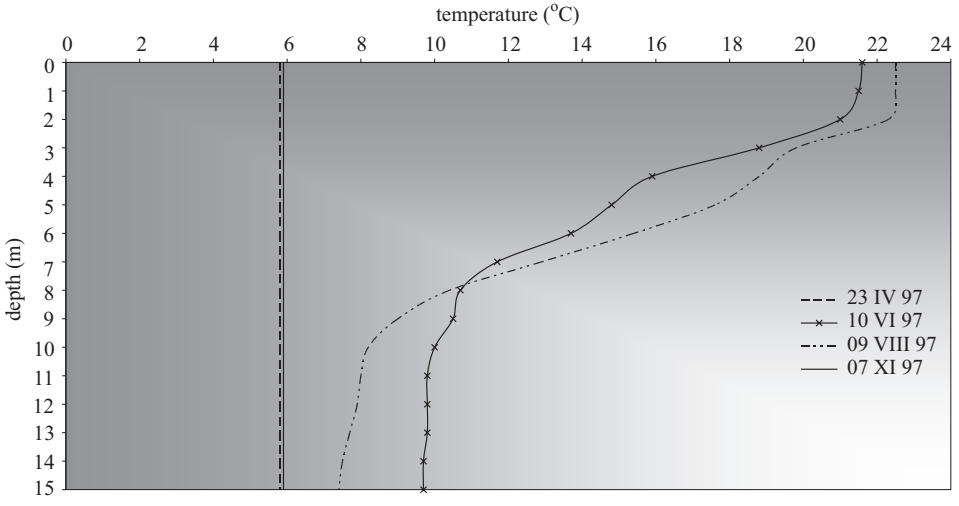


Fig. 2. Seasonal variation of water temperature in lake Sarąg

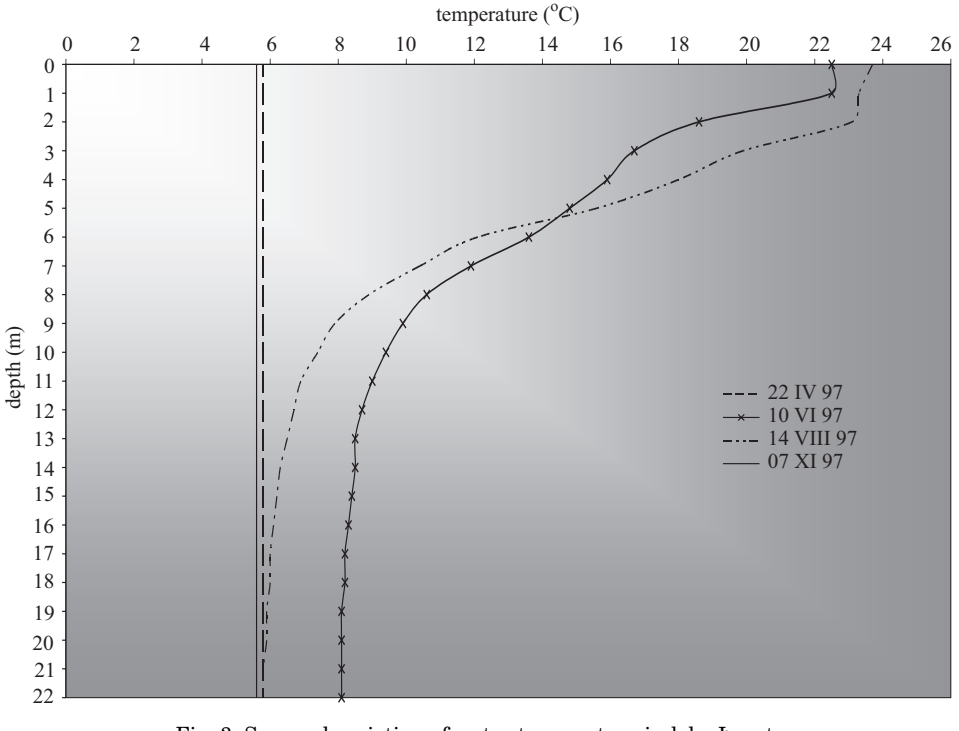


Fig. 3. Seasonal variation of water temperature in lake Łęguty

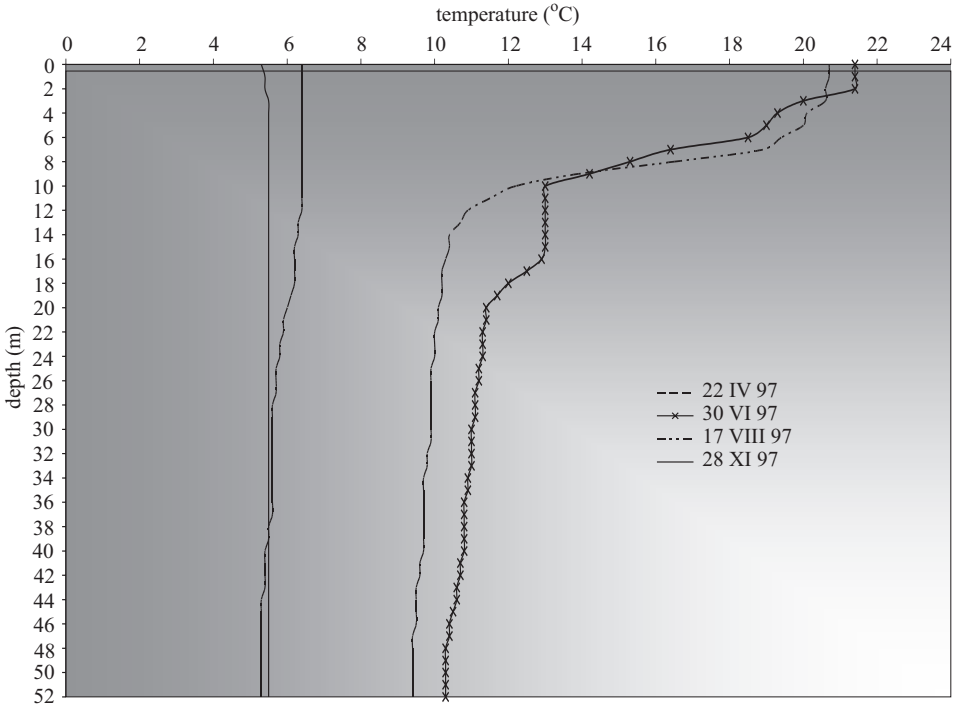


Fig. 4. Seasonal variation of water temperature in lake Isąg

In Lake Wymój the spring turnover was short. An early as May, the typical summer stratification was observed with a 2-m epilimnion (17.6-17.0°C), a thermocline between 2 and 6 m, and a hypolimnion at 8.7 to 6.8°C.

Early September was the peak of the summer stagnation in Lake Wymój. Epilimnion began to get shallow and was 3 m thick. Temperature of the water under the surface was identical to that in July (20.3°C) – Figure 1. Below the epilimnion, a thermocline was observed with the max. gradient of 3.5°C/m and a hypolimnion with 6.3°C near the bottom.

In Lake Sarąg, the stable 3-layer thermal stratification was not set up until June. Along with the air warming up, a constant increase of the temperature in the whole water mass was observed. In the early summer stagnation, the epilimnion was shallow (2 m) and quite consistent thermally (21.6-21.0°C). In the metalimnion, water temperature was 18.8-10.70C (max. gradient 2.7°C/m) – Figure 2. The hypolimnion below 8 m was relatively warm (9.7°C).

At the end of August in the summer peak, the epilimnion temperature slightly increased (22.5°C), and its thickness remained unchanged. Deeper down, a 7-m thick thermocline was observed (max. gradient 2.5°C/m) and a hypolimnion with the temperature 7.4°C near the bottom (Figure 2).

In Lake Łęguty in June, the summer stratification was observed with a shallow (1 m) epilimnion with the temperature of 22.5°C. Deeper, the thermocline occurred with the max. gradient of 3.9°C/m. Below the depth of 8 m, the hypolimnion was observed with the temperature 10.6-8.1°C (Figure 3).

In August, the 2-m thick epilimnion was still well warmed; i.e., from 23.7 to 23.1°C. The thermocline was set between 3 and 8 m (max. gradient 2.4°C/m) with a cold hypolimnion below, of 5.8°C near the bottom (Figure 3).

In the deepest of the examined lakes, Lake Isąg, the increase of the atmospheric temperature was accompanied by the growth of the water mass temperature with very distinct differences between the upper and lower layers. Stable, 3-layer summer stratification occurred in June. The mixing layer was 6-m thick (21.4-18.5°C), the thermocline was 5-m thick and gentle with the max. gradient 2.1°C/m, and the hypolimnion was warm with the temperature 10.3°C near the bottom (Figure 4). In August, slight cooling of the epilimnion was observed, down to approximately 20.7°C, and increase of its thickness to 7 m. In that period, thickness of the metalimnion decreased to 4 m (max. gradient 2.6°C/m). The hypolimnion spread out below 10 m with the temperature 12.2-9.4°C near the bottom.

The autumn turnover occurred in the lakes of the Pasłęka River catchment in November. Waters of lakes Wymój and Łęguty were mixed at 5.6°C, Saręg at 5.9°C, and Isąg at 8.4°C (Figures 1, 2, 3, 4).

No examinations of the thermal and oxygen conditions were possible in the winter because of the very thin ice cover. The temperature conditions in the lakes affected the oxygen conditions.

In Lakes Wymój, Saręg and Łęguty in the spring, complete homooxygeny was observed at the DO 12.2 mg $O_2 \cdot dm^{-3}$, 12.5 mg $O_2 \cdot dm^{-3}$, and 10.7 mg $O_2 \cdot dm^{-3}$ (50%, 99.5%, 85.2% saturation) (Figures 5, 6, 7), respectively. In Lake Isąg oxygen was detected in the whole water mass, and small differences between its concentration in the surface and near-bottom waters were observed. DO in the surface water was 10.6 mg $O_2 \cdot dm^{-3}$ (85.7% saturation) and decreased along with the depth increase; near the bottom it equalled 5.3 mg $O_2 \cdot dm^{-3}$ (64.4% saturation) – Figure 8).

Occurrence of stable thermal stratification stimulated changes in the oxygen structures.

In Lake Wymój, in June, the 3-m deep surface layer was well oxygenated; i.e., from 9.4 to 7.7 mg $O_2 \cdot dm^{-3}$ (98.5 to 79.1% saturation). Between 3 and 4 m a strong oxycline was observed with the 6.9 mg $O_2 \cdot dm^{-3}$/m gradient. Below 7 m, oxygen was not detected. Oxygen conditions deteriorated as the summer stagnation proceeded. In September, the high concentrations of oxygen were noted only at the surface (10.0 mg $O_2 \cdot dm^{-3}$ – 116.9% saturation) and at the first meter of the depth (9.6 mg $O_2 \cdot dm^{-3}$ – 104.7% saturation).

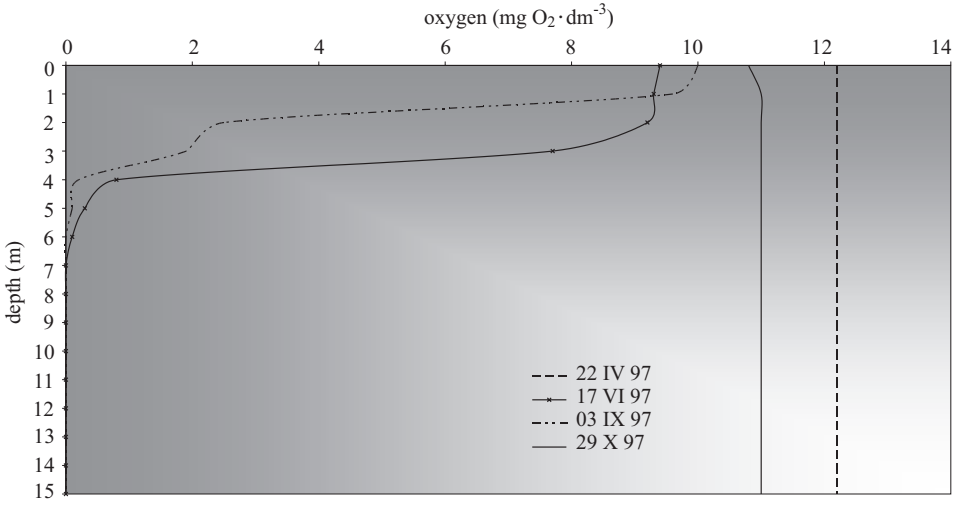


Fig. 5. Seasonal variation of oxygen content in water of lake Wymój

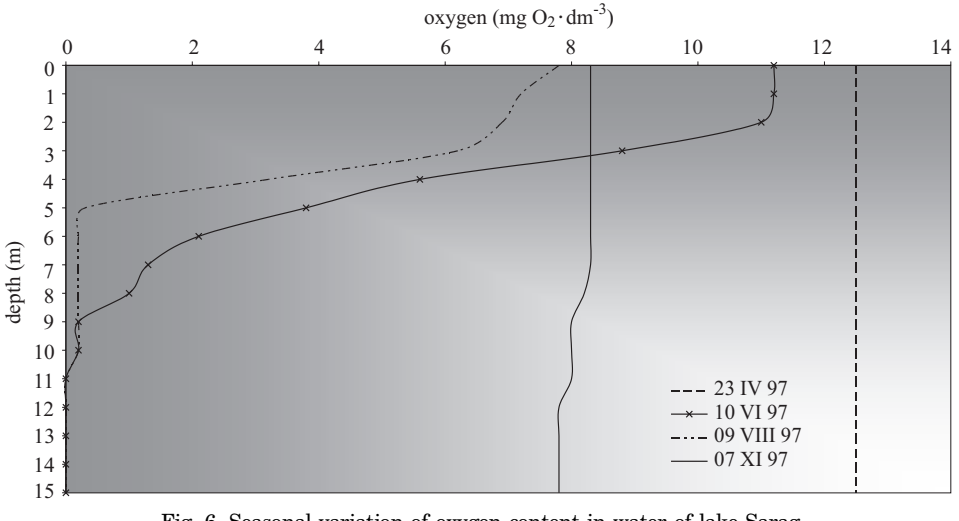


Fig. 6. Seasonal variation of oxygen content in water of lake Sarąg

Deeper, the amounts of oxygen were much lower; the complete deficit was observed below 6 m (Figure 5).

In Lake Sarąg, in the early summer, the epilimnion was super-saturated with oxygen (125.4-121.3% saturation). In the metalimnion, oxygen was slowly depleted and in the upper hypolimnion, it was fully consumed (Figure 6). In August, oxygenation of the lake waters deteriorated. At the surface, DO equalled 7.8 mg $O_2 \cdot dm^{-3}$ (88.8% saturation) and decreased along with the depth

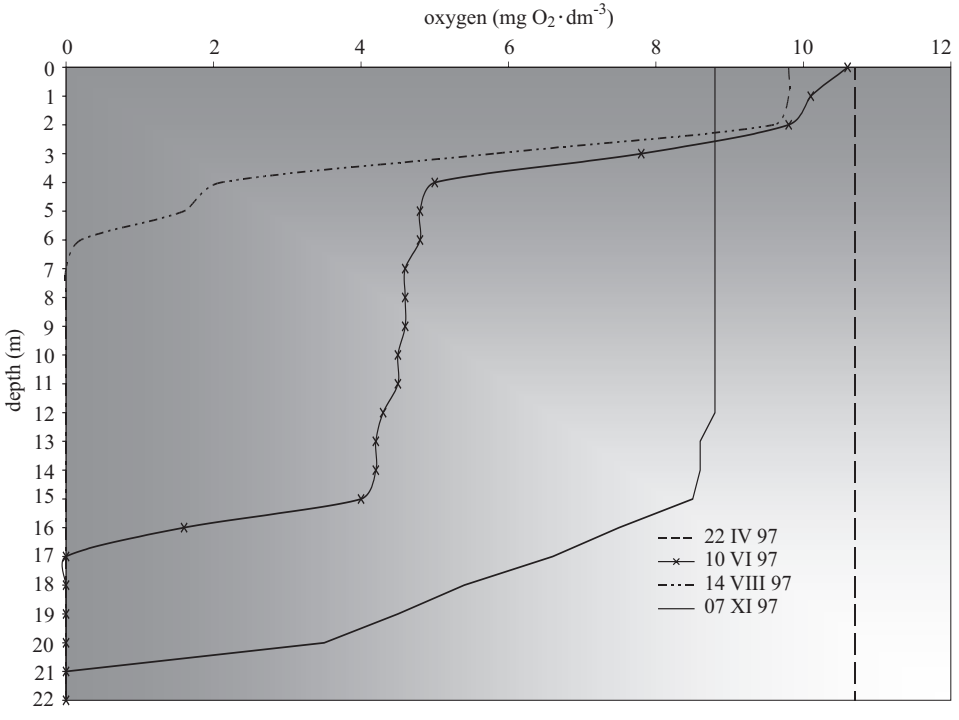


Fig. 7. Seasonal variation of oxygen content in water of lake Łęguty

increase. Below 5 m, oxygen was only detected in trace amounts (0.3-0.2 mg $O_2 \cdot dm^{-3}$), and below 10 m the water was oxygen-deficient (Figure 6).

In Lake Łęguty the surface water in the beginning of the summer stagnation was super-saturated with oxygen (120.6% saturation at DO 10.5 mg $O_2 \cdot dm^{-3}$). As the depth increased, oxygen was depleted until complete deficiency below 17 m. In August, oxygen conditions in the lake further deteriorated. The 2-m thick surface layer was super-saturated with oxygen (114.1-110. 6% saturation), and below 3 m, DO was rapidly reduced, reaching trace amounts (0.2 mg $O_2 \cdot dm^{-3}$) at 6 meters. Underneath, the water was completely deficient with oxygen (Figure 7).

In deep (54,5 m) Lake Isąg in the summer, a stable oxygen stratification was observed in the water column.

In June, the epilimnion down to 3 m was super-saturated with oxygen (162.8-127.1% saturation). Deeper down, the concentration fell, reaching 0.6 mg $O_2 \cdot dm^3$ near the bottom (Figure 8). In August, the oxygen conditions were different. The range of the super-saturated zone increased to 5 m (132.1-101.0% saturation). Likewise, in June, DO decreased towards the bottom, but

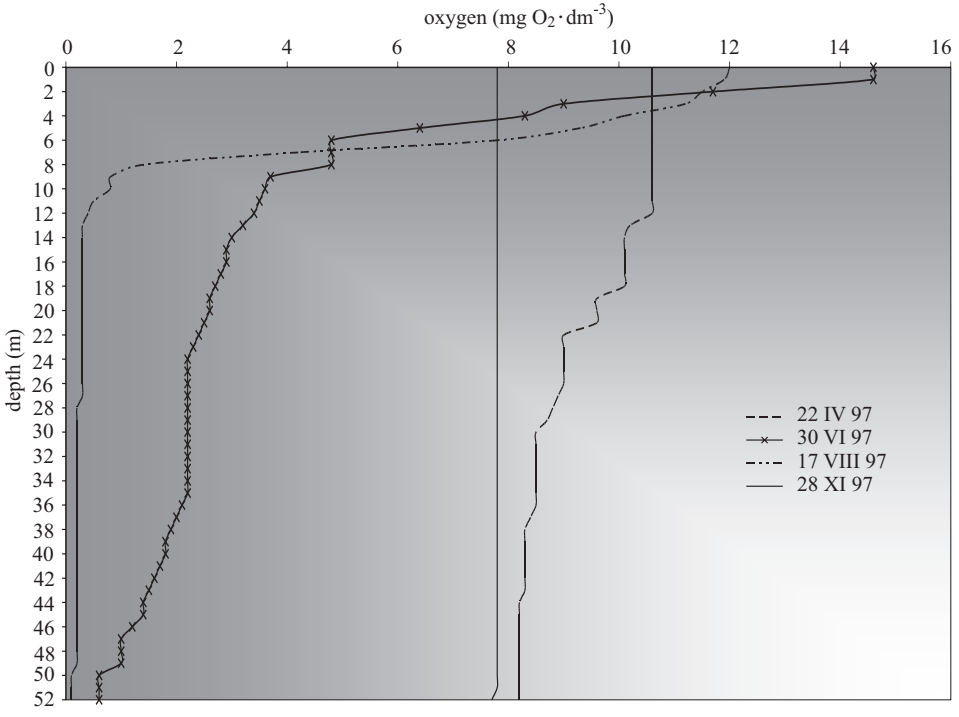


Fig. 8. Seasonal variation of oxygen content in water of lake Isąg

from 13 m down, it was detected only in trace amounts 0.3-0.1 mg $O_2 \cdot dm^{-3}$ (Figure 8).

The autumn turnover improved oxygen conditions in practically all the lakes discussed. The deficits from the summer were eliminated.

In Lake Wymój at the end of October, DO in the whole water column was similar and equalled 11.0 mg $O_2 \cdot dm^{-3}$ (87.1% saturation) – Figure 5.

In November in Lake Sarąg, complete homothermy was observed (5.9°C), yet the distribution of oxygen was not very diverse. The surface waters contained 8.3 mg $O_2 \cdot dm^{-3}$ (66.2% saturation), and the near bottom waters, 7.8 mg $O_2 \cdot$ $\cdot dm^{-3}$ (61.4% saturation) – Figure 6.

In Lake Isąg in November, the oxygen stratification was eliminated. DO then equalled 7.8 mg $O_2 \cdot dm^{-3}$ (61% saturation) – Figure 8.

Examinations in November in Lake Łęguty revealed that the summer oxygen deficits were not resupplied. The oxygen content slowly decreased until it reached 8.8 mg $O_2 \cdot dm^{-3}$ in the surface layers and 3.5 mg $O_2 \cdot dm^{-3}$ at 20 m. Below, the water was still oxygen-deficient (Figure 7).

## Discussion

Thermal stratification in lakes and particularly the changes they experience throughout the year depend on the climate, morphometry of lake (depth, shape), and setting in the area which in turn determines the intensity of mixing by wind (AMBROSETTI, BARBANTI 2002, LANGE, MAŚLANKA 1996, KALFF 2002, WETZEL 2001).

Lake Wymój is a small (47.3 ha) and not very deep (16 m) reservoir, situated in the land hollow and considerably shaded by the steep shores of pine forest. These factors effectively constrain the wind's impact on the lake. Calculated after Patalas (1960), the theoretical mixing range equals 4.2 m and is higher than the factual epilimnion thickness identified in the study of 1997. Although the spring turnover was complete (equal temperature in the whole water column, oxygen saturation above 50% $O_2$), it was rather short. As soon as May, the summer stratification was detected with a 3-m thick epilimnion, a thermocline with the max. gradient 3.5°C/m, and a cold hypolimnion of 6.3°C. The autumn turnover occurred at the end of October.

The short spring turnover, stable and long thermal stratification in the summer, and the low temperatures of the hypolimnion classify the examined lake as the 5$^{th}$ static degree (PATALAS 1960) according to OLSZEWSKI (1959), as bradymictic. Aberg and Rhode (1942) would have classified the lake as stable stratified.

OLSZEWSKI et al. (1978) investigating Lake Wymój in 1959 classified it as average eumictic. The current research has revealed that the lake is bradymictic. Such change is very possibly caused by the development in the close vicinity since the 1950s, intensive growth of the tree stand, and construction of the summer cottages.

Lake Sarąg has quite a large surface area (183 ha) and depth (16.5 m). The direct drainage basin is dominated by forests which obstruct wind penetration and thus, the water mixing. The theoretical thickness of the epilimnion, calculated after PATALAS (1960), is 5.9 m and is almost 3 times higher than the factual range of the mixing zone observed in this study. In spring, the lake was mixed in the whole water mass. Simple thermal stratification occurred in June. In the peak of the summer, stagnation epilimnion was observed down to 2 m depth, a metalimnion with the max. gradient 2.5°C/m, and a hypolimnion with the temperature 7.4°C near the bottom. In November, waters in Lake Sarąg were mixed at 5.9°C.

According to the criterion by PATALAS (1960), regarding the dependence between effective length of the lake's axis and the epilimnion thickness, Lake Sarąg can be classified as the 4$^{th}$ static degree which means that the water exchange intensity between the surface and the near bottom layers is low and

that the hypolimnion temperature is medium. According to FINDENEGG (1937), the lake can be classified as holomictic, periodically mixed in the whole water mass, characteristic for complete turnover in the spring and autumn and a summer thermal stratification with stagnant hypolimnion. OLSZEWSKI'S (1959) criteria classify the lake as eumictic, in between the easily and hardly mixing while ABERG and RHODE'S (1942) classify it as stratified stable.

The results of this study, regarding the water dynamics in Lake Saręg, are similar to those of OLSZEWSKI et al. (1978) who classified the lake as eumictic in 1962.

Lake Łęguty with a surface area of 60.9 ha and a max. depth of 22.7 m has two basins: western and eastern, parted distinctly by shallowness. On the eastern and western side, the lake is surrounded by forests. The other shores are adjacent to fields and meadows. The shores are flat, except for the eastern part where they are raised or even steep, and thus water mixing by the wind is obstructed, a fact confirmed by the results of the examinations. The spring turnover reached the bottom (5.8°C, saturation with oxygen 85%) but was short: in early June, thermal stratification was observed. As soon as the early June thermal stratification was observed. In the peak of the summer, the epilimnion was shallow (2 m) with a thermocline below with the max. gradient of 2.4°C/m and a cold hypolimnion with the temperature of 5.8°C near the bottom. The autumn turnover began in mid October.

Using Patalas' method of calculations (1960), the theoretical thickness of the epilimnion equal to 4.6 m is two times higher than the factual. With respect to the theoretical mixing depth and the max. depth of the lake as well as the relative temperature of the hypolimnion, Lake Łęguty can be classified as the 5$^{th}$ static degree (PATALAS 1960), or according to OLSZEWSKI (1959) as brady-mictic, and as stratified stable according to ABERG and RHODE'S criteria (1942). The results of OLSZEWSKI et al. (1978) obtained in 1962 were similar.

Lake Isąg is the largest (395.7 ha) and deepest of all examined in this study. It has mostly high or sporadically steep shores. The northern and southern edges are grown with forest. As in the other cases, the setting of Lake Isąg deters the wind's impact on the water table. The spring and autumn turnovers reached the bottom. In the peak of the summer stagnation, the epilimnion was 7 m thick, and the thermocline below had the max. gradient of 2.6°C/m. The temperature in the hypolimnion near the bottom equalled 9.4°C. The theoretical thickness of the epilimnion calculated after PATALAS (1960) is 7.5 m and is practically similar to the factual value. With regard to the PATALAS' (1960) classification, Lake Isąg is the 4th static degree reservoir, or according to OLSZEWSKI (1959), eumictic, or stratified stable following ABERG and RHODE'S criteria (1942). Willer (after OLSZEWSKI 1951) also classified the lake as a reservoir with average wind activity.

As many authors report (GROCHOWSKA, GAWROŃSKA 2005, KUBIAK, TÓRZ 2005, WETZEL 2001), water mixing and degree of eutrophication in lakes influence the oxygen stratification.

In the surface waters of all the studied lakes, oxygen saturation in some periods was much higher than 100% which indicates intensive production processes. In the deep layers, especially near the bottom, oxygen depletion was quite fast and ended with complete deficit. The reasons, no doubt, were the intensive processes of matter decomposition produced in the lakes or deposited in the bottom sediments (SEHGAL, WELCH 1991).

The clinograde curves observed in lakes in the summer (ABERG, RHODE 1942) evidence their highly eutrophic condition.

One of the main reasons for the poor quality of water in the Lakes Wymój, Saręg, Łęguty and Isąg is undoubtedly the Pasłęka River which carries high load of nutrients.

The results obtained in this study imply that all the lakes of the Pasłęka River catchment experience average or hindered mixing of the water masses. This is mainly due to their setting in the undulating area, formed in the last glacial period. The steep slopes surrounding the lakes, grown with forests, obstruct wind access to the water table. From the viewpoint of their vulnerability to degradation, the high percentage of the waters stratification, representing the hypolimnion's contribution to the whole water mass, is a positive feature. KUDELSKA et al. (1994) report that the reservoirs where production and decomposition processes of organic matter run in the distinctly isolated layers, are characterised by lower intensity of matter turnover and lower productivity. In such lakes nutrients released from the bottom sediments are transferred to euphotic zone with more difficulty. However, the current studies reveal that the analysed lakes are heavily eutrophic, as indicated by the unfavourable oxygen conditions reflected by the oxygen deficits in the near bottom waters throughout the summer stagnation. Such a situation calls for intensive protection of the lakes of the Pasłęka River catchment against degradation.

Translated by MONIKA SZEWCZYK

Accepted for print 9.03.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 307-324, Y. 2006

# COMPOSITION OF WASTEWATER ORIGINATED FROM THE PARTICULAR SECTIONS OF DAIRY PRODUCTION

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K e y w o r d s: composition of dairy wastewater, biological wastewater treatment, organic compounds, nutrients.

A b s t r a c t

Development and implementation of new technologies influence directly composition of wastewater from dairy industrial plants. Production units do not carry out a detailed characterization of wastewater generated by particular process lines. Therefore, there is a necessity of actualisation of the data concerning composition of wastewater produced currently by operating dairies. The aim of the investigation was to determine basic pollutants concentration of dairy wastewater. Wastewater was collected from outlets of all sections of a dairy industrial plant. The highest pollutants concentration expressed as organics and nutrients concentrations were determined in case of cottage cheese whey and cheese whey. Among other kinds of wastewater, these ones from cottage cheese section, apparatus room and cheese section characterized by the highest pollutant concentrations. From the point of view of a biological wastewater treatment the best proportion between organic compounds concentration and nutrients concentrations was in wastewater from apparatus room and wastewater from butter section.

## SKŁAD ŚCIEKÓW POCHODZĄCYCH Z POSZCZEGÓLNYCH DZIAŁÓW PRODUKCJI MLECZARSKIEJ

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S ł o w a k l u c z o w e: skład ścieków mleczarskich, biologiczne oczyszczanie ścieków, związki organiczne, związki biogenne.

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Abstrakt

Rozwój i wdrażanie nowych technologii wpływają bezpośrednio na zmianę składu ścieków z zakładów przemysłu mleczarskiego. Jednostki produkcyjne nie prowadzą jednak na ogół szczegółowych pomiarów jakości ścieków z poszczególnych linii produkcyjnych. Istnieje zatem konieczność zaktualizowania danych dotyczących ich charakterystyki w obecnie funkcjonujących mleczarniach. Celem badań było określenie charakterystyki podstawowych zanieczyszczeń ścieków mleczarskich. Ścieki pobierano z kratek ściekowych wszystkich działów przetwórczych zakładu mleczarskiego. Najwyższą koncentrację zanieczyszczeń – wyrażoną stężeniem związków organicznych oraz substancji biogennych – stwierdzono w serwatkach z produkcji sera i twarogu. Spośród pozostałych ścieków najwyższe wartości zanieczyszczeń stwierdzono w ściekach z twarożkowni, aparatowni oraz serowni. W aspekcie biologicznego oczyszczania ścieków najkorzystniejszą proporcję między ilościami substancji organicznych (wyrażonych wartością ChZT) i pierwiastkami biogennymi (wyrażonymi jako suma azotu ogólnego i fosforu ogólnego) miały ścieki z aparatowni i masłowni.

## Introduction

The amount and composition of wastewater from dairy industrial and processing plants is changing noticeable. High development, water-saving technologies and common use of modern methods of production are the main and direct factors influencing these changes. Of course, dairy wastewater content depends on production profile and offered assortment.

Dynamics of the changes of this kind of industry section can be showed on the basis of the investigations carried out in the U.S.A. in the 60$^{ths}$ and the 90$^{ths}$. HARPER and BLAISDELL in the 60$^{ths}$, on the basis of literature data and after visiting 10% of dairy plants working on the area of the U.S.A., prepared an analysis of dairy wastewater characterization and a review of used wastewater treatment technologies. The authors revealed that dairy sector in the U.S.A. is poor as for the knowledge concerning wastewater composition, and wastewater treatment plants operating then had low treatment effectiveness. It was shown that dairy processing characterized by high water consumption, and remainders of the products were lead into the wastewater stream. Later literature data supported the information about dairy waste contents and about possibility of wastewater treatment (EROGLU et al. 1991, GORONSZY 1989, OZTURK et al. 1993).

DANALEWICH et al. in 1995-1996 revised 15 dairy industrial plants on the area of the U.S.A., all with similar production assortment. It was noted that the volume of generated wastewater is 2-3 times lower than in the 60$^{ths}$ (HARPER, BLAISSDELL 1971), and it resulted from using of cooling water closed circuits, equipment of high technology, and recovering of parts of chemical substances and materials from wastewater. Wastewater flow ranged from 170 m$^3$/d to 2081 m$^3$/d. In most industrial plants high seasonal, daily and

hourly fluctuations of wastewater flow were observed: minimal flow was on the level of 4-170 $m^3$/d, and maximal one ranged from 257 $m^3$/d to 2650 $m^3$/d. Daily and hourly changes in wastewater generation resulted from procedures of devices and rooms washing at the end of each producing cycle, however, seasonal changes from higher quantity of processed milk during summer time and lower during winter period.

Taking into account dynamics of the changes in wastewater composition and the amount of generated wastewater, some questions concerning effectiveness of the wastewater treatment technologies arise. Treatment plants repose to activated sludge method and aerobic biodegradation. However, in many cases these techniques can appear ineffective and should be modernized or replaced by other more effective wastewater treatment plants. Dairies all over the world generally do not measure the amount of wastewater generated through the particular dairy sections. Moreover, it does not take care about monitoring of pollutants level in the environment (Pesta et al. 2003). Thus, there is a high necessity of actualisation and systematisation of the date concerning characterization and composition of wastewater generated by operating industrial dairy plants. Obtained knowledge could let to determined the possibility of pollutants degradation by known treatment technologies and by new, more effective methods of dairy wastewater treatment.

The aim of the study was to determine base dairy wastewater composition. Analysed wastewater was coming from the particular production processes in operating dairies.

## Materials and Methods

Wastewater was collected from outlets of all sections of dairy industrial plant. Cottage cheese whey and cheese whey composition were analysed. Samples of wastewater were taken one time a month in the period from September 2003 to August 2004. Wastewater samples at the volume of 2.0 $dm^3$ were taken every half an hour during working hours of the dairy from $6^{30}$ a.m. to $2^{30}$ p.m. (17 samples). Wastewater was collected in vessels kept and transported in the temperature of 4°C.

Wastewater used in the experiment comes from the following processes:

- wastewater from milk reception point – generated due to cisterns, lines, floors and approach roads washing. This wastewater is similar to diluted milk.
- wastewater from apparatus room – generated due to washing of centrifuges, devices used for thermal milk processing, devices for homogenization, concentration and degassing. Composition of this kind of wastewater depends on washing chemical agents used.

– wastewater from butter section – generated due to washing of rooms, devices and installations for butter production. Wastewater contains high concentration of fats.
– wastewater from cottage cheese section – generated due to washing of rooms, devices and installations for cottage cheese production. Wastewater contains high concentration of organic compounds.
– wastewater from cheeses section – generated due to washing of rooms, devices and installations for cheese production. Wastewater contains high concentration of organic compounds.
– cheese whey – waste product after cheese production (sweet whey).
– cottage cheese whey – waste product after cottage cheese production (sour whey).
– mixed wastewater – mixture of all kinds of wastewater generated in the dairy collected to one stream (wastewater from pumping station).

The following physicochemical analyses were done:

– chemical oxygen demand – COD [PN 74/C-04578/03]
– biological oxygen demand – $BOD_5$ [PN 72C-04545]
– total nitrogen – $N_{tot}$ [PN 73/C-04576/12]
– ammonium nitrogen – $N\text{-}NH_4$ [PN 73/C-04576/02]
– total phosphorus – $P_{tot}$ [PN 91/C-04537/09]
– phosphates – $P\text{-}PO_4$ [PN 88/C-04537/04]
– pH (electromagnetic method)

Statistical analysis of the obtained results was done using variance analysis, at the assumpted accuracy level ($p < 0.05$). Normal distribution was confirmed by Szapiro – Wilk test, and hypothesis concerning variance homogeneity inside the groups were verified on the basis of Leveney's test. Analysis of the differences between means from the particular groups was done using Tukey's test.

# Results

## Wastewater from milk reception point

Organic compounds concentration (expressed as COD) in wastewater from milk reception point ranged from 784 mg $O_2/dm^3$ to 5000 mg $O_2/dm^3$, and the average value was $2020 \pm 1388$ mg $O_2/dm^3$ (Figure 1). Biological oxygen demand ($BOD_5$) was on the level from 217 mg $O_2/dm^3$ to 2150 mg $O_2/dm^3$, averagely $850 \pm 652$ mg $O_2/dm^3$ (Figure 2). Concurrently, $BOD_5$/COD ratio varied from 0.15 to 0.87, at the average value of $0.46 \pm 0.24$ (Table 1).

Nitrogen concentration (determined as total nitrogen) ranged from

Table 1

Dairy wastewater chemical parameter ratios for particular production sections

| Section | | $BOD_5/COD$ mg $O_2$/ mg $O_2$ | $P\text{-}PO_4/P_{tot.}$ mg $P\text{-}PO_4$/mg P | $COD/(TKN+TP)$ mg $O_2$/(mg N + mg P) | COD/TP mg $O_2$/mg P | COD/TKN mg $O_2$/mg N | $BOD_5$/TKN mg $O_2$/mg N | $BOD_5$/ $NH_4$ mg $O_2$/mg $NNH_4$ | $BOD_5$/TP mg $O_2$/mg P | $BOD_5/(TP+TKN)$ mg $O_2$/(mg P+ mg N) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| milk reception point | mean | 0.46 | 0.51 | 43.23 | 228.07 | 56.06 | 24.44 | 317.14 | 99.45 | 17.22 |
| | Min | 0.15 | 0.45 | 9.50 | 36.15 | 9.70 | 2.93 | 62.50 | 13.46 | 2.86 |
| | max | 0.87 | 0.65 | 128.18 | 654.30 | 199.72 | 136.76 | 1153.75 | 207.41 | 76.56 |
| | SD | 0.24 | 0.06 | 41.73 | 207.67 | 59.68 | 36.28 | 350.79 | 63.95 | 19.87 |
| Apparatus room | mean | 0.29 | 0.66 | 67.66 | 391.02 | 84.98 | 25.71 | 607.00 | 121.91 | 20.59 |
| | min | 0.19 | 0.57 | 24.76 | 78.40 | 33.97 | 7.13 | 115.48 | 25.66 | 5.75 |
| | max | 0.55 | 0.82 | 324.60 | 2010.69 | 387.09 | 140.64 | 1352.00 | 730.56 | 117.94 |
| | SD | 0.12 | 0.07 | 83.74 | 545.73 | 98.81 | 36.61 | 359.59 | 201.93 | 31.01 |
| Butter section | mean | 0.34 | 0.46 | 59.45 | 1061.91 | 70.50 | 20.02 | 521.32 | 156.62 | 16.64 |
| | min | 0.07 | 0.06 | 5.21 | 30.26 | 6.30 | 2.77 | 62.65 | 13.30 | 2.29 |
| | max | 0.59 | 0.80 | 199.81 | 9173.82 | 246.97 | 83.08 | 2190.00 | 613.64 | 73.17 |
| | SD | 0.17 | 0.26 | 61.94 | 2582.80 | 72.36 | 22.29 | 651.04 | 217.51 | 19.38 |
| Cottage cheese section | mean | 0.21 | 0.54 | 26.24 | 80.25 | 40.55 | 8.51 | 147.86 | 15.12 | 5.37 |
| | min | 0.05 | 0.07 | 20.34 | 48.68 | 32.06 | 2.11 | 46.44 | 9.18 | 1.72 |
| | max | 0.42 | 0.99 | 38.17 | 170.65 | 66.43 | 21.25 | 436.07 | 28.69 | 12.21 |
| | SD | 0.10 | 0.30 | 4.98 | 30.70 | 9.65 | 5.25 | 110.77 | 6.99 | 3.01 |
| Cheese section | mean | 0.21 | 0.75 | 27.87 | 138.20 | 35.58 | 8.04 | 550.34 | 31.49 | 6.33 |
| | min | 0.14 | 0.46 | 10.13 | 90.94 | 11.27 | 1.70 | 83.38 | 14.96 | 1.53 |
| | max | 0.35 | 0.97 | 62.46 | 334.66 | 76.79 | 27.03 | 2923.33 | 117.80 | 21.98 |
| | SD | 0.07 | 0.16 | 12.88 | 68.57 | 16.62 | 6.32 | 870.55 | 27.83 | 5.14 |

cont. Table 1

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cottage cheese whey (sour whey) | mean | 0.51 | 0.74 | 34.13 | 80.73 | 63.81 | 31.76 | 644.96 | 41.03 | 17.27 |
| | min | 0.21 | 0.51 | 23.75 | 61.43 | 33.84 | 9.54 | 134.15 | 15.97 | 5.97 |
| | max | 0.74 | 0.99 | 54.23 | 105.78 | 160.93 | 61.63 | 1566.41 | 61.58 | 24.40 |
| | SD | 0.15 | 0.15 | 8.11 | 12.09 | 32.79 | 13.95 | 430.64 | 12.30 | 5.38 |
| Cheese whey (sweet whey) | mean | 0.41 | 0.61 | 37.00 | 137.68 | 50.68 | 20.98 | 2481.27 | 56.77 | 15.30 |
| | min | 0.30 | 0.49 | 30.11 | 120.02 | 39.88 | 14.93 | 225.80 | 42.71 | 11.13 |
| | max | 0.62 | 0.80 | 44.64 | 174.38 | 61.33 | 37.79 | 14173.57 | 93.67 | 26.92 |
| | SD | 0.09 | 0.10 | 4.67 | 15.56 | 6.96 | 6.66 | 3918.38 | 16.26 | 4.70 |
| Pumping station | mean | 0.43 | 0.64 | 43.30 | 120.87 | 91.65 | 46.23 | 306.18 | 49.32 | 18.82 |
| | min | 0.24 | 0.49 | 19.55 | 45.01 | 31.24 | 7.62 | 44.22 | 12.20 | 5.19 |
| | max | 0.74 | 0.80 | 86.82 | 317.80 | 420.75 | 290.63 | 870.71 | 93.73 | 54.60 |
| | SD | 0.18 | 0.11 | 22.70 | 72.12 | 107.96 | 77.64 | 270.48 | 25.26 | 13.09 |

9.1 mg N/dm$^3$ to 188.8 mg N/dm$^3$, averagely 60 ± 47 mg N/dm$^3$ (Figure 3). The average value of ammonium nitrogen was low, and was ranging from 0.8 mg N-$NH_4$/dm$^3$ to 5.6 mg N-$NH_4$/dm$^3$, averagely 3.65 ± 1.23 mg N-$NH_4$/dm$^3$ (Figure 4). It confirms that organic nitrogen (proteins) made up most of total nitrogen concentration, and conversion of amino groups to ammonium was not completed.

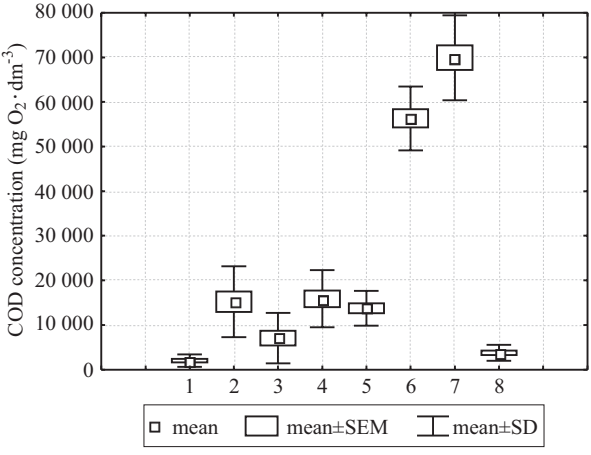


Fig. 1. COD concentration in dairy wastewater for particular production sections: 1 – reception point, 2 – apparatus room, 3 – butter section, 4 – cottage cheese section, 5 – cheeses section, 6 – cheese whey, 7 – cottage cheese whey, 8 – mixed wastewater

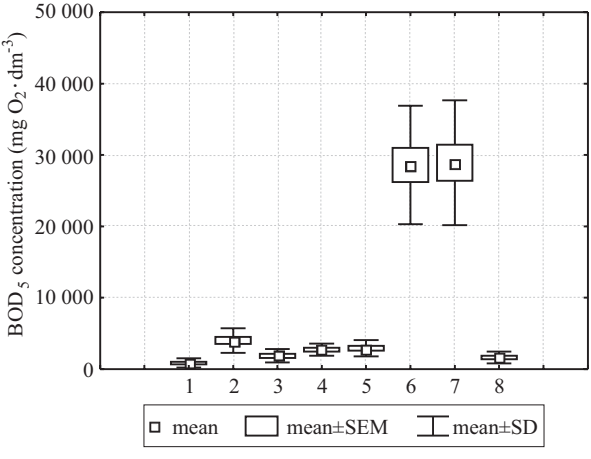


Fig. 2. $BOD_5$ concentration in dairy wastewater for particular production sections: 1 – reception point, 2 – apparatus room, 3 – butter section, 4 – cottage cheese section, 5 – cheeses section, 6 – cheese whey, 7 – cottage cheese whey, 8 – mixed wastewater

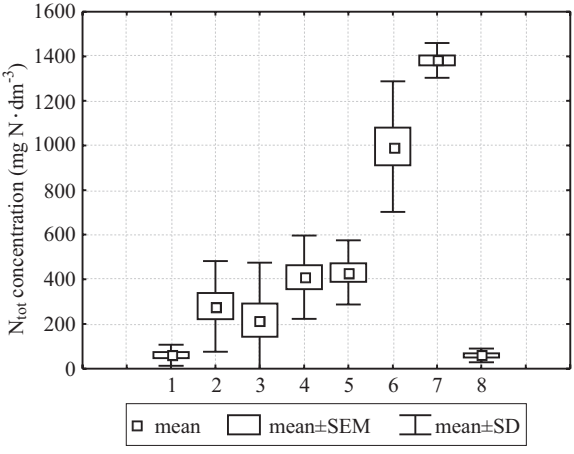


Fig. 3. Total nitrogen concentration in dairy wastewater for particular production sections: 1 – reception point, 2 – apparatus room, 3 – butter section, 4 – cottage cheese section, 5 – cheeses section, 6 – cheese whey, 7 – cottage cheese whey, 8 – mixed wastewater

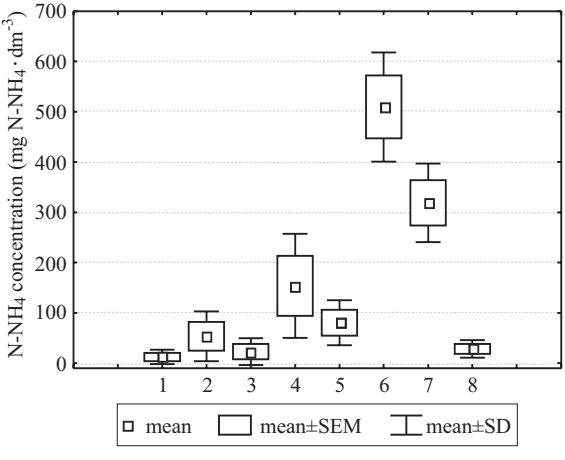


Fig. 4. Ammonium nitrogen concentration in dairy wastewater for particular production sections: 1 – reception point, 2 – apparatus room, 3 – butter section, 4 – cottage cheese section, 5 – cheeses section, 6 – cheese whey, 7 – cottage cheese whey, 8 – mixed wastewater

Phosphorus concentration (expressed as total phosphorus) in wastewater coming from milk reception point and cisterns washing was on the level from 1.2 mg P/dm$^3$ do 44.0 mg P/dm$^3$, at the average value of 13.9 ± 13,1 mg P/dm$^3$ (Figure 5). Concurrently, orthophosphate concentration ranged from 0.6 do 28.4 mg P-$PO_4$/dm$^3$, averagely 7.56 ± 8.18 mg P-$PO_4$/dm$^3$ (Figure 6). Orthophosphates made up 45-65% of total phosphorus, at the average value on the

level of 51%. Probably, organic phosphorus and polyphosphates made up remaining 49% of the total phosphorus. Both these form of phosphorus may come from milk, alkaline chemical washing agents or emulsifiers.

Wastewater reaction ranged from pH=3.58 to pH=10.11. Wide range of the reaction resulted from using alkaline and acid washing and disinfection chemicals (Figure 7). Concurrently, in 7 on 12 samples of wastewater pH ranged from 7.0 to 8.4, what shows that wastewater is mostly alkaline.

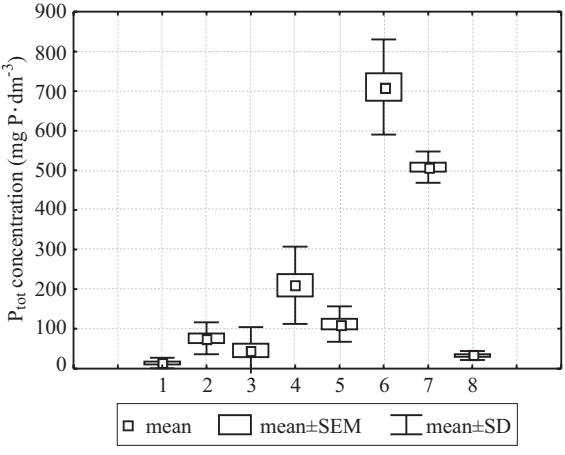


Fig. 5. Total phosphorus concentration in dairy wastewater for particular production sections: 1 – reception point, 2 – apparatus room, 3 – butter section, 4 – cottage cheese section, 5 – cheeses section, 6 – cheese whey, 7 – cottage cheese whey, 8 – mixed wastewater

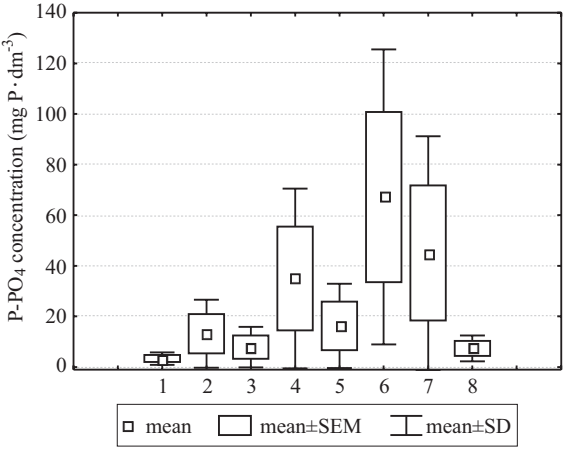


Fig. 6. Orthophosphates concentration in dairy wastewater for particular production sections: 1 – reception point, 2 – apparatus room, 3 – butter section, 4 – cottage cheese section, 5 – cheeses section, 6 – cheese whey, 7 – cottage cheese whey, 8 – mixed wastewater

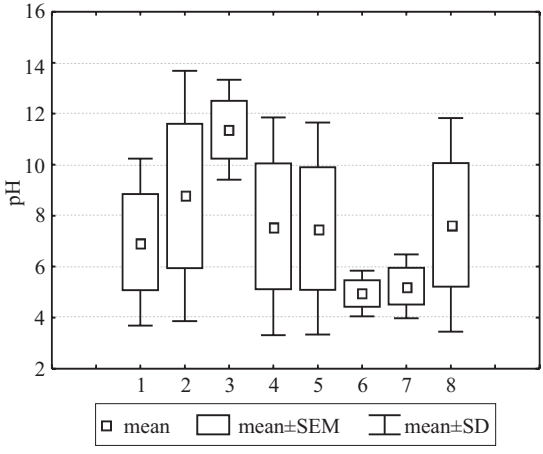


Figure 7. The reaction of dairy wastewater for particular production sections: 1 – reception point, 2 – apparatus room, 3 – butter section, 4 – cottage cheese section, 5 – cheeses section, 6 – cheese whey, 7 – cottage cheese whey, 8 – mixed wastewater

## Wastewater from apparatus room

In case of wastewater from apparatus room organics concentration COD varied from 6272 mg $O_2/dm^3$ do 34 090 mg $O_2/dm^3$ (Figure 1). The average value of this parameter was $15228 \pm 7952$ mg $O_2/dm^3$. Biochemical oxygen demand $BOD_5$ concentration was on the level from 2540 mg $O_2/dm^3$ to 5260 mg $O_2/dm^3$, at the average value of $4001 \pm 1710$ mg $O_2/dm^3$ (Figure 2). However, the ratio of $BOD_5$/COD ranged from 0.19 to 0.55, averagely $0.29 \pm 0.12$ (Table 1).

Total nitrogen concentration was ranging from 37.4 mg $N/dm^3$ to 725.6 mg $N/dm^3$, at the average value on the level of $279.6 \pm 203.3$ mg $N/dm^3$ (Figure 3). Ammonium nitrogen concentration was 2.2-28.1 mg $N\text{-}NH_4/dm^3$, averagely $9.1 \pm 6.7$ mg $N\text{-}NH_4/dm^3$ (Figure 4). Results show that organic nitrogen (proteins) made up almost total nitrogen concentration, and amino groups conversion was insignificant.

Total phosphorus concentration in wastewater from apparatus room was ranging from 7.2 mg $P/dm^3$ to 131.7 mg $P/dm^3$, at the average of $76.4 \pm 40.3$ mg $P/dm^3$ (Figure 5). Orthophosphates concentration varied from 4.9 to 104.2 mg $P\text{-}PO_4/dm^3$, averagely $51.3 \pm 30.5$ mg $P\text{-}PO_4/dm^3$ (Figure 6). Orthophosphates made up 56-82% of total phosphorus, at the average value on the level of 66%. Probably, organic phosphorus and polyphosphates, coming from milk, alkaline chemical washing agents, made up remaining 34% of the total phosphorus.

Wastewater reaction varied from pH=3.39 to pH=13.00. Wide range of the reaction resulted from using alkaline and acid washing and disinfection chemicals (Figure 7). Concurrently, the average value of the reaction 9.93 ± 2.55 pH shows that wastewater are mostly alkaline.

## Wastewater from butter section

In wastewater from butter section the lowest value of COD concentration was 940 mg $O_2/dm^3$ and the highest one was 20 720.6 mg $O_2/dm^3$ (Figure 1). The average value of COD was on the level of 7058.6 ± 5656 mg $O_2/dm^3$. $BOD_5$ concentration was ranging from 213 mg $O_2/dm^3$ to 2766 mg $O_2/dm^{-3}$, averagely 1854 ± 938 mg $O_2/dm^3$ (Figure 2). The ratio of $BOD_5$/COD varied from 0.07 do 0.59, but the average value was 0.34 ± 0.17 (Table 1).

Total nitrogen concentration ranged from 17.6 mg N/$dm^3$ to 812.6 mg N/$dm^3$, averagely 217 ± 258 mg N/$dm^3$ (Figure 3). Ammonium nitrogen concentration was low, in the range of 0.3-16.2 mg N-$NH_4/dm^3$, at the average value of 7.0 ± 4.97 mg N-$NH_4/dm^3$ (Figure 4).

The lowest value of total phosphorus in wastewater from butter section was on the level of 1.1 mg P/$dm^3$ but it reached the concentration of 169.2 mg P/$dm^3$ (Figure 5). The average value was 45.6 ± 58.7 mg P/$dm^3$. In the same samples orthophosphates concentration ranged from 0,6 to 52,6 mg P/$dm^3$, averagely 15.67 ± 17. 41 mg P/$dm^3$ (Figure 6). Orthophosphates made up 30-74% of total phosphorus but the average value was about 44%.

The reaction of wastewater from butter section was alkaline and ranged from pH = 9.29 to pH = 13.19 (Figure 7). If this kind of wastewater was treated separately, in all cases the reaction would be the limited factor for activated sludge growth (optimal rang is from 6,0 to 8.0 pH).

## Wastewater from cottage cheese section

Organic compounds concentration in wastewater from cottage cheese section was ranging from 3488 mg $O_2/dm^3$ to 23 915 mg $O_2/dm^3$ (Figure 1). The average value of COD was 15 877 ± 6422 mg $O_2/dm^3$. $BOD_5$ concentration varied from 1186 mg $O_2/dm^3$ do 4234 mg $O_2/dm^3$, averagely 2713 ± 868 mg $O_2/dm^3$ (Figure 2). The ratio of $BOD_5$/COD changed from 0.0 to 0.42 but the average value was 0.21 ± 0.1 (Table 1).

Total nitrogen concentration ranged from 107.8 mg N/$dm^3$ to 670.5 mg N/$dm^3$, the average was 409.8 ± 186.6 mg N/$dm^3$ (Figure 3). Ammonium nitrogen concentration was ranging from 4.4 mg N-$NH_4/dm^3$ to 73.9 mg

N-$NH_4$/$dm^3$ (the average – 26.5 ± 18.1 mg N-$NH_4$/$dm^3$) – Figure 4. Total nitrogen concentration was higher than in wastewater from milk reception point, apparatus room and butter section.

Total phosphorus concentration in wastewater from cottage cheese section was 58-378 mg P/$dm^3$, but the average value was on the level of 210 ± ± 97.7 mg P/$dm^3$ (Figure 5). Orthophosphates concentration ranged from 48.8 to 256.2 mg P/$dm^3$, averagely 156.1 ± 67.8 mg P/$dm^3$ (Figure 6). Orthophosphates made up 67-88% of the total phosphorus, the average value was 76%. It was the higher value of orthophosphate contents in contrary to other kinds of wastewater.

The ratio of wastewater ranged from pH=3.66 to pH=12.13 (Figure 7). Wide range of the reaction resulted from using alkaline and acid washing and disinfection chemicals. Low values of pH can be caused by coagulation performing during cottage cheese production, using lactic, hydrochloric and sulphuric acids.

## Wastewater from cheese section

In wastewater from cheese section the lowest value of COD concentration was on the level of 7208 mg $O_2$/$dm^3$, the highest one was 20 045 mg $O_2$/$dm^3$ (Figure 1). The average value of COD concentration was 13 760 ± 3901 mg $O_2$/$dm^3$. $BOD_5$ concentration ranged from 1086 mg $O_2$/$dm^3$ to 5030 mg $O_2$/$dm^3$, averagely 2923 ± 1150 mg $O_2$/$dm^3$ (Figure 2). The ratio of $BOD_5$/COD varied from 0.14 to 0.35 but the average value was 0.21 ± 0.06 (Table 1).

Total nitrogen concentration ranged from 186.1 mg N/$dm^3$ to 689.2 mg N/$dm^3$, at the average value on the level of 431.3 ± 143.8 mg N/$dm^3$ (Figure 3). The level was similar to total nitrogen content in wastewater form cottage cheese section. Ammonium nitrogen concentration was low on the level of 0.6-33.7 mg N-$NH_4$/$dm^3$, averagely 14.4 ± 10.2 mg N-$NH_4$/$dm^3$ (Figure 4).

Phosphorus concentration varied from 42.7 mg P/$dm^3$ to 187.8 mg P/$dm^3$, at the average value of 112.1 ± 45 mg P/$dm^3$ (Figure 5). In the same wastewater samples orthophosphates concentration was ranging from 35.8 to 125.4 mg P/$dm^3$, averagely 79.7 ± 24.9 mg P/$dm^3$ (Figure 6). Orthophosphates made up 46-97% of total phosphorus concentration (the average value was 75%). Similarly as for wastewater from cottage cheese section, such high orthophosphate share may confirm the possibility of this nutrient removal in EBPR process.

The reaction of this wastewater was ranging from pH = 3.29 to pH = 11.61 (Figure 7). The reaction resulted from using alkaline and acid washing and disinfection chemical. Concurrently, only in two samples pH was below 6.5, what proves that wastewater are lightly alkaline or strongly alkaline.

## Cheese whey

Chemical oxygen demands concentration was ranging from 53 000 mg $O_2/dm^3$ to 87 659 mg $O_2/dm^3$ (Figure 1). The average value of COD concentration was 69 948 ± 9514 mg $O_2/dm^3$ (Figure 2). As for $BOD_5$ concentration it ranged from 18 967 mg $O_2/dm^3$ to 49 000 mg $O_2/dm^3$ (averagely – 28 911 ± ± 8744 mg $O_2/dm^3$). The ratio of $BOD_5$/COD varied in the range of 0.30 to 0.62, at the average 0.41 ± 0,09 (Table 1). This parameter was lower than in case of cottage cheese whey, what may cause some problems with its biodegradability.

Total nitrogen concentration ranged from 1264 mg $N/dm^3$ to 1502 mg $N/dm^3$, averagely on the level of 1382.3 ± 78 mg $N/dm^3$ (Figure 3). However, ammonium nitrogen concentration varied from 1.4 mg $N\text{-}NH_4/dm^3$ to 93.4 mg $N\text{-}NH_4/dm^3$ (average value was 40.3 ± 31.7 mg $N\text{-}NH_4/dm^3$) – Figure 4.

Total phosphorus concentration was ranging from 431 mg $P/dm^3$ to 546.2 mg $P/dm^3$, at the average of 546.2 ± 39.3 mg $P/dm^3$ (Figure 5). Orthophosphates concentration changed in the range of 245.8 to 401.6 mg $P/dm^3$, averagely 309.3 ± 49 mg $P/dm^3$, and it made up 49-80% of total phosphorus concentration (averagely 61%) – Figure 6.

The reaction of cheese whey ranged from pH = 3.82 to pH = 6.2 (Figure 7). Acid character of wastewater resulted from the conditions of lactic fermentation performing during cheese production.

## Cottage cheese whey

Chemical oxygen demand concentration was ranging from 45 198 mg $O_2/dm^3$ to 70 502 mg $O_2/dm^3$ (Figure 1). The average value of COD concentration was on the level of 56 338 ± 7140 mg $O_2/dm^3$. $BOD_5$ concentration varied from 13 200 mg $O_2/dm^3$ to 41 890 mg $O_2/dm^3$, averagely – 28 586 ± 8294 mg $O_2/dm^3$ (Figure 2). The ratio of $BOD_5$/COD changed in the range from 0.21 to 0.74, at the average value on the level of 0.51 ± 0.15 (Table 1). It may confirm the possibility of biodegradation by microorganisms.

Total nitrogen concentration ranged from 438.1 mg $N/dm^3$ to 1507 mg $N/dm^3$, averagely 995.5 ± 293 mg $N/dm^3$ (Figure 3). High ammonium concentration was observed in the range of 12.1-128 mg $N\text{-}NH_4/dm^3$, at the average of 61.2 ± 33.47 mg $N\text{-}NH_4/dm^3$ (Figure 4).

Total phosphorus concentration changed from 460.9 mg $P/dm^3$ to 862 mg $P/dm^3$ (average value was 710.1 ± 120 mg $P/dm^3$) – Figure 5. Concentration of orthophosphates was ranging from 4 to 615 mg $P/dm^3$, at the average of 483.4 ± 164.9 mg $P/dm^3$ (Figure 6). Orthophosphates made up from 50 to 99% of total phosphorus concentration (averagely – 74%).

The reaction of cottage cheese whey ranged from pH = 4.01 to pH = 5.8 (Figure 7). Strongly acid character of wastewater resulted from using highly concentrated acids at cottage cheese production.

## Mixed wastewater

COD concentration of this wastewater was in the range of 1044.2--6737.3 mg $O_2/dm^3$, at the average value of 3762.9 ± 1769.6 mg $O_2/dm^3$ (Figure 1). The value of $BOD_5$ concentration ranged from 283 mg $O_2/dm^3$ to 2790 mg $O_2/dm^3$, and the average value was 1622.4 ± 851.8 mg $O_2/dm^3$ (Figure 2). The ratio of $BOD_5$/COD changed from 0.24 to 0.74, averagely 0.43 ± 0.18 (Table 1).

Total nitrogen concentration was ranging from 9.6 mg N/$dm^3$ to 121.2 mg N/$dm^3$, and the average value was 59.4 ± 31 mg N/$dm^3$ (Figure 3). However, ammonium nitrogen concentration was in the range of 2.5-12.6 mg N--$NH_4/dm^3$, averagely 7.0 ± 3,0 mg N-$NH_4/dm^3$ (Figure 4).

Phosphorus concentration in mixed wastewater ranged from 21.2 mg P/$dm^3$ to 60.2 mg P/$dm^3$ (Figure 5). The average value of phosphorus was 32.9 ± 11.6 mg P/$dm^3$. Orthophosphates made up from 49 to 80% of total phosphorus concentration (averagely – 64%).

Mixed wastewater reaction varied from pH=3.28 to pH=11.64 (Figure 7). Wide range of the value of the reaction proves that particular kinds of wastewater produced at different section determine the final reaction.

## Discussion

Dairy wastewater treatment involves the necessity to provide high effective technologies with simultaneous carbon, nitrogen and phosphorus removal. It results from this kind of wastewater composition (CHUDZIK 1997, BARTKIEWICZ 2000, LEMAN 2001), moreover, from law demands concerning treated wastewater from this branch of food industry (*Regulation of Minister of Environmental Protection*... 2004).

In tank with activated sludge at the wastewater treatment plant, simultaneous processes of carbon removal, nitrification, denitrification and biological phosphorus removal (EBPR) take place. The effectiveness of nitrogen and phosphorus removal depends on the accessibility of easily biodegradable organic compounds. These compounds are used not only by polyphosphates accumulating bacteria under anaerobic conditions, but also by denitrifying microorganisms at the reduction of nitrate to gas nitrogen under anoxic and anaerobic conditions.

Easily biodegradable organic compounds concentration presents in wastewater with relation to total organics concentration directly influences on the effectiveness of nitrate and phosphorus removal.

According to the stechiometric equation in order to reduce 1 g $N\text{-}NO_3$ – 0.646 g COD is needed, it means 2.86 g COD/g N (GRADY et al. 1999). In accordance with NARKIS et al. (1979) a complete denitrification is possible that the ratio of organics to nitrate is 2.3 g $BOD_5$/g $N\text{-}NO_3$ (independently on carbon source). Mentioned values do not include organic substrate used by microorganisms of activated sludge as energy source and to new cells synthesis. Moreover, part of organic substrate is slowly or nonbiodegradable and can not be used by denitrifiers and consequently is oxidized in nitrification tank (or during aeration phase). Taking into account all the processes and the experience of operating wastewater treatment plants, it is known that in order to obtain completed denitrification the ratio of COD : $N\text{-}NO_3$ should be ranging from 5 to 10 (HENZE 1991).

More reliable determination of possibility of completed biological nitrogen removal from wastewater (the amount of nitrogen removed is obtained mainly due to denitrification) in activated sludge tank is the ratio of supplied substrate concentration (expressed as COD) to total nitrogen concentration. According to the literature date (GRADY et al. 1999) In case of the ratio of COD : N below 5 the effectiveness of nitrogen removal is really poor. However, the ratio above 9 can guarantee very high nitrogen removal efficiency.

Experimental results of dairy wastewater composition revealed that regardless of the place of generating, the average value was above 9. In case of mixed wastewater from the dairy plant the ratio of COD : N was on the level of $91.65 \pm 107.96$ g COD/g N. Minimal value of the ratio was 31.24 g COD/g N and was three-fold higher than the value of the ratio required to obtain completed denitrification in activated sludge. It proved that denitrification process performing in activated sludge tank would not be limited by organic compounds.

In case of phosphorus removal organic substrate concentration essential at dephosphatation process depends on using of technological system. Technology A/O, requiring the ratio of $BOD_5$ : P ($BOD_5$ and P concentration determined before filtration of wastewater) in the range of 30 – 40 g $BOD_5$/g P, guarantees effluent phosphorus concentration below 1 mg P/dm$^3$ (TETREAULT at al. 1986). Modified Bardenpho system at the ratio from 15 to 25 g $BOD_5$/g P lets obtain final phosphorus concentration from 0.7 to 2.5 mg P/dm$^3$.

Other literature date (GRADY et al. 1999) shows that, depending on the technology for nutrient removal, recommended ratios of $BOD_5$ : $\Delta$P and COD : $\Delta$P are given ($\Delta$P this is the difference between total phosphorus concentration P in the influent, in the wastewater samples before filtration, and soluble phosphorus, in the effluent samples after filtration). In case of Bardenpho

system, operating with low phosphorus removal effectiveness, in order to remove 1 g P at least 43 g ChZT (> 25 g $BZT_5$/g P) is required. However, as for A/O™ and $A^2$/O™ systems (both with nitrification) in order to achieve medium effectiveness of phosphorus removal COD is required in the range from 34 to 43 g COD/g P (20 – 25 g $BOD_5$/g P). In high effective technologies e.g. A/O™ (without nitrification) and UCT to remove 1 g P we have to provide from 26 to 34 g COD (15 – 25 g $BOD_5$/g P).

According to BERNARD (2000) at wastewater treatment plant for biological phosphorus removal the ratio of COD : Ptot over 40 : 1 is much more reliable than the ratio of $BOD_5$ : Ptot on the level of 20 : 1 (at the assumption that total phosphorus concentration in the effluent is below 1.0 mg P/$dm^3$. There is lack of the literature date concerning COD requirements at the higher concentration than 43 g COD/g P and 25 g $BOD_5$/g P. All cited values are given for municipal wastewater treatment.

From the analysis of presented results it can be concluded that from mixed wastewater from dairy industry the average value of the ratio of $BOD_5$ : COD was $0.43 \pm 0.18$ (minimal value – 0.24). Biodegradable organics concentration was lower than in municipal wastewater (KLIMIUK, ŁEBKOWSKA 2003). For this reason $BOD_5$ is more reliable parameter, as for accessibility of easily biodegradable organic fraction used at biological phosphorus removal. But at the same time we have to remember that in sequencing batch reactors SBR that after shorter phase of mixing there is longer phase of aeration (the most common conditions), and at the beginning of the next cycle nitrate are present in the reactor. Nitrate concentration can be higher than in municipal wastewater because dairy wastewater does not undergo a sedimentation process, and simultaneously, in wastewater high total nitrogen concentration can be observed (over 121 g $N_{tot}$/$dm^3$, the value id higher than in municipal wastewater before fermentation, (KLIMIUK, ŁEBKOWSKA 2003) depending on chemicals using at devices and installation washing. It means that in order to determined minimal ratio of $BOD_5$ : P for this wastewater treatment technology, guarantying phosphorus concentration in the effluent below 1.0 mg P/$dm^3$, we have to assume the values higher than for A/O™ and $A^2$/O™ systems with nitrification i.e. over 25 g $BOD_5$/g P.

The average value of the ratio of $BOD_5$ : $P_{tot}$ in dairy wastewater was on the level of $49.32 \pm 25.26$ g $BOD_5$/g P. In some cases it can be a limiting parameter what is confirmed by the value of standard deviation (SD). This vale means that required organics concentration will be not sufficient to remove phosphorus below 1.0 mg P/$dm^3$. It is also confirmed by the minimal value of the ratio 12.2 g $BOD_5$/g P obtained during the investigations. Additional limiting parameter can be nitrate presented at the beginning of the mixing phase. Then organic compounds would be used by denitrifying microorganisms.

## Conclussions

The highest concentration of organic compounds was obtained in whey of cheese and cottage cheese production, on the level of COD over 69 000 and 56 000 mg $O_2/dm^3$, respectively. Among other kinds of wastewater the highest value of COD was observed in wastewater from cottage cheese section and apparatus room (COD concentration over 15 000 mg $O_2/dm^3$), and from cheese section (COD concentration over 13 000 mg $O_2/dm^3$). Concurrently, the highest value of $BOD_5$ was determined in these kinds of wastewater.

The highest total nitrogen concentration was in cheese whey (over 1300 mg $N/dm^3$) and in cottage cheeses whey (almost 1000 mg $N/dm^3$). In case of wastewater the highest value of nitrogen concentration was noted in wastewater from cheese section (over 430 mg $N/dm^3$), cottage cheese section (over 400 mg $N/dm^3$) and apparatus room (over 270 mg $N/dm^3$).

As for phosphorus concentration, cottage cheese and cheese whey characterized by the highest phosphorus concentration on the level over 700 mg $P/dm^3$ and over 500 mg $P/dm^3$, respectively. The highest phosphorus concentration was in wastewater from cottage cheese section (over 210 mg $P/dm^3$), cheese section (over 110 mg $P/dm^3$) and apparatus room (over 75 mg $P/dm^3$).

The most appropriate proportion between organic substances (expressed as COD) and other nutrients (expressed as a sum of total nitrogen and phosphorus concentration) was observed in wastewater from apparatus room (67.6 mg COD/(mg N + mg P)) and wastewater from butter section (59.5 mg COD/(mg N + mg P)). The value of this proportion of wastewater from milk reception point and pumping station was about 43 mg COD/(mg N + mg P). As for other kinds of wastewater and whey this parameter was ranging from 26.3 mg COD/(mg N + mg P) (cottage cheese section) to 37.0 mg COD/ /(mg N + mg P) (cheese whey).

In case of organic compounds expressed as $BOD_5$ the most appropriate proportion with relation to sum of total nitrogen and phosphorus concentration was observed in wastewater from apparatus room (20.59 mg $BOD_5$/(mg N + mg P)), wastewater from pumping station (18.82 mg $BOD_5$/(mg N + mg P)), from cottage cheese section (17.72 mg $BOD_5$/(mg N + mg P)), from milk reception point (17.22 mg $BOD_5$ /(mg N + mg P)) and from butter section (16.64 mg $BOD_5$/(mg N + mg $P_{tot}$)).

Translated by Marcin Dębowski

Accepted for print 9.03.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 325-343, Y. 2006

# CONDITIONS FOR THE GROWTH AND DEVELOPMENT OF THE A POPULATION OF THE NEW CLAM SPECIES TO POLAND *SINANODONTA WOODIANA* (LEA, 1834) IN ANTROPOGENICALY TRANSFORMED ECOSYSTEM

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K e y w o r d s: *Unionidae*, *Sinanodonta woodiana*, ecological structure, annual growth, heated waters.

A b s t r a c t

In the mid 1980s, the clam *Sinanodonta woodiana* (Lea, 1834) from southeast Asia was accidentally introduced to the Konin heated lake system (central Poland) from Hungary along with stocking material. The heterogeneous conditions of various stations of the Konin ecosystem had a significant impact on the spatial, morphophysiological, age, and sex structures of the *Sinanodonta woodiana* population. Specimens with shell lengths of 70-115 mm dominated in the cooler zones, those measuring from 90 to 125 mm dominated in the moderately heated zones, and in the warmest areas the dominants were 125-160 mm in length. The most intense growth was noted in one- and two-year-old specimens, while that of older specimens did not exceed 2 cm annually.

The *Sinanodonta woodiana* population was comprised of specimens ranging in age from three to five years. The largest age-size group occurred in the zone with the strongest flow of heated water. The oldest specimens were aged ten years and had attained lengths of 230-240 mm. *Sinanodonta woodiana* did not exhibit sexual dimorphism. Males, which comprised more than 70% of the specimens, dominated the sex structure of the population.

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**WARUNKI WZROSTU I ROZWOJU POPULACJI MAŁŻY SINANODONTA WOODIANA (LEA, 1834) W ANTROPOGENICZNIE PRZEKSZTAŁCONYM EKOSYSTEMIE**

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S ł o w a k l u c z o w e: *Unionidae, Sinanodonta woodiana,* struktura ekologiczna, przyrost roczny, wodny podgrzane.

A b s t r a k t

Małże *Sinanodonta woodiana* (Lea, 1834) pochodzące z południowo-wschodniej Azji zostały zawleczone z materiałem zarybieniowym z Węgier do antropogenicznie przekształconego systemu podgrzanych jezior konińskich (centralna Polska).

Duża heterogenność siedlisk tych małży w ekosystemie konińskim wpłynęła znacząco na strukturę przestrzenną, morfofizjologiczną, wiekową i płciową populacji *Sinanodonta woodiana.* W chłodniejszych strefach dominowały osobniki, u których długość muszli wynosiła 70-115 mm, w umiarkowanie podgrzanych – 90-125 mm, a w najcieplejszych – 125-160 mm. Najintensywniejszy wzrost stwierdzono u osobników jednorocznych i dwuletnich. Przyrost starszych małży nie przekraczał 2 cm rocznie. W podgrzanych jeziorach konińskich populację *Sinanodonta woodiana* tworzyły osobniki w wieku 3-5 lat. Najwięcej grup wiekowo-wymiarowych występowało w strefach o najsilniejszym wpływie wód podgrzanych, szczególnie widoczne to było w zbiorniku wstępnego schładzania. Najstarsze osobniki, w wieku 10 lat, osiągały 230-240 mm długości.

U *Sinanodonta woodiana* nie stwierdzono dymorfizmu płciowego. W strukturze płciowej populacji wykazano ponad 70% udział osobników męskich.

## Introduction

*Sinanodonta woodiana* (Lea, 1834) is classified to the order Unionoidea and is a new species to Polish malacological fauna. According to the latest systematics conducted as part of the European-wide CLECOM project, the goal of which is to unify the nomenclature and classification of European inland water mussels, the appropriate genus name appears to be *Sinanodonta* (BOETERS et al. 2001).

Natural habitat of this clam is the ecosystems of two, large Asian rivers – the Amur and Yangtze. Activities related to the spawning and rearing of cultivated fish have impacted the spread of this species not only in southern Asia, but also in Central America and in some European countries (WATTERS 1997, KRASZEWSKI 2003).

*Sinanodonta woodiana* was accidentally introduced into Poland from Hungary in the mid 1980s along with stocking material of herbivorous fish – silver carp *Hypophthalamichthys molitrix* (Valenciennes, 1844) and grass carp *Ctenopharyngodon idella* (Valenciennes 1844) (PROTASOV et al. 1993, ZDANOWSKI et al. 1996, KRASZEWSKI, ZDANOWSKI 2001). The advantageous

environmental conditions provided to this species in the Konin heated lake system, especially higher temperature and water turbulence, allowed for their intensive growth and development. The clams abundantly inhabit the littoral zone of the five lakes in the system, the initial cooling basin, and the majority of both intake and discharge canals. Currently, the *Sinanodonta woodiana* population appears to be stable, and this species is the dominant among benthic fauna aggregations. In addition to the Konin system, *Sinanodonta woodiana* has also settled in Poland in the discharge canal of the Dolna Odra Power plant in Nowy Czarnów (DOMAGAŁA et al. 2004). Natural, unheated basins under Polish climatic conditions do not provide advantageous habitats for this clam. There is, however, one exception, five live specimens were found in fish ponds near Sieraków (MIZERA, URBAŃSKA 2003).

The aim of the study was to analyze the population of the clam *Sinanodonta woodiana* (Lea, 1834) with regard to its morphophysiological and age structures and the conditions for growth and development provided by the various habitats in the Konin heated lake system and associated canals.

## Study area

The Konin lakes[1] system is situated in central Poland in the Wielkopolsko-Kujawski Lakeland 20 km north of Konin in the Lake Gopło catchment area (KONDRACKI 1998). With a total surface area of 13 km$^2$, the Konin lake system is comprised of five lakes connected by canals that serve as the cooling system for the Konin and Pątnów electric power plants. Lakes of Konin are characterized by varied morphology, trophic status, mixing, and thermal regimes – Table 1 (HILLBRICHT-ILKOWSKA, ZDANOWSKI 1988, ZDANOWSKI 1989, 1994). The network of the cooling system canals that intakes and discharges power plant water is 26 km in length (Table 2). Since 1976, the system has been connected to the artificial initial cooling basin that is supplied by post-cooling waters from the Konin power plant (Table 1). The water level in the system is maintained by two chamber locks located on the Warta-Gopło Canal that supplies water to the system, and water flow is regulated by four intermediate pumping stations.

The Konin lakes have two water circulation systems. During periods of lower temperatures from September to May the "short" system is operational, while in the summer season, the "longer" water system is put into operation, and Lake Ślesińskie and the northern section of Lake Mikorzyńskie are included.

[1] This commonly used name refers to the complex of heated lakes in the vicinity of Konin – Ślesińskie, Mikorzyńskie, Pątnowskie, Licheńskie, and Gosławskie.

Table 1

Limnological, morphometric, and hydrological characteristics of the Konin heated lake system and the initial cooling basin (according to data from Inland Fisheries Institute (IFI) and Pątnów-Adamów-Konin Electric Power Plants (ZE PAK)

| Index | Unit | Lake Ślesińskie | Lake Mikorzyńskie | Lake Pątnowskie | Lake Licheńskie | Lake Gosławskie | Preliminary cooling reservoir |
|---|---|---|---|---|---|---|---|
| Limnological type | – | eutrophy, dymixis | β-mezo/eutrohy, dymixis | eutrophy, polymixis | eutrophy, monomixis | eutrohy, polimixis | eutrophy, polimixis |
| Surface area | ha | 152.3 | 251.8 | 282.6 | 147.6 | 454.5 | ~ 75 |
| Maximal depth | m | 24.5 | 36.5 | 5.5 | 12.6 | 5.3 | 4.2 |
| Average depth | m | 7.6 | 11.5 | 2.6 | 4.5 | 3.0 | 2.5 |
| Shore-line development | m | 11 500 | 15 520 | 11 910 | 12 660 | 11 330 | – |
| Water retention* | d | **14** (6-30) | **9** (5-16) | **4** (3-6) | **3** (2-5) | **5** (4-6) | **3** (3-4) |
| Water temperature* | °C | **14.1** (0.6-27.0) | **14.5** (2.5-31.5) | **14.8** (2.3-30.0) | **16.2** (4.2-31.5) | **16.4** (5.5-28.5) | **22.0** (6.0-33.0) |

* Data from 1999-2002; values of water retention in the Ślesińskie and Mikorzyńskie lakes during operation of "long" water system May-September.

Table 2

Morphometric and hydrological characteristics of the canals of the Konin system (according to authors' own data and that of ZE PAK)

| Canal | Length (km) | Depth (m) | Total width (m) | Average width of bottom (m) | Nominal value of water flow* ($m^3 \cdot s^{-1}$) | Flow velocity ($m \cdot s^{-1}$) | Water temperature** (°C) |
|---|---|---|---|---|---|---|---|
| Konin Power Plant intake canal | 1.50 | 3.8 | 15-30 | 6.0 | 25 | 0.32 | 14.7 (1.5-27.8) |
| West Pątnów Power Plant intake canal | 2.64 | 2.6 | 20-35 | 16.0 | 23 | 0.14 | 15.3 (2.5-27.5) |
| East Pątnów Power Plant intake canal' | 1.87 | 4.5 | 20-30 | 10.0 | 28 | 0.90 | 20.1 (10.8-26.8) |
| Konin Power Plant discharge canal | 0.75 | 3.8 | 15-30 | 13.5 | 25 | 0.12 | 22.5 (10.6-35.9) |
| Pątnów Power Plant discharge canal | 4.25 | 4.0 | 20-40 | 16.0 | 53 (30) | 0.28 | 23.5 (9.6-35.0) |
| Licheński | 5.21 | 4.0 | 15-40 | 14.0 | 55 (29) | 0.17 | 21.2 (9.4-33.8) |
| Licheńsko-Pątnowski | 0.50 | 3.0 | 15-25 | 6.0 | 6 | 0.11 | (4.2-23.5) |
| Wąsoski | 5.57 | 3.3 | 15-30 | 12.0 | 15 (10) | 0.09 | (9.4-24.5) |
| Piotrkowicki | 6.20 | 3.2 | 15-40 | 15.0 | 23 | 0.05 | 24.8 (18.0-30.5) |
| Ślesińsko-Mikorzyński | 0.25 | 2.8 | 25-30 | 14.0 | 20 | 0.02 | (3.1-24.0) |
| Warta-Gopło | 8.50 | 3.5 | 25-50 | 11.5 | 30 | 0.10 | (4.5-25.6) |

* water flow in the last canal segment is in parentheses

** data from the 1999-2002 period; range of values in parentheses

## Methods

The study of the clam *Sinanodonta woodiana* population was conducted in the 1999-2002 period at stations located in the littoral zones of five lakes, the initial cooling basin of the Konin power plant, and the intake and discharge canals of the system (Figure 1). The clam study stations were determined with the scuba diving method described in Protasov et al. (1982).

After the bottom morphometry of various sections of canals and the littoral zone of the lakes (width, depth, bottom profile) had been described initially, measurements of water flow rate were taken and typical habitat types and the variety of substrata that the clams settled were identified. This information was used to choose stations with representative characteristics of the sites inhabited by the clams in the lakes, canals, and initial cooling basin. Continuous observations of the clams (several times in a year) were conducted in lakes Ślesińskie and Licheńskie, in the post-cooling water discharge and outflow zones of the initial cooling basin, and in the Licheńsko-Pątnowski, Konin power plant intake and discharge, Warta-Gopło, Licheński, and Piotrkowicki canals (Figure 1).

The habitats where the clams occurred were characterized based on the analyses of water flow rates in the canals and the initial cooling basin, the granulometry and organic matter content of bottom sediments, water temperature, and seston content.

Clams were collected for analysis using a frame with a surface area of 0.25 $m^2$ (0.5 x 0.5 m) from a bottom surface area of at least 1 $m^2$. Periphyton and sediments were cleaned off the specimens in the laboratory, and they were left to dry on filtration tissue for ten minutes. A slide caliper was used to take measurements of the specimens to the nearest 1 mm.

Clam age was determined by reading the annual growth increments, this method is commonly applied in malacology (Crowley 1957, Piechocki 1969, Sadykhova 1972, Neves, Moyer 1988, Abraszewska-Kowalczyk 2002).

Clam growth and the maximum theoretical life span was described with the Ludwig von Bertalanffy equation using the Walford method (1946) adapted to malacological studies (Afanasjev et al. 2001).

Clam growth rate was studied experimentally (*in situ*) based on seasonal and annual growth increments in the heterogeneous habitats – Konin power plant intake and discharge canals, initial cooling basin (Figure 1). Marked *Sinanodonta woodiana* specimens from various size groups were placed in wire pens with a surface area of 0.3 $m^2$, and deployed in these habitats at depths of 2 m. These were left for two years from July 1999 to July 2001.

The sex of *Sinanodonta woodiana* was determined with the method described in Vlastov (1956) in clam specimens from habitats with varied

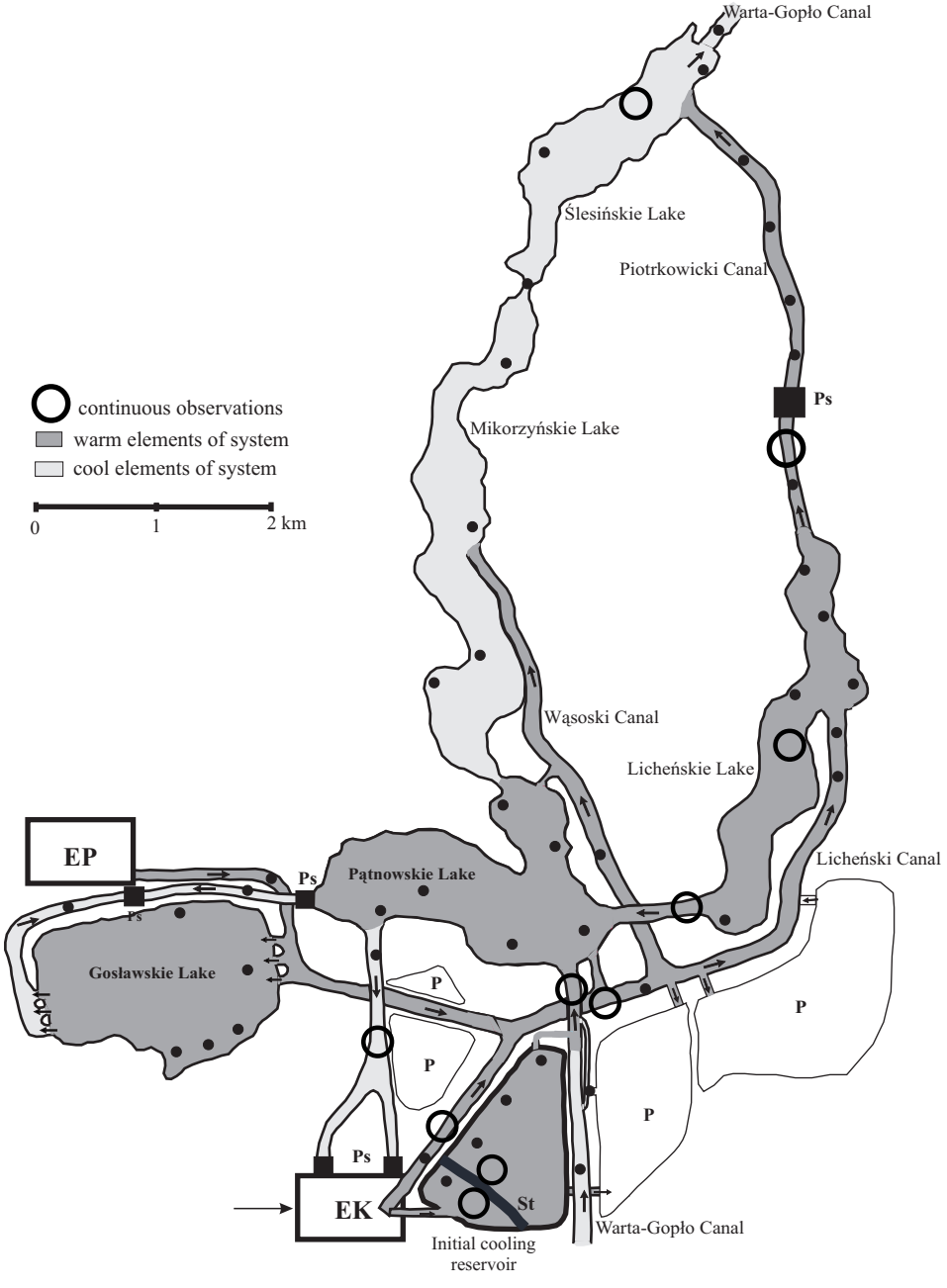


Fig. 1. Study stations of the calm *Sinanodonta woodiana* (Lea, 1834) in the Konin heated lakes: EP – Pątnów power plant, EK – Konin power plant, Ps – pump station, P – ponds

environmental conditions. A section of the gonad was excised *in vivo* from specimens from the littoral zone of lakes Licheńskie and Ślesińskie, the Warta-Gopło Canal, and the Konin power plant discharge canal. The sections were excised in June when mature reproductive cells found in the gonads. The specimens were sexed based on the presence of either oocytes or sperm. The specimens which were shown to have two types of reproductive cells – eggs and sperm – were categorized as hermaphrodites. The clam sex ratio at given sites was analyzed with the $\chi^2$ test. The morphological differences between male and female specimens (sexual dimorphism) was determined with variance analysis (ANOVA).

# Results

## Environmental conditions

The clam habitats in the Konin heated lake system differed significantly with regard to thermal conditions. Less prefer for Chinese mussels were cooler littoral of lakes Ślesińskie and Mikorzyńskie (22-27°C, summer season), than littoral in lakes Pątnowskie, Gosławskie, and Licheńskie (25-30°C) – Figure 1. Temperature differences between these lakes are higher in the winter season, and they are even higher between the intake and discharge canals, reaching as much as 8-10°C (Table 2). The maximum water temperature in the intake canals did not exceed 26°C, and in the discharge canals it was not higher than 35°C.

High water temperatures of up to 33°C were also noted in the Konin initial cooling basin. The difference in water temperature between the immediate post-cooling water discharge zone, the area in the middle of the basin, and that at the outflow was approximately 5°C.

The clam habitats also differed with regard to water flow rate. In the initial cooling basin this was recorded in the immediate post-cooling water discharge zone. Zones farther away from the water discharge were of a lentic character, as they were in the lakes. The differences in water flow in the canals was much more significant (0.02-0.90 $m \cdot s^{-1}$) – Table 2. The strongest water turbulence occurred in the immediate vicinity of the intermediate pumping stations. Those places were avoided by mussels. The fastest water flowed in the Pątnów power plant eastern intake canal and the slowest was in the cool canals that connected lakes Ślesińskie and Mikorzyńskie and the Piotrkowicki Canal (Figure 1). In most of the canals, weak currents or stagnant areas occurred nearest the banks.

The depth of the canals at the study stations did not exceed 3.5 m. The

banks of some of them were reinforced with fascine, including, among others, the Licheńsko-Pątnowski, Licheński, Piotrkowicki, and Warta-Gopło canals (Figure 1). The profile of the bottoms was gently sloped. There were rocks on the bottom in many places, including the intake and discharge canals of the Konin power plant, which made it difficult for clams to settle there.

The littoral zone of lakes Pątnowskie and Gosławskie had the widest range, enabling for *Sinanodonta woodiana* numerous settling places. A narrow littoral zone to a depth of 2.5 m and with a much steeper slope occurred in the northern area of Lake Licheńskie and throughout lakes Ślesińskie and Mikorzyńskie. The bottom profile of the initial cooling basin, which was 2.5 m deep, has a gentle, graduated slope.

The clams usually occurred on fine-grained and muddy substrata, as these substrate types dominated in the system. The organic matter content did not exceed 20% of the dry weight, even when filamentous algae and plant detritus occurred. The shells of dead zebra mussel *Dreissena polymorpha* Pallas 1771 or clams from the family Unionidae were frequent components of the substrata. Gravel or rocky bottoms made it difficult for mussels to settle, occurred mainly where there was the fastest water flow, *i.e.*, in the intake and discharge canals of the Konin power plant and the Licheński Canal.

Due to significant water circulation, there were only small differences in water salinity among the areas inhabited by the clams. Slightly higher concentrations of seston were noted occasionally in the initial cooling basin and in the Licheński Canal.

The canals were well oxygenated, and the oxygen content in the lake littoral did not fall below 6 $mg \cdot dm^{-3}$.

## Clam growth and development and the ecological structure of the population

The *Sinanodonta woodiana* population of the Konin system was comprised of several age classes. Most frequently, the clams reached an age of six to nine years, while approximately 70% of the population was comprised of specimens that were three to five years old. Specimens older than six years were the least numerous, a fact that was connected to natural mortality. The average age at the ten monitored study stations was 3.7 years, with the minimum average of 2.9 years in Lake Licheńskie and a maximum average of 4.1-4.2 years in the discharge canals of the Konin power plant and the Licheńsko-Pątnowski and Licheński canals (Table 3).

Lentic and lotic environments differed with respect to the share of clam age groups that inhabited them (Figure 2). The majority of clams in the lakes were

Table 3
Arrangement of increasing likelihood of clam age (mean values) occurring at monitored stations

| Station | Age* |
|---|---|
| Licheńskie Lake | 2.961$^{a}$ |
| Piotrkowicki Canal | 3.577$^{b}$ |
| Initial cooling reservoir | 3.686$^{b}$ |
| Ślesińskie Lake | 3.710$^{b}$ |
| Warta-Gopło Canal | 3.805$^{bc}$ |
| Initial cooling reservoir | 3.885$^{bc}$ |
| Konin power plant discharge canal | 4.147$^{c}$ |
| Licheńsko-Pątnowski Canal | 4.203$^{c}$ |
| Licheński Canal | 4.206$^{c}$ |

* The same indexes signal a significant likelihood (T-Tukey test, $\alpha = 0.05$).

three years old (Lake Licheńskie) or three and four years old (Lake Ślesińskie). Four-year-old clams dominated in the warmest canals (Warta-Gopło, Licheński and the Konin power plant canals). In the Konin power plant intake canal and the moderately warm Piotrkowicki and Licheńsko-Pątnowski canals, the largest share of clams was comprised of four- and five-year-old specimens, and the age structure was very similar within the range of 1-7 years. In the initial cooling basin, three, four-, and five-year-old specimens were at a decided advantage.

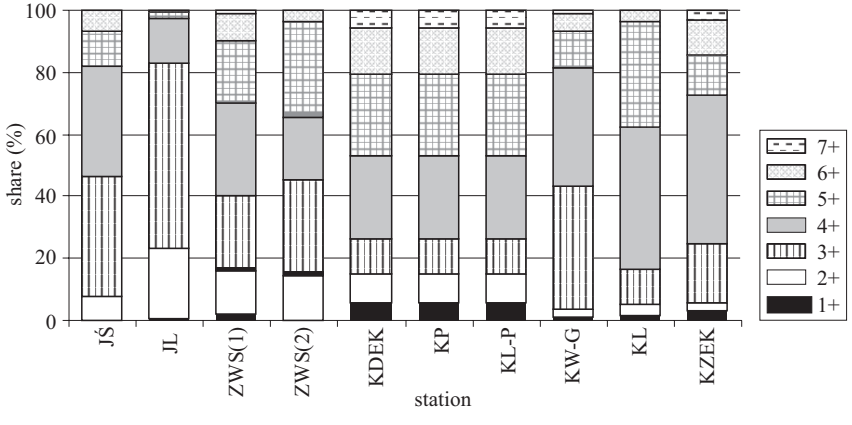


Fig. 2. Percentage share of various age groups of the clam *Sinanodonta woodiana* at the study stations monitored in the Konin system: JS – Lake Ślesińskie, JL – Lake Licheńskie, ZWS(1) – reservoir of initial cooling – discharge zone, ZWS(2) – reservoir of initial cooling – outflow zone, KDEK – Konin power plant intake canal, KP – Canal Piotrkowicki, KL-P – Canal Licheńsko-Pątnowski, KW-G – Canal Warta-Gopło, KL – Canal Licheński, KZEK – Konin power plant discharge canal

The area in the direct vicinity of the post-cooling water discharge in the initial cooling basin had the most clam size groups (Figure 3). The length range of shells from all the size classes was almost 200 mm (42-241 mm) in this location. The fewest size classes were noted in the middle and outflow zones of the initial cooling basin where the shell length range was barely 75 mm. The smallest clam size classes with shells of up to 40 mm were not noted as the youngest specimens were buried in the sediments.

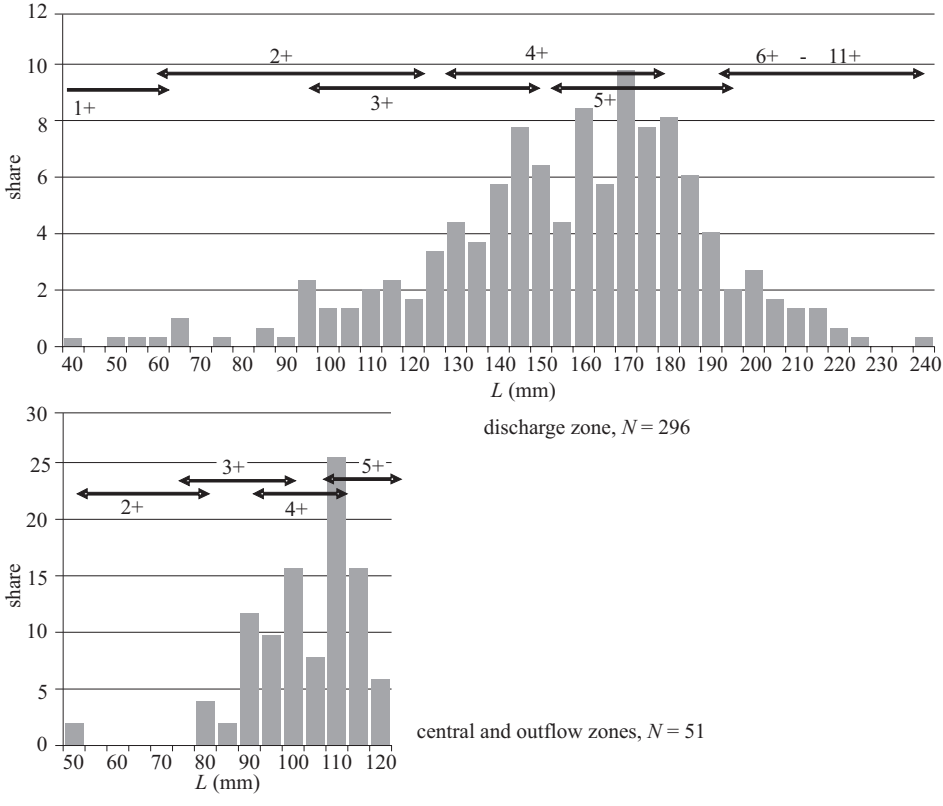


Fig. 3. Size-age structure and the share of various sizes of the clam *Sinanodonta woodiana* in the initial cooling basin

The three- and four-year-old specimens, which were the most numerous, differed as regards size at the various sites they inhabited. For example, the length range of three- and four-year-old clams in the warm Lake Licheńskie was 75-125 mm, while that of the cooler Lake Ślesińskie was 65-115 mm. Three- and four-year-old specimens from the warmest canal system were characterized by a shell length range of 90 to 150 mm, and in the moderately

heated canals from 85 to 135 mm. In the initial cooling basin the shell length range of three- to five-year-old specimens inhabiting the discharge zone was 90-175 mm, while in the middle and outflow zones it was 75-115 mm.

Increases in the individual weight of *Sinanodonta woodiana* were slower in comparison to shell length growth in the group of smaller specimens (up to 100 mm). The clams exhibited significantly more intense weight gain beginning at the shell length range of 140-150 mm.

The lowest annual growth was confirmed in the clams from the cooler Lake Ślesińskie, and the highest in specimens that inhabited the immediate vicinity of the post-cooling water discharge into the initial cooling basin (Figure 4). The first ring was deposited by specimens from Lake Ślesińskie at a shell length of 38.6 mm and in those from the initial cooling basin at 58.9 mm. The sixth increment was deposited at shell lengths of 123.8 mm and 180.3 mm, respectively.

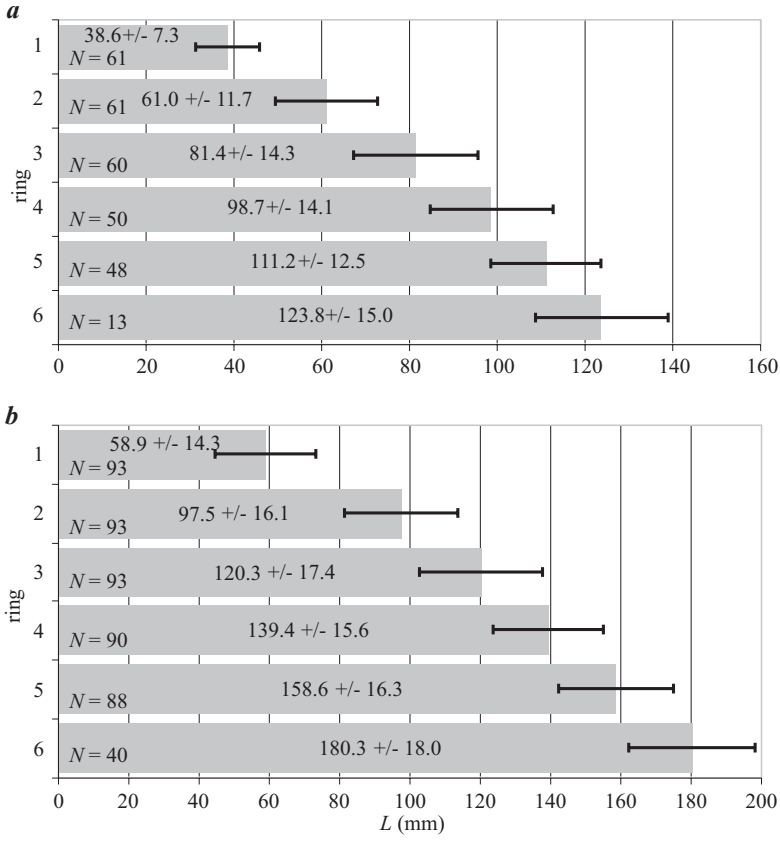


Fig. 4. Annual shell length growth of clams *Sinanodonta woodiana* (average ± SD) occurring in Lake Ślesińskie (*a*) and in discharge zone of the initial cooling basin (*b*)

The most intense growth was recorded in the youngest clams of one and two years and was approximately more than 20 mm annually. The fastest growth was confirmed in one-year-old specimens inhabiting the warmest canals of the system (approximately 34 mm) and the initial cooling basin (approximately 39 mm). Older clams grew slightly slower, and their growth did not exceed 20 mm annually.

The most intense clam growth occurred in the "summer" period (May--October) and accounted for 80% of the annual, individual clam growth in terms of both morphologic parameters (*L, H, W*) and weight (*M*).

The clams grew at a rate that was appropriate to the conditions prevailing in habitats. The most intense growth was exhibited by the clam *Sinanodonta woodiana* in the zone immediately adjacent to the post-cooling water discharge in the initial cooling basin, the Konin power plant discharge canal, and the Warta-Gopło Canal. The slowest growth was recorded in specimens that inhabited the coolest of the lakes – Lake Ślesińskie, and the middle and outflow sections of the initial cooling basin. The fastest growth was seen in the youngest specimens of two to three years of age.

It was demonstrated experimentally that the average annual growth of the clam *Sinanodonta woodiana* was 25.8 mm in the initial cooling basin, 17.6 mm in the Konin power plant discharge canal, and only 5.8 mm in the cool Konin power plant intake canal (Figure 5). Growth was also slower with age here. Concurrence with the variability of the morphological parameters of clams inhabiting natural conditions was confirmed.

The maximum theoretical shell size (up to 290 mm) can be achieved by clams inhabiting the zone in the immediate vicinity of the heated water discharge into the initial cooling basin, somewhat smaller sizes (245 mm) were achieved in the discharge canal, and the smallest (180-195 mm) – in the middle and outflow sections of the basin, lakes Ślesińskie and Licheńskie, and the Piotrkowicki, Licheńsko-Pątnowski and Licheński canals.

The maximum theoretical life span of *Sinanodonta woodiana* in the Konin heated lake system was eleven years, but the clams generally lived for a shorter time – nine years.

The sex structure of the *Sinanodonta woodiana* population in the Konin system was clearly dominated by males specimens (70-80%). Males comprised from 73.0 to 74.3% of the overall abundance of clams in lentic environments and 80.0% in the Licheński canal (Figure 6). Female specimens and hermaphrodites comprised from 20 to 30% of the population, with varying shares at different stations. In lentic environments, the share of hermaphrodites was high and ranged from 6.8 to 20.2%. The $\chi^2$ test indicated that the sex distribution at the various stations was random and independent ($\chi$emp 10.138 < $\chi$tab 12.592).

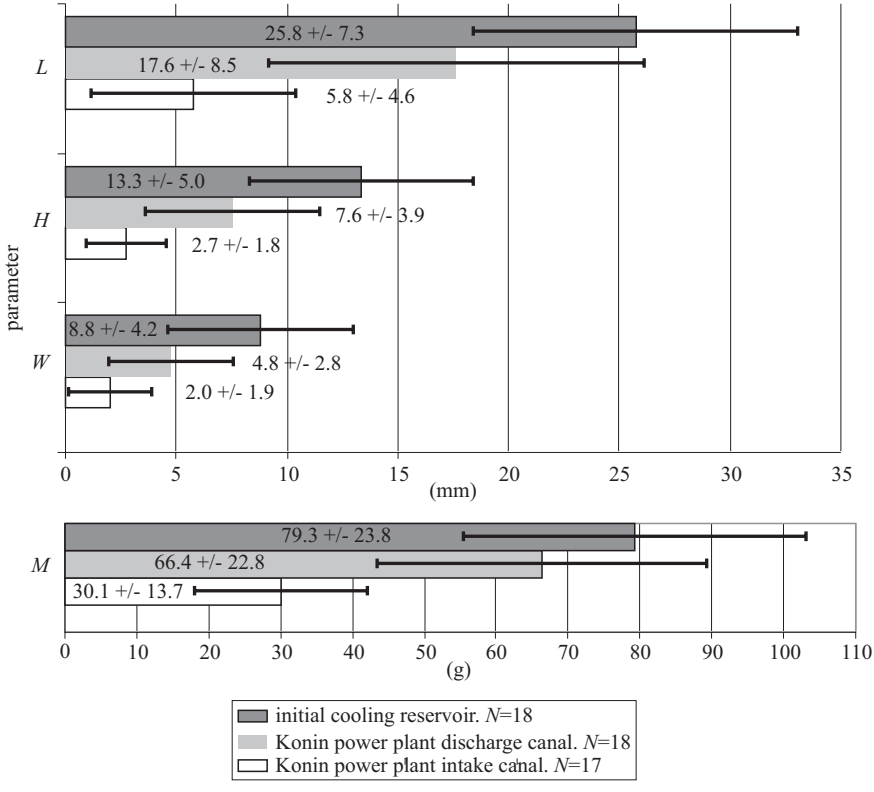


Fig. 5. Average, annual growth (L, H, W, M) of clams *Sinanodonta woodiana* at experimental study stations

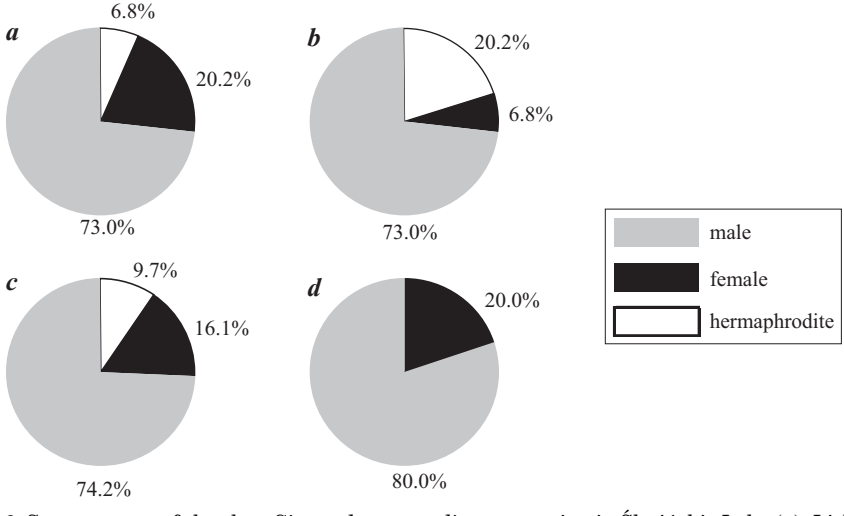


Fig. 6. Sex structure of the clam *Sinanodonta woodiana* occurring in Ślesińskie Lake (*a*), Licheńskie Lake (*b*), initial cooling reservoir (*c*) and Licheński Canal (*d*)

## Discussion

Apart from the process of reproduction itself, the larval stage is key in the development of clam populations. The settling of clam larvae depends on environmental conditions and access to suitable habitat substrata (WALZ 1975, LEWANDOWSKI 1976, 2001, STAŃCZYKOWSKA 1977, MELLINA, RASMUSSEN 1994, LEWANDOWSKI, STAŃCZYKOWSKA 2000). The settling of individual habitats shapes the distribution of clams throughout the ecosystem.

Larvae of mussels from the Unionidae family are attaching to a fish's body, where they stay dozen of days. KISS (1995) determined the time of remaining Chinese mussels glochidiosis about 220-250 daydegrees, which in the Hungarian ecosystems was 8-12 days. In the Konin heated lake system glochidia remained a shorter time from 4 to 9 days, bacause of higher water temperature prevailing there. There was observed from a few to dozen glochidia the most often attached to a dorsal and caudal fin of a several, various fish species. The other authors found mussel larvae from the Unionidae family on many, various fish species (ZALE, NEVES 1982, DUDGEON, MORTON 1984, KONDO 1989, BAUER 2001).

Information regarding transforming clam larvae that inhabit bottom sediments until sexual maturity is achieved is fragmentary (JANSEN et al. 2001). KISS (1995), who observed Chinese clam behavior under experimental conditions, confirmed that during the first month of sediment dwelling 30-40% of the larvae survived and attained a size range of 1.0-1.5 mm. Immature *Sinanodonta woodiana* specimens were noted infrequently in the Konin heated lake system. They probably dwelled buried several centimeters under the surface of the bottom sediments. According to PIECHOCKI (1969) and MÜLLER, PATZNER (1996), they remain there until reaching sexual maturity. Due to their small mobility, they do not move to other zones (AMYOT, DOWNING 1997, ALDRIDGAE 2000).

*Sinanodonta woodiana* grew more intensively in the initial cooling basin and in the warmest discharge canals than it did in the lakes. The growth of mussels was four-fold faster in summer than in the fall-winter season. This general biological truth regarding faster growth in more advantageous conditions is not limited only to malacological fauna (NEGUS 1966, BAUER 1992, HRUSKA 1992, OSTROVSKY et al. 1993). NEGUS (1966) confirmed faster growth in specimens of *Anodonta anatina* in the heated water discharge zone as the vegetation season continued. GHENT et al. (1978) proved that the clams *Anodonta grandis* and *Elliptio complanata* were larger in the shallow littoral where the water temperature was higher. HANSON et al. (1988) also reported that there was a dependence between clam growth and temperature.

The largest and heaviest specimens in the Konin heated lake system were

noted in the warmest habitats with the highest water flow rates. The basis of the population was formed here by specimens with a shell length of up to 160 mm, while those from moderately heated zones were up to 125 mm, and those from the coolest were up to 115 mm. The maximum individual weight of the oldest clams reached as much as 900 g. As reported by FILLIPPOV and GERASIMOVA (1992) and ALDRIDGE (1999), the youngest specimens grew the fastest.

The effectivity of reproduction, which includes the anchoring of new cohorts in the sediments and the natural mortality of the generations, impacts population distribution and structure. Mortality might have been the highest in the first stages of the lives of larvae and juveniles, as was observed in other clams (LEWANDOWSKI 1991, 2001), as well as when there were large temperature fluctuations. AFANASJEV et al. (1996) indicated that the oldest specimens died out the fastest. Massive clam deaths were noted in the spring before the long water cooling system was put into operation.

The populations of *Sinanodonta woodiana* in the Konin system were comprised of clams of several age classes. They usually reached an age of six to nine years. The basis of the population (approximately 70%) studied in the current research was comprised of three- to five-year-old specimens, as was the case in the second half of the 1990s (AFANASJEV et al. 2001). Specimens of six or more years were the least numerous, a fact that is related to the natural mortality of the clams. A similar age distribution was noted in a population of indigenous Unionidae (glochidia) that occur in a different, unheated ecosystem (LEWANDOWSKI, STAŃCZYKOWSKA 1975, LEWANDOWSKI 1976, 1990, DUSOGE et al. 1999, ABRASZEWSKA-KOWALCZYK 2002).

The most age differentiation in the clam *Sinanodonta woodiana* was noted in the warm discharge canals and in the heated water discharge zone of the initial cooling basin. Specimens seven years old and older occurred in these habitats, and the average age of these clams was the highest here.

NEGUS (1966), TUDORANCEA (1968), and LEWANDOWSKI (1991) maintained that a wider age spectrum, as well as the occurrence of older specimens, are characteristic of populations that inhabit environments that offer them the most advantageous conditions. LEWANDOWSKI (1991) observed a decided decline in the age of Unionidae in Lake Mikołajskie along with an increase in this basin's trophic status. A wider range of shell lengths, more size classes, and larger clam sizes occurring in the warmest zones of the Konin system, *i.e.*, the initial cooling basin and the warm discharge canals, might be evidence of the more advantageous conditions in these locations for the Chinese clam.

One of the reproductive strategies that simplified fertilization was that the clam *Sinanodonta woodiana* formed dense aggregations. This was emphasized by PIECHOCKI (1969), BAUER (1994), FUKUHARA and NAGATA (1995). In their

study of the reproductive process of the Canadian *Elliptio complanta*, DOWNING et al. (1993) reported that females were not fertilized when population concentrations were lower than 15 ind · $m^{-2}$.

## Conclusions

The high degree of heterogenity in the prevailing environmental conditions of the Konin heated lake system, principally with regard to temperature, water flow rate, food supply, and substrate type, had an impact on the ecological structure of the clam *Sinanodonta woodiana* population. This not only affected clam distribution in the various habitats of this system, and thus the spatial structure, but also the morphophysiological, age, and sex structures. This is confirmation of the hypothesis that environmental conditions determine the rate of growth and the direction of changes in a population. They have an immediate impact on growth and reproduction and through this shape the appropriate population status.

The wide age and size spectrum and the highest annual growth in the most heated zones of the system was a reflection of the advantageous conditions prevailing in these habitats. The population base was comprised of specimens from three to five years of age. Cohorts comprised of a few specimens from the youngest age and size were noted in most of the habitats. It is most probable that the clams settled a given station on a massive scale a few years previously, and currently the transformed clam larvae do not reach the settled sites in exactly the same place and do not enrich the population with a younger generation. The lower abundance of older specimens (above six years) was caused by natural mortality.

Translated by JENNIFER ZIELIŃSKA

Accepted for print 12.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 345-354, Y. 2006

# QUALITATIVE COMPOSITION OF BACTERIAL MICROFLORA OF TANK WATER AND A HYBRID OF SIBERIAN STURGEON (*Acipenser baeri* Br.) WITH RUSSIAN STURGEON (*Acipenser gueldenstaedti* Br.) FROM INTENSIVE REARING IN A CLOSED WATER SYSTEM

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K e y w o r d s: intensive rearing, closed water system, bacteria, sturgeon hybrid, water.

A b s t r a c t

Investigations were carried out at the Experimental Stocking Station Dgał, Institute of Inland Fisheries in Olsztyn. The bacteria under study were cultured in nutritive agar at a temperature of 22°C (TVC 22°C) and 37°C (TVC 37°C), after being isolated from tank water and fish (mucus and gastrointestinal digesta). All habitats examined were predominated by Gram-negative bacteria, of which rods of the genus *Flavobacterium* sp., *Aeromonas* sp. and *Pseudomonas* sp. and those of the family *Enterobacteriaceae* occurred in the highest percentage. In the case of the Gram-positive bacteria, *Micrococcus* sp. and *Corynebacterium* sp. were found to prevail.

## SKŁAD JAKOŚCIOWY MIKROFLORY BAKTERYJNEJ WODY BASENOWEJ I HYBRYDA JESIOTRA SYBERYJSKIEGO (*Acipenser baeri* Br.) Z JESIOTREM ROSYJSKIM (*Acipenser gueldenstaedti* Br.) Z INTENSYWNEGO CHOWU W OBIEGU ZAMKNIĘTYM

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S ł o w a k l u c z o w e: intensywny chów, zamknięty obieg wody, bakterie, hybryd jesiotra, woda.

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Abstrakt

Badania prowadzono w Ośrodku Zarybieniowym Dgał, Instytutu Rybactwa Śródlądowego w Olsztynie. Identyfikowano bakterie wyhodowane na agarze odżywczym w temp. 22°C (TVC 22°C) i 37°C (TVC 37°C), wyizolowane z wody basenowej oraz ryb (śluzu i treści przewodu pokarmowego). We wszystkich badanych środowiskach dominowały bakterie Gram-ujemne, wśród których najliczniej występowały pałeczki należące do rodzaju *Flavobacterium* sp., *Aeromonas* sp. i *Pseudomonas* sp. oraz rodziny *Enterobacteriaceae.* W przypadku bakterii Gram-dodatnich dominowały *Micrococcus* sp. i *Corynebacterium* sp.

## Introduction

During intensive rearing of fish, a vast amount of metabolic products are excreted to the water, including mircoorganisms that constitute the microflora of the gastrointestinal tract. This may alter the quantitative ratios in the qualitative composition of bacterial microflora of waters (ZMYSŁOWSKA et al. 2000). Contamination of the aquatic environment and the resulting presence of bacteria indicative of the sanitary status as well as pathogenic bacteria of the gastrointestinal tract of humans and hematothermal animals in that habitat are reflected in the bacteriological status of the fish inhabiting it (ZMYSŁOWSKA et al. 2003a,b).

Microflora of the gastrointestinal tract of fish is predominated by genera: *Aeromonas, Pseudomonas,* representatives of the family *Enterobacteriaceae*, and obligatory anaerobes – *Bacteroides, Fusobacterium* and *Eubacterium* (FELDHUSEN 2000, ZMYSŁOWSKA et al. 2003a). Such bacteria as: *Aeromonas, Bacterioides*, *Pseudomonas* and those of the family *Enterobacteriaceae* occur in all sections of the gastrointestinal tract, and the per cent of aerobic and facultatively aerobic bacteria changes with an increase in the total bacteria count (SUGITA et al. 1990). This phenomenon, affecting increased oxygen utilization, is also likely to cause enhanced secretion of substances inhabiting the growth of aerobes by *Bacterioides*. Under specified environmental conditions and at the occurrence of factors determining their pathogenicity, multiple species of bacteria from the genus *Aeromonas* and *Pseudomonas,* commonly isolated from aquatic ecosystems, may induce infections in fish (ESTEVE et al. 1993).

Due to their taste attributes, the sturgeons may become a valuable additive, making the market offer of fish farms attractive. Hence, taking into account requirements referring to fish material as well as the safety of the aquatic environment, it is vital to carry out complex microbiological analyses of both fish, water and feedstuffs. Constant improvement and implementation of new, highly effective methods of fish health protection are also of crucial importance. It is one of prerequisites determining the economic effects and advances in the rearing and culture of fish.

The study was aimed at isolating and identifying bacterial microflora of a sturgeon hybrid and environment during intensive tank culture in a closed water system based on its morphological, physiological and biochemical traits.

## Material and Methods

Investigations were carried out at the Experimental Stocking Station Dgał, Institute of Inland Fisheries in Olsztyn during intensive culture of hybrids of Siberian sturgeon (*Acipenser baeri* Br.) with Russian sturgeon (*Acipenser gueldenstaedti* Br.) in tanks with a closed water system. The experiment was carried out in 14 flow tanks 2.0 m x 1.0 m x 0.9 m in size. In addition, the system was equipped with a fluid biofilter, preliminary horizontal filter with a settling tank, and 2 storage reservoirs – an upper and a bottom one. The technological design of the experiment was presented in a previous work (KRAUSE 2005). Over the experimental period, the basic physicochemical examinations of water were carried out at the laboratory of the Institute of Inland Fisheries. The values of the parameters assayed accounted for 15-24.2°C in the case of temperature and for more than 6 mg $O_2$ per $dm^3$ of water in the case of oxygen saturation.

Over the rearing period of the hybrid (Siberian sturgeon with Russian sturgeon), samples of water and fish (mucus from skin surface and intestinal digesta) for microbiological analyses were collected from March 2001 to October 2001 in ca. one-month intervals. Water samples (250 $cm^3$) were collected directly to sterile glass-stoppered bottles and transported to the laboratory in thermal bags at a temperature of 4°C.

At each sampling of the tank water, 3 fish were collected at random from rearing tanks. The fish were transported to the laboratory in bags filled with tank water and oxygen.

The time span from the sampling of water and fish to their microbiological analyses did not exceed 6 hours.

In the experiment, a total of 24 water samples and 24 fish specimens were examined.

Microbiological analyses, with the incubation method on nutrient agar medium at temperatures of 22°C and 37°C, were carried out for water, mucus from skin surface of fish and their intestinal digesta. After the incubation, single colonies differing from one another were isolated for identification, and were then passaged two times on the same medium and incubated under the same conditions. The monocultures obtained were subjected to the identification process, by determining their morphological traits: macroscopic (shape and color of colonies) and microscopic traits (shape of cells, arrangement of agglomerations, reaction to Gram staining, motion capacity), as well as

enzymatic traits (capacity for producing catalase and cytochrome oxidase and for oxidation or fermentation of glucose on Hugh-Leifson medium). Further identification of the bacteria was based on BERGEY'S key (1984), identification schemes provided by HENDRI et al. (1964) and LECHEVALLIER et al. (1980), as well as the identification tests Api 20E and Api 20NE by BioMérieux.

## Results

Table 1 contains the results for the number of bacterial strains isolated, on nutritive agar at a temperature of 22°C and 37°C, from water and fish samples examined in a closed system. Both in water and fish (mucus from skin surface and intestinal digesta) the Gram-negative bacteria occurred in higher amounts, whereas the Gram-positive bacteria – in lower ones. Over the experimental period, 65 strains of Gram-negative bacteria and 35 strains of Gram-positive bacteria were isolated out of 24 water samples. In the mucus from the skin surface and intestinal digesta, the number of isolated strains of

Table 1
The number of strains of Gram-negative and Gram-positive bacteria isolated from samples of tank water and fish (a hybrid of Siberian sturgeon with Russian sturgeon) from intensive culture in a closed system

| Bacterial strains | | Water (1 $cm^3$) | Mucus from the surface 1 $cm^2$ of fish skin | Intestinal digesta (1 g) of fish |
|---|---|---|---|---|
| | | number of bacterial strains | | |
| Gram--negative bacteria | *Achromobacter* sp. | 1 | 1 | 10 |
| | *Aeromonas caviae* | 2 | 3 | 7 |
| | *Aeromonas hydrophila* | 12 | 13 | 12 |
| | *Aeromonas sobria* | 2 | 2 | 1 |
| | *Alcaligenes* sp. | 5 | 4 | 5 |
| | *Enterobacteriaceae* | 6 | 5 | 8 |
| | *Flavobacterium* sp. | 12 | 13 | 4 |
| | *Pseudomonas aeruginosa* | 2 | 2 | 5 |
| | *Pseudomonas fluorescens* | 8 | 2 | 7 |
| | *Pseudomonas putida* | 2 | 1 | 2 |
| | *Vibrio* sp. | 1 | 2 | 4 |
| | Unidentified strains | 12 | 11 | 7 |
| | **Total** | **65** | **59** | **72** |
| Gram--positive bacteria | *Arthrobacter* sp. | 6 | 7 | 8 |
| | *Bacillus* sp. | 1 | 1 | 11 |
| | *Corynebacterium* sp. | 9 | 9 | 9 |
| | *Micrococcus* sp. | 10 | 11 | 10 |
| | *Staphylococcus* sp. | 2 | 3 | 3 |
| | *Streptococcus* sp. | 1 | 1 | 5 |
| | Unidentified strains | 6 | 7 | 6 |
| | **Total** | **35** | **39** | **52** |

Gram-negative bacteria accounted for 59 and 72, whereas that of the Gram-positive bacteria were 39 and 52, respectively.

In the samples of tank water, the isolated and identified Gram-negative bacteria appeared to be predominated by *Flavobacterium* sp., *Aeromonas hydrophila*, each constituting 17%, and by *Pseudomonas fluorescens*, constituting 13.4%. A lower percentage was observed for bacteria of the family *Enterobacteriaceae* and strains *Alcaligenes* sp. which constituted respectively 8.6% and 6.8% of all the identified Gram-negative bacteria. In the case of the Gram-positive ones, *Micrococcus* sp. was found to prevail, followed by *Corynebacterium* sp. and *Arthrobacter* sp., which constituted: 28.6%, 25.7% and 17.1%, respectively. The contribution of bacteria from the genera: *Staphylococcus* sp., *Streptococcus* sp. and *Bacillus* sp. did not exceed several per cents and accounted for: 5.7%, 2.9% and 2.9%, respectively, of all the Gram-positive bacteria identified in the study (Figure 1).

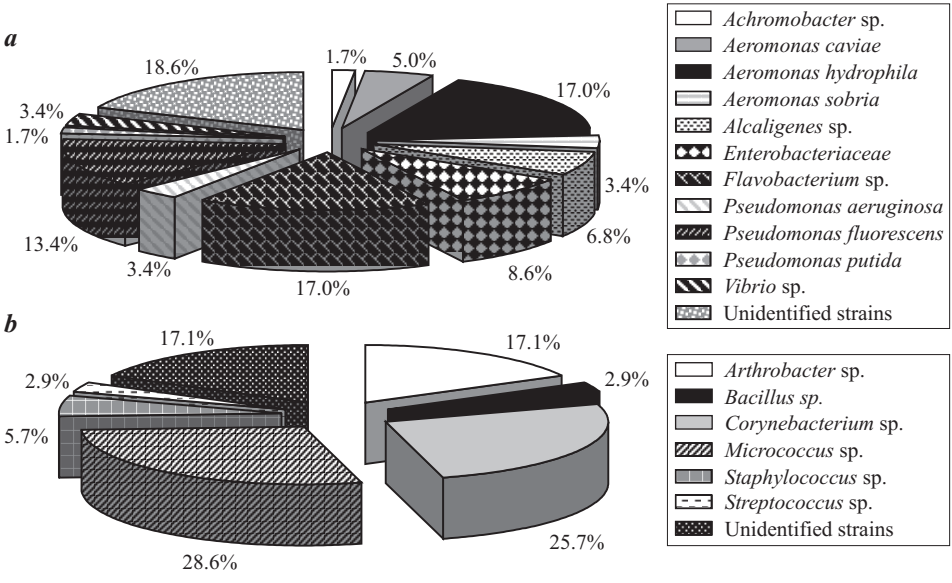


Fig. 1. Percentage of identified genera and species of Gram-negative bacteria (*a*) and Gram-positive bacilli and cocci (*b*) isolated from 1 $cm^3$ of tank water

In fish mucus, likewise in water, the isolated Gram-negative bacteria were predominated by *Flavobacterium* sp. and *Aeromonas hydrophila*, each constituting 20.0% of all the identified Gram-negative strains. Bacteria of the family *Enterobacteriaceae* constituted 10.6%, whereas the contribution of other identified microorganisms, such as: *Achromobacter* sp., *Pseudomonas putida*,

*Vibrio* sp., *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Aeromonas caviae*, *Aeromonas sobria* and *Alcaligenes* sp., did not exceed 7% of all Gram-negative bacteria determined. The Gram-positive bacteria isolated from the mucus of skin surface were represented in the highest numbers by: *Micrococcus* sp., *Corynebacterium* sp. and *Arthrobacter* sp., whose contribution accounted for: 28.2%, 23.1% and 17.9%, respectively. Bacteria of *Streptococcus* sp. and *Bacillus* sp. constituted 2.6% of all the identified Gram-positive bacteria each (Figure 2).

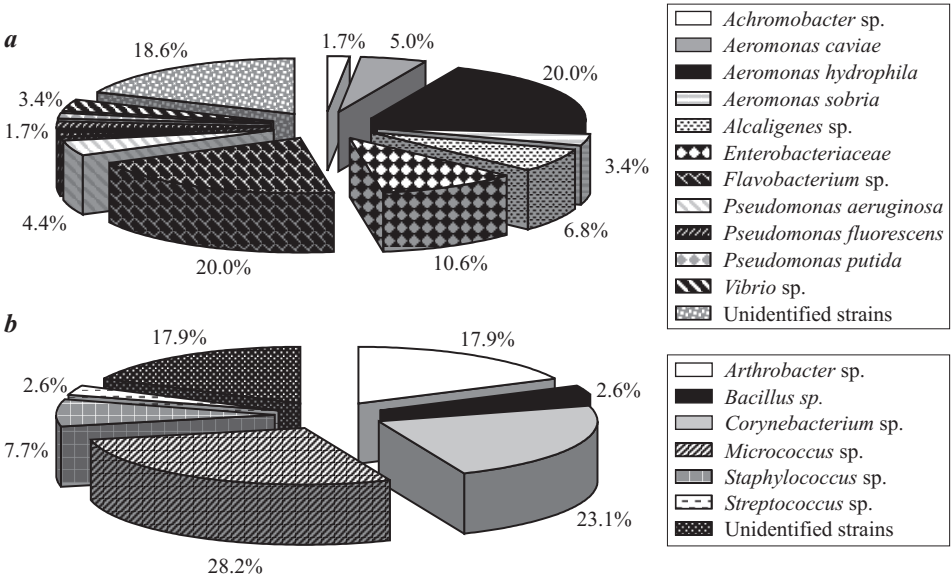


Fig. 2. Percentage of identified genera and species of Gram-negative bacteria (*a*) and Gram-positive bacilli and cocci (*b*) isolated from 1 $cm^3$ of fish mucusi

Among the Gram-negative bacteria isolated from intestinal digesta of fish, the most common were: *Aeromonas hydrophila* – 16.7%, *Achromobacter* sp. – 13.9%, bacteria of the family *Enterobacteriaceae* – 11.1% as well as *Pseudomonas fluorescens* and *Aeromonas caviae*, each constituting 9.7%. The other Gram-negative strains identified, including: *Pseudomonas aeruginosa*, *Alcaligenes* sp., *Vibrio* sp., *Flavobacterium* sp., *Pseudomonas putida*, and *Aeromonas sobria,* constituted from 1.4 to 6.9%. The Gram-positive bacteria isolated from intestinal digesta were predominated by: *Bacillus* sp., *Micrococcus* sp. and *Corynebacterium* sp. that constituted: 21.1%, 19.2% and 17.3% of the strains identified, respectively, whereas *Arthrobacter* sp., *Staphylococcus* sp. and *Streptococcus* sp. bacteria constituted from 5.8 to 15.4% (Figure 3).

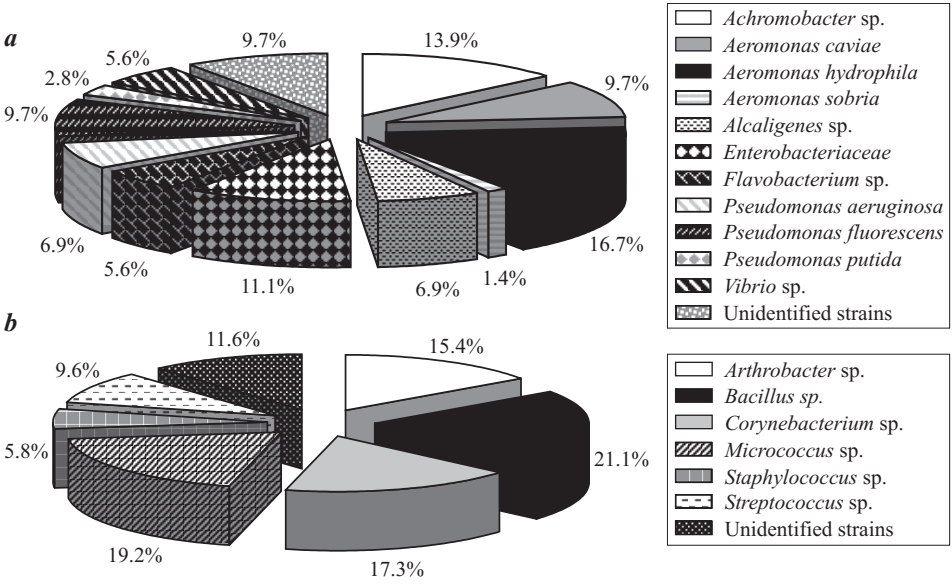


Fig. 3. Percentage of identified genera and species of Gram-negative bacteria (*a*) and Gram-positive bacilli and cocci (*b*) isolated from 1 g of intestinal digesta of fish

## Discussion

The study found that bacteria isolated on nutritive agar medium at a temperature of 22°C and 37°C, from water and the hybrid of Siberian sturgeon (*Acipenser baeri* Br.) with Russian sturgeon (*Acipenser guldenstaedti* Br.) were usually predominated by bacteria of the genera *Flavobacterium* sp., *Aeromonas* sp., *Pseudomonas* sp. and those of the family *Enterobacteriaceae*. Investigations into the qualitative composition of microflora of water and various hybrids of the sturgeons, cultured in diverse technological systems supplied with both fresh or sea water, have demonstrated similar dependencies (ZMYSŁOWSKA et al. 2003c, KRAUSE, ZMYSŁOWSKA 2004, KRAUSE 2005). In addition, quantitative prevalence of Gram-negative bacteria of the genera: *Pseudomonas* and *Aeromonas* and those of the family *Enterobacteriaceae* has been observed. In both water and fish samples, the predominating species of the genus *Pseudomonas* appeared to be: *P. fluorescens*, *P. aeruginosa*, *P. putida*, whereas those of the genus *Aeromonas* were represented by: *A. hydrophila*, *A. caviae*, *A. sobria*, and those of the family *Enterobacteriaceae* – by: *Stenotrophomonas maltophila*, *Citrobacter braaki*, *C. youngae*, *Proteus mirabilis*, *P. vulgaris*, *Enterobacter* sp. and *Serratia ficaria*, *S. plynuthica*, *S. marcescens*. Among the Gram-positive bacteria identified in the same

habitats, the bacteria of the genera *Micrococcus* and *Bacillus* were the most dominant. Apart from these, bacteria of the genera *Staphylococcus* or *Streptococcus* were reported as well, usually in lower numbers. The genus *Staphylococcus* was represented by species: *S. aureus*, *S. sciuri*, and *S. hominis* (KRAUSE 2005) which are considered, next to *Aeromonas* and *Pseudomonas* genera, as an indicator of water contamination with organic matter (NIEWOLAK, OPIEKA 2000). According to SUGITA et al. (1988), who investigated the gastrointestinal tract of crucian carp (*Carassius auratus*), bacteria of the genus *Aeromonas* sp. constitute autochthonous microflora of the gastrointestinal tract of fish, whereas those of the family *Enterobacteriaceae* are claimed to be transient microflora. In a study on Baltic herrings (*Clupea harengus*), ZMYSŁOWSKA (2001) detected *Enterobacter* sp., *Escherichia coli* as well as *Staphylococcus sciuri* and *Staphylococcus aureus* in the microflora of their intestinal digesta. A similar bacteriological composition was demonstrated in the intestinal digesta of wels (*Silurus glanis* L.), (ZMYSŁOWSKA et al. 2004). CAMPBELL and BUSWELL (1983) claim that the microflora of fish is determined by the type and microflora of food uptaken. The current study demonstrated frequent occurrence of bacteria from the genus *Flavobacterium* sp. in the habitat of tank water during culture of sturgeon hybrids. According to BECKER et al. (2001), they are considered to be human pathogens. Investigations by ESTEVE and GARAY (1991) have shown high population numbers of bacteria from the genus *Flavobacterium* in post-production waters from piscicultures. KOZIŃSKA (2004) claims that apart from bacteria of the genera *Aeromonas*, *Plesiomonas* and *Pseudomonas,* fish can also be colonized by *Edwardsiella* sp., *Mycobacterium* sp. which are well recognized as fish pathogens. Currently, they are in the focus of interest among ichtiopathologists, both in routine test and scientific research, due to relatively high incidence of mixed bacterial infections, which greatly hinders appropriate diagnostics. An increased number of these bacteria in fish is probably linked with the occurrence of environmental conditions being favorable to their growth. Their frequent occurrence in fish displaying health disorders points to their relative pathogenicity that may be manifested more distinctively under unfavorable conditions and/or in fish with lowered resistance. As reported by SIWICKI et al. (2004), there has recently emerged a new disease entity referred to as atypical Bacterial Gill Disease or, in intensive rearing, as a hatching syndrome of rainbow trout. It is induced by bacteria of the genus *Flavobacterium psychrophilum*, commonly occurring in inland and sea waters. These bacteria are claimed to be constituents of normal bacterial flora of the mucus of healthy fish.

An increasing yield of fishery production should be accompanied by concurrent improvement of the quality of fish material mainly due to consumer safety. Intensive fish culturing triggers physicochemical and biological changes

in the aquatic system. During rearing and fattening of fish, the bacterial microflora of waters is subject to quantitative and qualitative changes. This is affected, to a large extent, by the feed administered to fish, which in turn constitutes a good culture medium for the growth of bacteria by fish excreta through which a vast number of microorganisms are discharged to waters. Taking into account the requirements for fish material and environmental safety, it is crucial to carry out detailed quantitative and qualitative microbiological assays which would simultaneously characterize the quality of fish and water constituting natural habitat of their existence and development.

## Conclusions

1. During intensive culture of Siberian sturgeon x Russian sturgeon hybrid, Gram-negative bacteria were isolated in higher amounts than the Gram-positive ones, irrespective of sample type (water or fish).

2. The Gram-negative bacteria isolated from water, mucus and intestinal digesta of the fish were predominated by bacteria of the genera *Flavobacterium* sp., *Aeromonas* sp., *Pseudomonas* sp. and those of the family *Enterobacteriaceae*, whereas the Gram-positive bacteria were predominated by the genera *Micrococcus* sp. and *Corynebacterium* sp.

Translated by JOANNA JENSEN

Accepted for print 31.03.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 355-374, Y. 2006

# PHOSPHORUS REMOVAL FROM WASTEWATER USED FOR RECYRCULATING AQUACULTURE SYSTEM ON THE GRAVEL ACTIVE MEDIUM

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K e y w o r d s: phosphates removal, active filter, iron hydroxide, recirculating system, aquaculture.

A b s t r a c t

An attempt was made to investigate the effectiveness and usefulness of a filter medium used for underground water deironing (termed active medium) in removing phosphorus compounds from aquaculture effluent of a recirculating system. This paper presents the results of the experiments conducted in two phases: phase one was conducted in a static mode in the laboratory conditions and phase two on the flow filters treating water from fish breeding in the recirculating system. The process of phosphates removal was examined for various technological variants. Additionally, the effect of the active medium was analysed on numerous water quality parameters.

It has been concluded that the active medium has the capacity to bind phosphates from water and can be successfully used for aquaculture effluents treatment. Phosphates reduction depends on the time of contact with the medium, the initial concentration of phosphates, the water temperature, and the quantity of the medium and amounted to 33.3 ÷ 93.9% and the reduction intensity was the highest in the first 30 ÷ 45 minutes.

## USUWANIE FOSFORU ZE ŚCIEKÓW POCHODZĄCYCH Z HODOWLI RYB W OBIEGU ZAMKNIĘTYM NA WYPEŁNIENIU AKTYWNYM

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Abstrakt

W doświadczeniu wykorzystano wypełnienie filtracyjne z układu odżelaziającego wodę gruntową (zwane wypełnieniem aktywnym) w procesie usuwania fosoforu z wody pochodzącej z hodowli ryb w obiegu zamkniętym. Doświadczenie przeprowadzono w skali laboratoryjnej w dwóch etapach. W pierwszym etapie testowano układ statyczny, w drugim analizowano skuteczność wypełnienia aktywnego w układzie dynamicznym, oczyszczającym wodę pochodzącą z hodowli ryb w obiegu zamkniętym. Podczas eksperymentu analizowano różne warianty technologiczne. Stwierdzono, iż testowane wypełnienie aktywne jest bardzo skuteczne w procesie usuwania fosforu z wód pohodowlanych. Sprawność usunięcia ortofoforanów zależała od czasu kontaktu z medium, początkowego stężenia ortofosforanów, temperatury wody, i mieściła się w zakresie 33,3% – 93,9%. Najwyższą sprawność oczyszczania obserwowano podczas pierwszych $30 \div 45$ min zatrzymania oczyszczanej wody w układzie.

## Introduction

Due to the rapid aquaculture expansion throughout the world, the development of effluent treatment systems gains special importance to reduce its environmental impact and also to provide the bred fish with the optimal growth conditions in recirculation systems (LAMAIRE at al. 1988, DORITH, EITAN 1998, HANNESSON 2003, BARAK at al. 2003).

On the grounds of numerous surveys, phosphorus can be regarded as the factor determining eutrophication rate in most water reservoirs. It stimulates increase of water fertility but on the other hand limits the production in water bodies (NESBITT 1968, STEFFENS 1979, YEOMAN at al., 1988). Having in mind that the most common are technically simple methods and not too costly in terms of investment and operation, attention was paid to the possibility to remove phosphorus on active medium such like gravel coated with iron compounds taken from water deironing filters (KRZEMIENIEWSKI 1995, KRZEMIENIEWSKI STRACZYNSKA-KNYSAK 1999, 2000).

The aim of the experiments was to characterise the process of phosphorus removal from aquaculture effluents (from fish breeding) on the active medium. An assumption was made that phosphorus reduction regards mainly the dissolved mineralised fraction and that water quality after the contact with gravel should not deteriorate which in fish breeding has a great effect on the fish condition.

## Materials and Methods

The active medium comprised gravel coated with iron oxides with small addition of battery manganese ($MnO_2$). It was taken from an underground water treatment plant, from a single-stage pressure filter removing iron and

manganese from water (Table 1). The surface area of the gravel grains was calculated from the following formula:

$$\omega = \frac{6 \cdot \alpha (1 - \varepsilon)}{d_r},$$

where:

$\omega$ – surface area of grains contained in volume unit;
$\alpha$ – gravel shape coefficient, assumed was $\alpha = 1.25$;
$\varepsilon$ – porosity, $\varepsilon = 0.35$.
$d_r$ – equivalent diameter, assumed was $d_r = 0.15$ cm.

Table 1

Characteristics of the experimental gravel

| Physical form | Brown grains |
|---|---|
| Bulk density ($g \cdot dm^{-3}$) | |
| static mode | 1.94 |
| dynamic mode | 1.23 |
| Mean number of grains in 1 $cm^3$ | |
| static mode | 308 |
| dynamic mode | 265 |
| Graining range (mm) | |
| static mode | 1-2 |
| dynamic mode | 2-3 |
| Mean iron content | |
| static mode (mg Fe $\cdot g^{-1}$) | 43.43 |
| (mg Fe $\cdot cm^{-3}$) | 84.25 |
| dynamic mode (mg Fe $\cdot g^{-1}$) | 59.28 |
| (mg Fe $\cdot cm^{-3}$) | 72.75 |
| Filter porosity | |
| static mode | 0.350 |
| dynamic mode | 0.425 |

The examinations of phosphorus compounds removal were carried out in two phases. Phase one comprised laboratory-scale surveys in a static mode whereas phase two was conducted on the flow filters treating water from a recirculating fish breeding system.

In the static-mode tests water from various tanks was used. The stock in the tanks were: carp and goldfish with their hybrids, whitefish, ide and chub. The fishes were fed with KarpStarter granulate. Water in the tanks was not replaced, only filled up to compensate for the evaporation and cleaning losses. The circulating water was treated on shelf biofilters and disinfected with UV radiation. The reason why water was drawn from various tanks was not the different species, age and population size but rather the different content of phosphorus and other chemical elements and compounds, such as nitrogen in

various forms, sulphates, chlorides, chemical compounds causing water hardness, as well as various permanganate value, pH and alkalinity of the water (Table 2).

Table 2

Quality characteristics of the water from fish tanks

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Initial concentration of orthophosphate P | (mg P-$PO_4$ · $dm^{-3}$) | 0.19 | 0.26 | 0.28 | 0.54 | 0.26 | 0.58 | 1.03 | 1.14 |
| Total P | (mg P · $dm^{-3}$) | 0.26 | 0.30 | 0.36 | 0.65 | 0.95 | 1.43 | 1.61 | 1.68 |
| Orthophosphates content in total P | (%) | 71.64 | 88.23 | 78.98 | 82.96 | 27.52 | 40.91 | 63.74 | 68.08 |
| Temperature (during tests) | (°C) | 20.1 | 22.0 | 21.9 | 21.7 | 10.6 | 10.0 | 9.8 | 11.5 |
| DO | (mg $O_2$ · $dm^{-3}$) | 5.8 | 6.2 | 6.3 | 6.2 | 5.1 | 7.7 | 7.7 | 5.8 |
| Water pH | (Ph) | 8.7 | 8.65 | 8.21 | 7.92 | 8.39 | 8.29 | 8.51 | 8.40 |
| TSS | (mg · $dm^{-3}$) | 20.0 | 12.0 | 16.5 | 13.5 | 18.0 | 12.5 | 13.5 | 12.0 |
| COD-$KMnO_4$ | (mg $O_2$ · $dm^{-3}$) | 12 | 21 | 23 | 28 | 17 | 16 | 20 | 29 |
| Total hardness | (mval · $dm^{-3}$) | 6.92 | 6.48 | 7.06 | 6.10 | 5.82 | 5.48 | 6.34 | 6.42 |
| Total alkalinity | (mval · $dm^{-3}$) | 8.2 | 6.5 | 6.6 | 5.0 | 7.2 | 6.8 | 7.0 | 6.9 |
| Total N | (mg N · $dm^{-3}$) | 14.0 | 6.0 | 8.0 | 17.0 | 6.0 | 4.0 | 120.0 | 63.0 |
| Ammonium N | (mg N-$NH_4$ · $dm^{-3}$) | 0.20 | 0.12 | 0.15 | 0.19 | 0.07 | 0.11 | 0.10 | 0.10 |
| Nitrates | (mg N-$NO_3$ · $dm^{-3}$) | 12.1 | 5.3 | 7.1 | 16.3 | 5.3 | 3.5 | 113.6 | 54.0 |
| Nitrites | (mg N-$NO_2$ · $dm^{-3}$) | 0.005 | 0.013 | 0.012 | 0.017 | 0.013 | 0.025 | 0.019 | 0.075 |
| Sulphates | (mg $SO_4^{2-}$ · $dm^{-3}$) | 47 | 27 | 24 | 29 | 21 | 26 | 25 | 51 |
| Chlorides | (mg $Cl^-$ · $dm^{-3}$) | 9.4 | 4.8 | 3.8 | 3.5 | 6.3 | 4.6 | 7.2 | 5.9 |
| Iron | (mg Fe · $dm^{-3}$) | 0.02 | 0.06 | 0.11 | 0.10 | 0.02 | 0.08 | 0.03 | 0.03 |
| Manganese | (mg Mn · $dm^{-3}$) | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.1 | 0.1 | 0.1 |

Phosphates reduction was investigated for 5 different gravel volumes. The voluminal ratio between gravel and water/waste water equalled: 1:30, 1:20, 1:15, 1:10 and 1:5 (i.e. per 50 mL of water the quantities of gravel equalled: 1.67; 2.50; 3.33; 5.00; 10.00 mL). The examinations were carried out for 9 reaction periods: 1 min, 3 min, 5 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 min. The samples were marked in a conventional way e.g. 1:15/20 which means that the gravel/water voluminal ratio in the sample is 1:15 and the time of water mixing with gravel 20 minutes.

During the examinations water was mixed with the active gravel and the mixing intensity was adjusted in such way that only the liquid in the vessel was agitated and the gravel grains only slightly.

The examinations were done at ambient temperature 20°C ÷ 22°C and at 9.5°C ÷ 11.0°C.

Determined was the dependence between the reduced amount of phosphates and:
– contact time,
– size of the contact surface area,
– initial concentration,
– water temperature.

Before the physico-chemical measurements, each water sample after the defined contact time was filtered on a filter paper.

The flow tests conducted during fish breeding in the closed system were done in the fractional-commercial scale. The system for fish breeding consisted of three plastic tanks: one holding the fishes and two retention tanks, a filter for nitrification with plastic medium, and an active filter for phosphorus removal (Figure 1). The breeding tank was fed with water from the upper tank through four filtration columns with active media. If the filtration columns were disconnected from the system, water was flowing directly into the breeding tank. Liquid from this tank was directed by gravity to the bottom retention tank and next pumped up to the upper retention tank. Some water from the upper tank was sprinkled over the biofilter for nitrification and then it was flowing to the bottom tank.

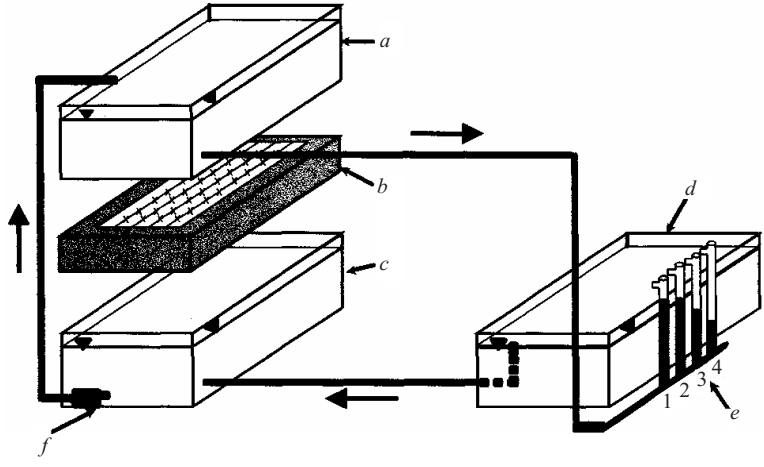


Fig. 1. Scheme of the fish breeding system with test stand: *a, c* – retention tanks, *b* – biofilter, *d* – breeding tank, *e* – test stand with filtration columns, *f* – return pump

In the upper retention tank a heater was fixed. The water temperature varied between 18.0°C and 23°C. Air was delivered to the breeding tank.

The test stand consisted of four filtration columns made of plastic, 1-m high and 50 mm in diameter. Flow direction in the columns was bottom-up and

regulated with valves fixed on each column. The filtrating medium comprised an active material with the grain diameter 2-3 mm and the characteristics as presented in Table 1. The columns were filled with gravel to different levels, i.e. 1st to 76 cm (1.20 $dm^3$ of gravel), 2nd to 60 cm (1.00 $dm^3$ of gravel), 3rd to 47 cm (0.75 $dm^3$), 4th to 29 cm (0.50 $dm^3$).

# Results

## Contact time

The size of phosphates reduction depended very clearly on the contact time between water and active gravel (Figures 2, 3, 4, 5).

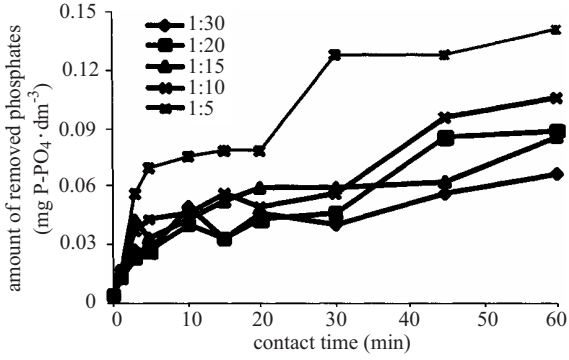


Fig. 2 Relation between phosphates reduction and contact time; $C_0$ = 0.19 mg P-$PO_4 \cdot dm^{-3}$, temp. 20.1°C

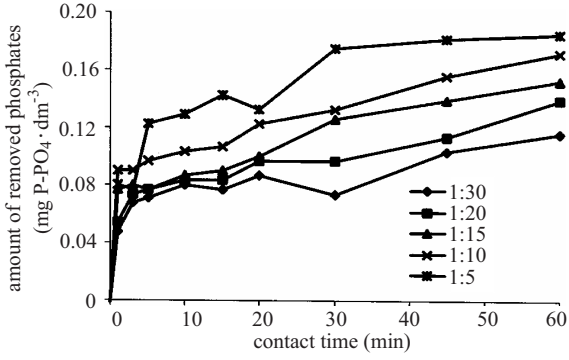


Fig. 3. Relation between phosphates reduction and contact time; $C_0$ = 0.26 mg P-$PO_4 \cdot dm^{-3}$, temp. 10.6°C

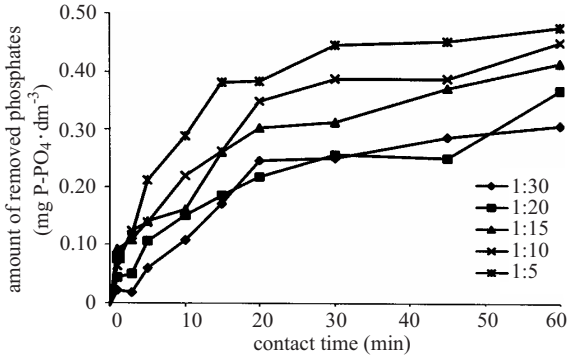


Fig. 4. Relation between phosphates reduction and contact time; $C_0$ = 0.58 mg P-$PO_4 \cdot dm^{-3}$, temp. 10.0°C

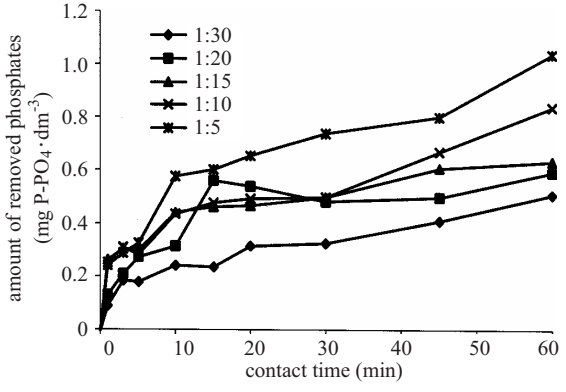


Fig. 5. Relation between phosphates reduction and contact time; $C_0$ = 1.14 mg P-$PO_4 \cdot dm^{-3}$, temp. 11.5°C

In the initial 10 minutes of the test, from 19.0% to 64.9% of phosphates were removed, depending on the initial concentration and the gravel volume. In the following 20 minutes, the reduction of phosphates reached from 19.3% to 86.7% (compared to the initial value), and finally after the 60-minute contact the efficiency varied between 33.3% and 93.9%.

## Initial concentration

On the grounds of the tests done at 20°C ÷ 22°C it may be concluded that the higher is the initial concentration, the higher the process efficiency (Figures 6, 7).

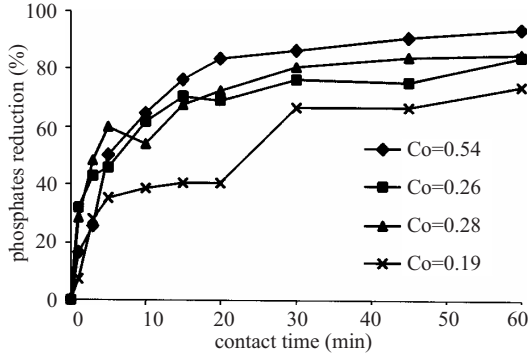


Fig. 6. Phosphates reduction (as percentage of initial concentration) in relation to contact time, at various initial concentrations; temp. 20-22°C, voluminous ratio gravel/water 1:5

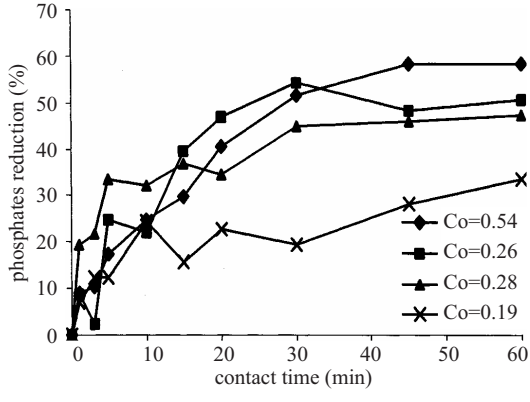


Fig. 7. Phosphates reduction (as percentage of initial concentration) in relation to contact time, at various initial concentrations; temp. 20-22°C, voluminous ratio gravel/water 1:30

Results of the investigations carried out by the authors in the past, concerning phosphates reduction with the same method in relatively concentrated solutions (3.4 ÷ 14.7 mg $P\text{-}PO_4/dm^{-3}$) (STRĄCZYŃSKA-KNYSAK 1999), have shown that the higher is the initial concentration of phosphates, the lower the per cent reduction at a defined gravel quantity and time. Therefore, it can be assumed that there is a limiting concentration of orthophosphates below which removal efficiency increases along with the concentration growth, whereas above this limit the rise of concentration causes a decrease of orthophosphates reduction. The value of such limiting concentration will depend on the process conditions.

## Active medium volume

The gravel surface area for the samples: 1:30, 1:20, 1:15, 1:10, 1:5 equalled: 54.27 $cm^2$; 81.25 $cm^2$; 108.23 $cm^2$; 162.5 $cm^2$; 325.0 $cm^2$ (respectively). It was determined that reduction of phosphates grows along with the increase of the contact surface area, particularly at longer contact periods. At the max. contact surface area of 325 $cm^2$ (1:5) a small dependence between the initial concentration and the final concentration was detected (Figure 8).

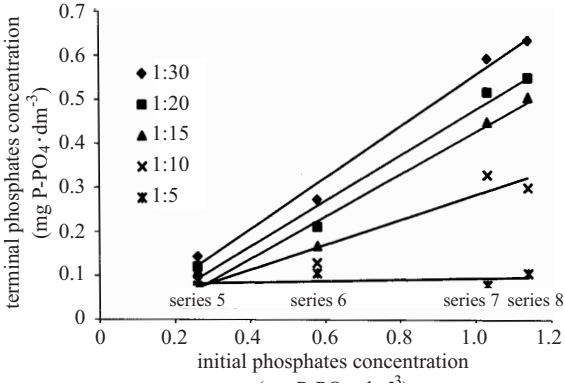


Fig. 8. Relation between initial and final concentration for different gravel volumes; temp. 9.5-11.5°C, contact time 60 min

Along with the increase of the gravel quantity, its efficiency decreases i.e. less phosphorus is removed in time unit per unit surface area of gravel. At the six-fold increase of the gravel quantity, the efficiency dropped by 2.7 ÷ 3.9 times.

## Temperature

Comparison of the phosphates reduction in solutions of similar concentration at 9.5°C ÷ 11°C and at 20°C ÷ 22°C reveals that the reduction rises together with the water temperature increase (Figures 9, 10).

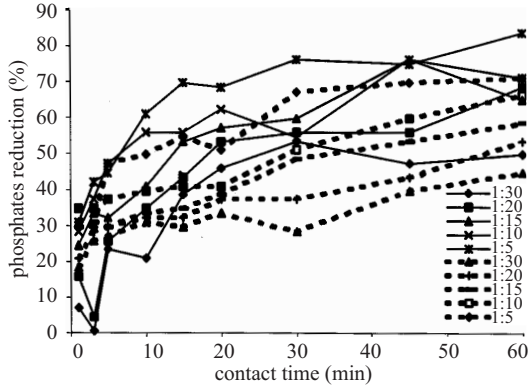


Fig. 9. Temperature effect on phosphates removal per 1 m · cm$^3$ of gravel – comparison of two test series at $C_0$ = 0.26 mg P-$PO_4$ · dm$^{-3}$; at 22°C – full line and at 10.6°C – broken line

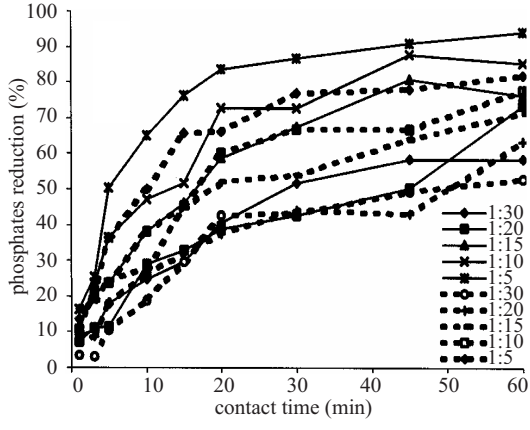


Fig. 10. Temperature effect on phosphates removal per 1 cm$^3$ of gravel – comparison between the test at 21.7°C ($C_0$ = 0.54 mg P-$PO_4$ · dm$^{-3}$) – full line, and the test at 10.0°C ($C_0$ = 0.58 mg P-$PO_4$ · dm$^{-3}$) – broken line

## Characteristics of other quality indicators

A decrease of the water pH was observed along with the increase of the active material quantity and the passing contact time. The max. pH decrease equalled 0.48 pH. However, it should be stressed that in the water after the contact with gravel, the reaction never dropped below 7.0 pH. The total water alkalinity was also subjected to a decrease which was growing together with the increase of the active material content in the water.

Water mixing with the gravel was increasing the iron content in the water (Figures 11, 12). The increase was the higher, the more gravel was contained in the water sample, nonetheless the concentration did not exceed 1 mg $Fe \cdot dm^{-3}$ in any sample. The rise of iron concentration in the water was observed during the first $5 \div 10$ minutes of the water and gravel contact. Past this period, no obvious dependence between iron concentration in the water and the process time was observed.

Sulphates in the water were contained in the range $21 \div 51$ mg $SO_4 \cdot dm^{-3}$. After 30 min contact, in the samples with little gravel (1:20) the concentration of sulphates increased by $2 \cdot 9$ mg $SO_4 \cdot dm^{-3}$ whereas in the samples with the highest gravel content (1:5) by $6 \div 14$ mg $SO_4 \cdot dm^{-3}$. After the next 30 min of contact, the concentration of sulphates increased again by 1-6 mg $SO_4 \cdot dm^{-3}$ (respectively). In comparison to the initial concentration the increase amounted to $31 \div 63\%$.

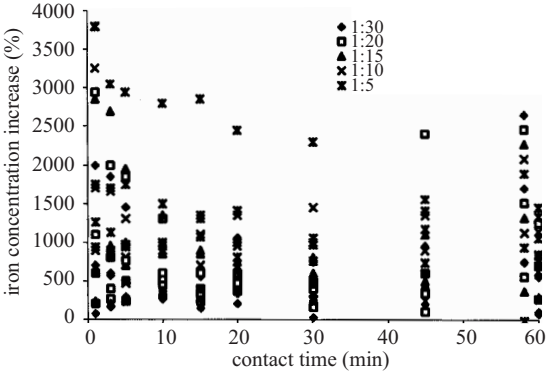


Fig. 11. Increase of iron concentration in water after mixing with gravel coated with iron oxides; initial iron concentration: 0.02 – 0.03 mg $Fe \cdot dm^{-3}$

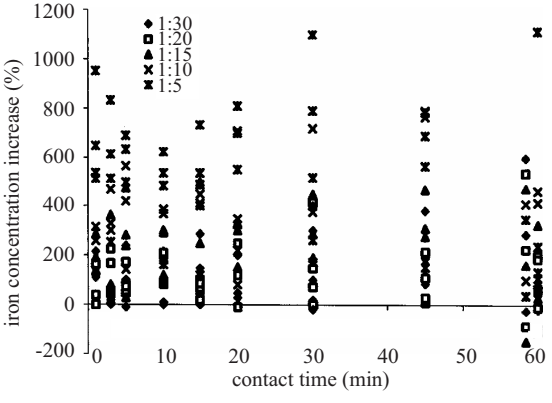


Fig. 12. Increase of iron concentration after mixing with gravel coated with iron oxides, initial iron concentration: 0.06 – 0.11 mg $Fe \cdot dm^{-3}$

The initial concentrations of chlorides in the water varied between 3.5 and 9.4 mg $Cl^- \cdot dm^{-3}$. They were growing along with the increasing amount of gravel in the water sample and extension of the contact time. The max. observed rise of chlorides amounted to 2.7 mg $Cl^- \cdot dm^{-3}$ at the high volume of gravel (1:10) which comprised 77% increase.

Changes of total N concentration in the water after mixing with gravel were examined for the following test settings: 1:10/20, 1:20/10, 1:20/20. In the samples (1:10/20) an increase of total N was observed in seven series (from 1 to 3 mg $N_{tot} \cdot dm^{-3}$) whereas in one series a decrease was determined of about 2 mg $N_{tot} \cdot dm^{-3}$. In the samples (1:20/10) the content of total N increased in five series from 1 to 3 mg $N_{tot} \cdot dm^{-3}$, diminished in one series by 1 mg $N_{tot} \cdot dm^{-3}$, and did not change in the others. In the samples (1:20/20) the concentration of total N was higher than the initial in six series by 1 to 3 mg $N_{tot} \cdot dm^{-3}$, lower in one series by 1 mg $N_{tot} \cdot dm^{-3}$, and did not change in one series.

The quantity of ammonium N in the water before contact with the gravel varied from 0.07 to 0.20 mg $N\text{-}NH_4 \cdot dm^{-3}$. The changes of ammonium N were examined at the following parameters: 1:5/10, 1:5/45, 1:20/45. At the above cited periods of water mixing with the gravel, the concentrations of ammonium N were generally increasing from 0.15 to 0.70 mg $N\text{-}NH_4 \cdot dm^{-3}$. It was determined that after the mixing ammonium N content in the water reached the max. value of 1.46 mg $N\text{-}NH_4 \cdot dm^{-3}$ at the max. volume of gravel and long contact time.

The concentration of nitrites in the water sampled for analyses was low and contained between $0.005 \div 0.075$ mg $N\text{-}NO_2 \cdot dm^{-3}$. Changes of the quantity of nitrites were analysed at the following test settings: 1:10/10, 1:10/45, 1:30/45. After the contact with gravel, concentration of this nitrogen fraction was increasing, except for two cases when the measurements revealed no changes. The increase was variable but most frequently from 0.003 to 0.015 mg $N\text{-}NO_2 \cdot dm^{-3}$, although in one case it exceeded 0.040 mg $N\text{-}NO_2 \cdot dm^{-3}$.

The concentrations of nitrates in the water varied in a wide range i.e. between 3.5 and 113.6 mg $N\text{-}NO_3 \cdot dm^{-3}$. These changes were examined at the following test settings: 1:15/10, 1:15/30, 1:30/30. After the contact with gravel, little increase was usually measured; maximally it reached only 2 mg $N\text{-}NO_3 \cdot dm^{-3}$. The increase was higher in the 1:15 samples than in 1:30.

## Flow tests

During the tests, the fish breeding tank contained 600 $dm^3$ water, the upper retention tank 350 $dm^3$, and the bottom tank 400 $dm^3$. The stock in the basin comprised of 14-month old sheatfish *(Silurus glanis)* of the average unit

weight 100 g. The water flow intensity in the columns with media equalled 26.3 $dm^{-3} \cdot h^{-1}$ which corresponds with the hydraulic loading of 13.42 $m^3 \cdot m^2 \cdot h^{-1}$ in a single column. After start-up of the active filter, the concentration of phosphorus in the tanks was decreasing for 12 hours by 33%, and afterwards remained constant in the range of $0.35 \div 0.39$ mg $P\text{-}PO_4 \cdot dm^{-3}$.

As for the individual columns, it was determined that the highest reduction was achieved in the column with the max. height of packing i.e. 76 cm. After the first hour of filtration the P-removal efficiency reached 68% whereas in column 4, at the packing height of 29 cm, the efficiency was by 26% lower. The phosphates removal capacity was decreasing in the following hours of the filtration (Figure 13).

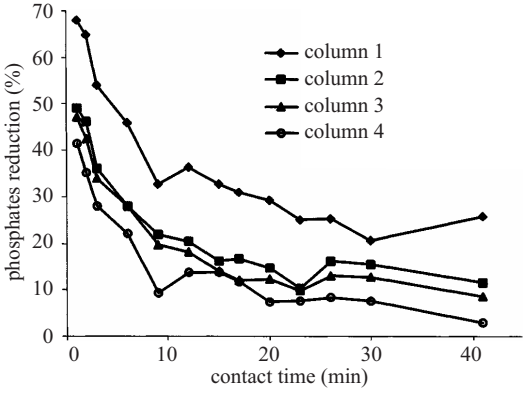


Fig. 13. Orthophosphates reduction in aquaculture effluent during filtration on individual columns with active media

Based on the comparison of the phosphates quantities retained per volume unit, mass unit, and medium surface area unit, it may be concluded that irrespective of the packing height, similar treatment effects can be obtained (Table 3).

Table 3

Quantity of phosphates retained per unit volume and mass volume of the filter, and per medium unit surface area, on individual filtration columns

| Column No | Quantity of retained phosphates | | |
|---|---|---|---|
| | (g $P\text{-}PO_4 \cdot dm^{-3}$ of filter) | (g $P\text{-}PO_4 \cdot kg^{-1}$ d.m. of filter) | (g $P\text{-}PO_4 \cdot m^{-2}$ of grains surface area) |
| 1 | 0.133 | 0.108 | 0.077 |
| 2 | 0.101 | 0.082 | 0.059 |
| 3 | 0.120 | 0.098 | 0.070 |
| 4 | 0.136 | 0.110 | 0.079 |

In the course of the examinations the following determinations were done: iron and ammonium N concentration, water pH. The measurement of iron concentration in the water was needed in order to control its potential washing out from the medium. Thus, the analyses were conducted every few hours in the water from the breeding tank, in the inflow and outflow from the filtration columns.

The results presented in the table (Table 4) show that during filtration iron is not washed out from the medium, neither in the first hours of filtration nor after a longer time of the process duration.

Table 4

Iron concentration in water from fish breeding in a closed system during water filtration on active medium

| Time (h) | Iron concentration (mg Fe · $dm^{-3}$) | | | | | Breeding basin |
|---|---|---|---|---|---|---|
| | inflow to filter | outflow | | | | |
| | | column 1 | column 2 | column 3 | column 4 | |
| 0 | | | | | | 0.02 |
| 3 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 |
| 9 | 0.02 | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 |
| 17 | 0.02 | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 |
| 26 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 0.02 |
| 41 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 |

The value of water reaction was determined once per day and it was observed that when the filtration columns are operating in the system, the water pH in the breeding tank decreased slightly i.e. by 0.13 pH. Analysis of the pH values in the inflow and outflow water revealed that reaction diminished by about 0.03 pH. It should be emphasized that during the experiment the pH in water from all tanks was not lower than 8.11 pH.

During the experiment on phosphorus compounds removal on the active medium, the concentration of ammonium N and nitrates increased slightly. These changes were as follows: start of the process 0.73 mg $N\text{-}NH_4 \cdot dm^{-3}$, 2.70 mg $N\text{-}NO_3 \cdot dm^{-3}$, end of the process 0.91 mg $N\text{-}NH_4 \cdot dm^{-3}$; 3.20 mg $N\text{-}NO_3 \cdot dm^{-3}$. Concentrations of the nitrite nitrogen did not change. During the experiments a minimal decrease of the total hardness was determined.

## Discussion

Application of various iron-containing products in the water and waste water treatment, beside the conventional method of coagulation with iron salts, was and still is the subject of research (JENKINS at al. 1971, GRØTERUD,

SMOCZYŃSKI 1986, EL-HOZ, APPERTEY 1996, GEORGE 2001). The by-product of the iron removal process that can be used in waste water treatment is the sludge contained in the washery liquid from the water deironing filters. Its use in waste water treatment is not a novelty. Due to the number of surveys conducted in Germany, the general scientific-technical use principles have been worked out (concerning mainly iron hydroxides sludge) in the phases of waste water discharge and treatment, as well as sewage sludge treatment (DAMMANN, BENZINGER 1999, HÄFELE et al. 1999, BENZINGER et al. 1996).

In the present investigations, gravel was applied as a filtration medium and adsorbent, used primarily as filtration medium in tap water deironing. As a result of the filtration of iron-rich water the gravel was coated with a layer of hydrated iron oxides thus when re-used as a filtration medium it can be regarded as active medium. Such terming can be justified by the previous studies of phosphates removal on this medium (KRZEMIENIEWSKI 1995, JANCZUKOWICZ et al. 1995, STRĄCZYŃSKA-KNYSAK 1999, KRZEMIENIEWSKI, STRĄCZYŃSKA-KNYSAK 2000). The analyses of phosphorus compounds regarded mainly orthophosphates. The effluent from fish ponds contains phosphorus mostly in the form of orthophosphates (BERGHEIM et al. 1984).

On the grounds of the tests carried out in the static mode it has been concluded that the efficiency of phosphates removal from water by gravel coated with hydrated iron oxides depends on:
– contact time,
– size of the contact surface area,
– initial concentration of phosphates,
– temperature.

Contact time comprised an important factor determining the amount of reduced phosphorus. In the described experiment, the max. reduction was observed in the first 30 min while in the following 15 min it generally differed only slightly.

The tested initial concentrations, adsorbent quantities, and contact times have not allowed to draw explicit conclusions regarding the dependence between the initial phosphates concentration, the volume of adsorbent, and the duration of the period after which phosphates binding stops.

The results obtained in the static tests have also shown that up to some initial phosphates concentration value the degree of reduction (%) increases along with the increase of this concentration. Above the value, the reduction does not increase.

It has been observed that together with the increase of the active medium volume, despite the obvious growth of phosphates reduction, the capacity of the medium diminishes i.e. less phosphates can be retained per time unit by the volume unit (or surface unit) of the adsorbent. Unfortunately, on such

grounds no conclusions can be drawn concerning the total capacity of the active medium for phosphate binding and the ratio between the amount of medium and water/waste water, as the state of equilibrium was not set up during the tests.

The efficiency of phosphates reduction depended also on temperature: in lower temperature phosphates removal per time unit was lower. The reference literature reports that the amount of adsorbed substance decreases along with the temperature rise (KÜMMEL, WORCH 1990). However, in the present survey, it has been determined that the efficiency of phosphates removal was higher in higher temperatures. It may be explained by the fact that the experiment was not run until the equilibrium state settlement, after which it might have been revealed that in higher temperature less phosphates were adsorbed than in lower temperature. In higher temperature adsorption can occur faster as also faster is the transport of particles to the adsorbent's surface. Beside that, removal of phosphates on the applied medium can also occur due to chemical precipitation whose efficiency increases with temperature rise, due to increased reaction rate.

During the static-mode tests, in the water after mixing with gravel coated with iron oxides, an increase of the iron amount was observed which depended on the gravel volume in water sample: the increase was higher, the higher was the active medium/water ratio. In the samples mixed with the max. gravel quantity, iron concentration in the water reached in few cases about 0.9 mg $Fe \cdot dm^{-3}$ whereas with the min. gravel amount only few times it exceeded 0.3 mg $Fe \cdot dm^{-3}$ and only once 0.4 mg $Fe \cdot dm^{-3}$.

Iron is regarded as little toxic, especially that it is a microelement necessary to plants and animals growth. In water, in the common concentrations it usually has no toxic impact on fish. However, in higher concentrations iron salts can in a short time cause damage to fishes and influence in a negative way the production conditions in waters (LUKOWICZ 1976, MÜLLER 1997).

Iron concentrations in the present experiments did not differ from the values typical in natural waters. In running waters these concentrations most often vary between $0.5 \div 1.0$ mg $Fe \cdot dm^{-3}$ (MÜLLER 1997), higher values can be measured in oxygen-deficient hypolimnion as well as in the boggy areas, acidic soils, and water effluents from spruce forests and brown coal deposits.

In the static tests, in each series, the mean iron concentrations after water mixing with the active medium amounted to $0.10 \div 0.25$ mg $Fe \cdot dm^{-3}$ (for the min. gravel quantity of 33.3 $cm^3 \cdot dm^{-3}$), and to $0.31 \div 0.73$ mg $Fe \cdot dm^{-3}$ (for the max. gravel quantity of 200 $mL \cdot dm^{-3}$). The concentration values in some cases, at high quantities of gravel, were higher than the most rigorous permissible values quoted in the references. Thorough rinsing of gravel before its use and – first of all – application as a medium in static filters, should prevent iron washing out.

One of the compounds which are given special attention in fish breeding is ammonia. In the dissociated form ($NH_4^+$) it is practically non-harmful. The main source of ammonia are the metabolic transformations: it is an excretion product (BERGHEIM et al. 1984, LAMAIRE at al. 1998, DORITH, EITAN 1998). It particularly threatens fishes bred in the circulating systems (KOLMAN 1992). Production of ammonia by fishes depends on the species, size and age (BERGERO at al. 2001).

During the static tests, an increase of the content of some nitrogen forms in the water after mixing with gravel was observed, in most cases rather small. The max. rise was determined in the case of ammonium N. The suspicions regarding increase of ammonium N in the water after the contact with gravel were not confirmed in further experiments in the flow system; the concentrations of this form of nitrogen in the effluent from the column with the active medium were not much different from those in the inflow, or even were much lower compared to the inflow.

In the test, small changes in the water pH were detected after the contact with gravel. They depended on the contact time and the gravel quantity. However, they were not so obvious as to have a negative effect on the fish condition; the pH value was contained in the range $7.4 \div 8.6$ which corresponds with the optimal value range to most species (SZCZERBOWSKI 1993).

Additionally, a small decrease of the total alkalinity was determined in the water after mixing with gravel. The initial value was however so high in most series that the reduction was not a negative phenomenon.

Total hardness of the tested water from fish breeding can be regarded as average. Changes in the total hardness in the water samples after mixing with gravel were not explicit thus the difficulty to define their reason. Generally the modifications were minimal.

The quantity of chlorides and sulphates in the water from fish breeding, after mixing with gravel was increasing but the concentrations were very low. Some quantity of chloride ions in the water from the recirculation systems is even required, as according to KOLMAN (1992) they prevent fish mortality at lethal concentrations of nitrites. The increase of chlorides concentrations (like in the case of iron) was more determined by the quantity of gravel than by its contact time with the water. Therefore, it seems correct to apply longer contact times and smaller gravel quantities which should guarantee similar reduction of phosphates like in the case of short mixing with large quantity of the active medium, yet the quantities of iron and chlorides migrating to water will be lower.

## Conclusions

In the study an attempt was made to investigate the efficiency and usefulness of a used medium from filters for underground water deironing, termed active medium, in phosphorus compounds removal from aquaculture effluents. For this purpose, a large-scale laboratory experiments were carried out.

In the laboratory static-mode experiments phosphates removal was investigated from water originating from fish breeding. Additionally, the effect of the active medium on a number of water quality parameters was determined.

The analysis of the obtained results comprised the ground to draw the following conclusions:

1. Active medium can adsorb phosphates from water. This medium can be successfully used in aquaculture effluents treatment.

2. Removal of phosphates in the static mode depends on the time of contact with the medium, the initial concentration of phosphates, the water temperature, and the quantity of the medium.

Phosphates reduction after 60-minute mixing amounted to $33.3 \div 93.9\%$ and the reduction intensity was the highest in the first $30 \div 45$ minutes.

Decrease of the process temperature by about $10^{o}C$ diminishes phosphates adsorption by a few up to more than ten per cent.

Along with the contact area increase (quantity of the medium), the reduction of phosphates rises but the medium's capacity decreases i.e. the amount of phosphates removed per time unit by the volume unit of the medium.

3. Contact of the water from fish breeding and the active medium in the mixing conditions causes increase of iron and ammonium N concentration, reduction of total alkalinity and small decrease of the water pH. Filtration on the static filter has no considerable effect on the iron concentration, water pH, nor ammonium N increase.

4. The obtained results comprise an inspiration for seeking other kinds of active media containing iron compounds. Higher effectiveness than observed in the present experiment can be expected e.g. in case of artificially prepared grains of iron hydroxide contained in the washery liquid from water deironing filters.

Translated by Monika Szewczyk

Accepted for print 08.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 375-388, Y. 2006

# THE INFLUENCE OF THE AIRPORTS AND THE PLANES DE-ICEINGON SURFACE WATER QUALITY

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K e y w o r d s: de-icing, rainwater, melt water, total organic carbon, total nitrogen, chlorine.

A b s t r a c t

The influence of the airport on the environment was analysed on the basis of the concentration of organic compounds (TOC), total nitrogen (TN) and chlorides ($Cl^-$) in the waters of the Służewiecki Stream, a receiving body of rainwater from Warsaw and the airport. The use of acetate- and glycol-based chemical agents to de-ice airport surfaces in the winter period caused an increase in the TOC concentration in the receiving water, even up to the level of 563.9 mg TOC $\cdot$ $dm^{-3}$. Additionally, the use of urea for de-icing runways in the winter period caused a long-term increase in the TN concentration in the waters of the Służewiecki Stream, up to the level of 149.8 mg TN $\cdot$ $dm^{-3}$. Increased concentrations of chlorides in the winter period in the waters of the Służewiecki Stream up to the level of 63.0 mg $Cl^-$ $\cdot$ $dm^{-3}$ resulted from the use of sodium chloride to de-ice roads outside the area of the airport. In the summer period, the influence of the airport on the quality of water in the Służewiecki Stream was not observed. In the winter period, the degree of pollution of the waters of the Służewiecki Stream was directly proportional ($p<0.05$) to the flow rate.

## WPŁYW PORTU LOTNICZEGO I ODLADZANIA SAMOLOTÓW NA JAKOŚĆ WÓD POWIERZCHNIOWYCH

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S ł o w a k l u c z o w e: odladzanie, woda deszczowa, związki organiczne, azot całkowity, chlorki.

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Abstrakt

Oddziaływanie portu lotniczego na środowisko przeanalizowano na podstawie stężeń związków organicznych (TOC), azotu całkowitego (TN) oraz chlorków ($Cl^-$) w wodach Potoku Służewieckiego – odbiornika wód deszczowych z Warszawy oraz lotniska. Stosowanie roztworów glikolu do odladzania samolotów w okresie zimowym powodowało wzrost stężenia TOC w wodach odbiornika nawet do wartości 563,9 mg $TOC \cdot dm^{-3}$. Wykorzystanie mocznika do odladzania pasów startowych w okresie zimowym powodowało długoterminowy wzrost stężenia TN w wodach Potoku Służewieckiego do wartości 149,8 mg $TN \cdot dm^{-3}$. Podwyższone stężenia chlorków w okresie zimowym w wodach Potoku Służewieckiego do wartości 63,0 mg $Cl^- \cdot dm^{-3}$ wynikały ze stosowania chlorku sodu i chlorku wapnia do odladzania dróg poza terenem lotniska. W okresie letnim nie stwierdzono oddziaływania lotniska na jakość wód w Potoku Służewieckim. W okresie zimowym stopień zanieczyszczenia wód Potoku Służewieckiego był wprost proporcjonalny ($p<0.05$) do wielkości przepływu.

## Introduction

Ice and snow pose an important safety problem for air transport. Iced runways are an obvious danger and a number of methods are available to treat them.

Mechanical methods of de-icing, such as the use of snow ploughs or sand spreaders, are not so efficient or convenient in usage as chemical agents, e.g. glycol or urea (O'CONNOR, DOUGLAS 1993). Urea and glycol are also less expensive and easier to use than less toxic de-icers based on potassium acetate (TURNBULL, BAVEN 1995). The usage of these agents increases a potential impact of airports on the environment. This can occur particularly in the vicinity of reservoirs or watercourses. The negative impact of both glycol and urea on the environment results from the proven toxicity (MCGAHEY, BOUWER 1992, PILLARD 1995) of these compounds, as well as of products of their biological decomposition (formaldehyde, ammonia). Therefore, it is necessary to monitor the occurrence of these compounds in the vicinity of sites where they are used, such as airports.

The aim of the study was to determine the impact of the operations performed at Warsaw Frederick Chopin Airport on the water quality in the Służewiecki Stream. The existing hydrological system, as well as the presence of a rainwater drainage network, makes it possible to determine the influence of the municipal drainage basin as well as the airport drainage basin on the water quality of the Służewiecki Stream.

# Materials and Methods

## Description of the object under analysis

Warsaw Frederick Chopin Airport has the capacity to handle about 3.5 million passengers per year and the Cargo terminal can handle 60.000 tons of freight per year. The airport is situated about 7 km from the city centre. In the winter season, in order to properly maintain planes, airport surfaces – particularly runways and taxiways – chemical agents are used. These are mainly glycol- and urea-based agents. Additionally, some agents based on acetates and formic acid are introduced. Technical roads dedicated for vehicles and car parks are de-iced with the use of calcium chloride. De-icing operations are carried out only in case of glazed frost, freezing drizzle, icing or intense snow. The airport has a system for discharging rain and melt water to a natural watercourse known as the Służewiecki Stream. The total length of the Służewiecki Stream is 13.540 m, with an average fall of 1.38%. Above the airport, the catchment basin of the Służewiecki Stream covers an area of about 420 ha, including housing and commercial areas of the south-west part of Warsaw. In the area of the airport, the catchment basin of the Służewiecki Stream is 760.5 ha and includes hardened surfaces of runways, taxiways and technical roads with a total area of 188.5 ha, green areas – 544.6 ha and ground support equipment facilities – 27.4 ha. This gives an average run-off coefficient of $\psi = 0.279$. The waters of the Służewiecki Stream, from their source to the outlet from the airport area, flow in a closed channel with numerous terminals of the rainwater drainage system. The Służewiecki Stream is a receiving body of rainwater from urbanized areas. This causes significant differences between the medium-low water level SNQ – 0.056 $m^3 \cdot s^{-1}$, and medium high water level SWQ = 0.57 $m^3 \cdot s^{-1}$, while the highest of high levels reaches the value of 26.71 $m^3 \cdot s^{-1}$. (Data from hourly measurements, carried out between 17.07.2000 and 23.10.2002 at the Rainwater Treatment Plant situated behind the airport at the Służewiecki Stream.)

The water quality of the Służewiecki Stream at the outlet from the area of the airport depends on two main factors. They include the quality of water carried by the Służewiecki Stream to the airport from the area of the city and the management and usage of the drainage basin in the area of the airport. Therefore, it was expected that as a result of de-icing the surface of runways, taxi-ways, parking aprons and planes, as well as of work related to refuelling, the concentration of organic compounds (TOC), nitrogen (TN) and chlorides ($Cl^-$) in the waters of the Służewiecki Stream that flows through the area of the airport would increase. In the areas of the city located above the airport, the only chemical agent used to de-ice the surfaces of streets and pavements is

calcium chloride. Pollutants found in the waters of the stream reaching the airport could originate from hardened surfaces washed by rain and from potential uncontrolled outlets of social and domestic sewage. It was also expected that the level of water pollution in the Służewiecki Stream would depend on the flow rate.

## Description of research

The study was conducted from February 2002 to December 2002. In this period, 31 samples of water were taken in two-week intervals. The sampling of water from the Służewiecki Stream was performed in the influent (I) and the effluent (E) from the area of the airport (Figure 1). Rainwater sewage flowing out of the area of the airport was divided into two types, depending on the technology used for maintaining the surface of the airport. The first type of rainwater sewage was observed only when de-icing agents are used (W), while the second type of rain sewage was observed in the period when de-icing agents were not used (S). With regard to the fact that the method of exploitation of the airport depended on weather conditions, sewage was additionally divided into

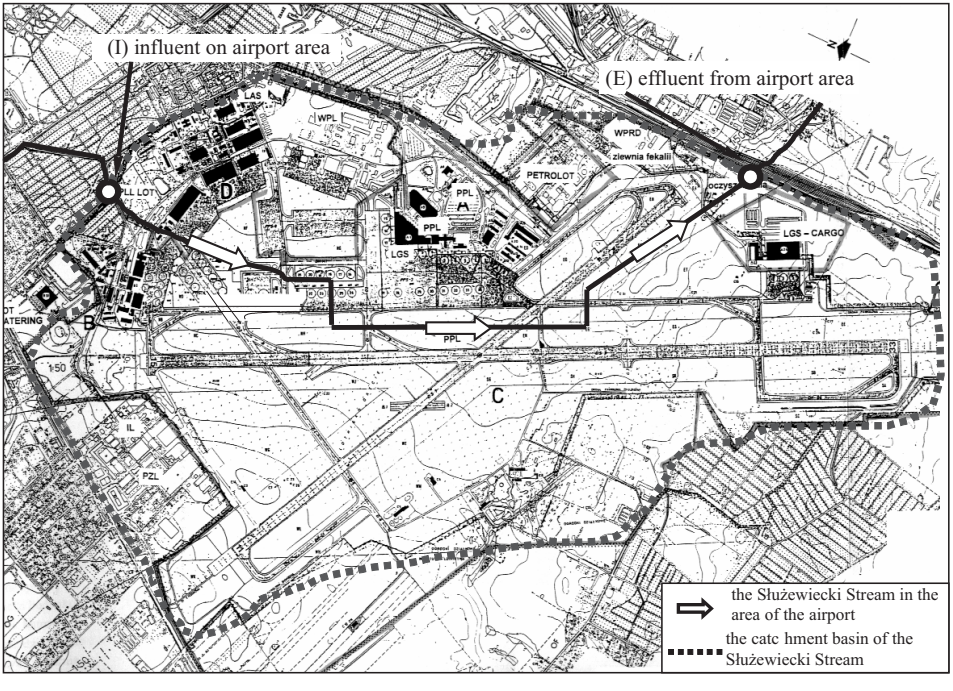



Fig. 1. Layout plan of the Służewiecki Stream in the area of Warsaw Frederick Chopin Airport

Table 1

Variants of the experiment

| Type of exploitation of the airport area | Using deicers (S) | Weather conditions | | | |
|---|---|---|---|---|---|
| | | dry (D) | | rainfall (R) | |
| | | SDI (summer dry influent) | SDE (summer dry effluent) | SRI (summer rain influent) | SRE (summer rain effluent) |
| | without using deicers (W) | WDI (winter dry influent) | WDE (winter dry effluent) | WRI (winter rain influent) | WRE (winter rain effluent) |
| | | influent (I) | effluent (E) | influent (I) | effluent (E) |
| | | point of sampling | | | |

two types: dry period sewage (D) and rain period sewage (R). Thus, the expressed assumptions made it possible to set apart eight variants of the experiment (Table 1).

The values determined in water samples included: total organic carbon (TOC) and total nitrogen (TN) (using the method of infrared radiation detection, with the use of TOC 1200 apparatus produced by Thermo Electron Corporation) and chlorides (Cl) (using Mohr method). The rate of water flow in the Służewiecki Stream was measured on-line, at the outlet from the area of the airport. Measurements at one-hour intervals were stored in the memory of a recording device (Enders-Hauser level sensor).

The obtained results were statistically analysed with the use of Statistica 6.0 software. The following parameters were determined: the arithmetic mean, size, standard deviation, coefficient of variation, minimum and maximum values. The analysis of variance was carried out with the use of ANOVA test at the significance level ($p<0.05$), and subsequently, a detailed analysis was carried out with the use of post-hoc RIR Tukey test for unequal sizes. The strength of the relationship between the flow rate of the Służewiecki Stream on the days when samples were taken and the values of concentrations of individual pollutants was determined at the significance level of $p<0.05$. In cases where a significant correlation existed ($p<0.05$), linear regression coefficients between analysed parameters were determined.

## Results

There were significant differences ($p<0.05$) observed between average values of TOC in the waters of the Służewiecki Stream (Table 3). In the period when de-icers were used, in rainy weather, a significant ($p<0.05$) (Table 4) increase of TOC condensation in waters of the Służewiecki Stream was

observed, from the value of 9.9 mg TOC · $dm^{-3}$ before the influent to the airport area to the value of about 564 mg TOC · $dm^{-3}$ at the mouth of the canal out of the area of the airport (Table 2). The occurrence of rainfall resulted in a substantial increase ($p<0.05$) – Table 4 in TOC concentration in the waters of the Służewiecki Stream, both in its influent as well as in its effluent from the area of the airport in the period when de-icers were not used, from average values of 1.9-13.0 mg TOC · $dm^{-3}$ to average values ranging from 45.8 to 56.9 mg TOC · $dm^{-3}$. However, in the period when de-icers were used, a significant increase ($p<0.05$) – Table 4 in TOC concentration was observed only at the outflow of the stream from the area of the airport, in rainy weather, from the level of about 9.2 to 564 mg TOC · $dm^{-3}$ (Table 2). In the period when de-icers were not used, the increase of TOC concentration in rainy weather at both measuring sites proves that the main sources of organic pollution in the waters of the Służewiecki Stream in this period were the areas situated above the area of the airport. However, in the period of using de-icing agents, the increase of pollutant concentration in rainy weather observed only in the effluent suggests the impact of the airport on the quality of water in the Służewiecki Stream.

Table 2
Parameters of the variance analysis of TOC variable with the use of ANOVA test at the significance level of $p<0.05$

| ANOVA parameters | | | | | | | |
|---|---|---|---|---|---|---|---|
| *SS* effect | *df* effect | *MS* effect | *SS* error | *df* error | *MS* error | *F* | *p* |
| 1 852 399 | 7 | 264 628.5 | 11 799.45 | 54 | 218.5083 | 1211.068 | 0.000000 |

Table 3
Calculated values of probability in post-hoc Tukey test at the significance level ($p<0.05$) of the diversification of average values of TOC

| Variant | SDI | SDE | SRI | SRE | WDI | WDE | WRI |
|---|---|---|---|---|---|---|---|
| SDE | 0.851127 | | | | | | |
| SRI | **0.000151** | **0.000134** | | | | | |
| SRE | **0.002884** | **0.000151** | 0.803136 | | | | |
| WDI | 0.998956 | 0.991360 | **0.000134** | **0.000249** | | | |
| WDE | 0.999733 | 0.982114 | **0.000134** | **0.000310** | 1.000000 | | |
| WRI | 0.999929 | 0.970598 | **0.000137** | **0.000884** | 0.999999 | 1.000000 | |
| WRE | **0.000134** | **0.000134** | **0.000134** | **0.000134** | **0.000134** | **0.000134** | **0.000134** |

* **bold** – the difference between means is significant

Table 4

Parameters of descriptive statistics of the values of TOC concentration in the waters of the Służewiecki Stream

| Parameter | Variant of the experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SDI | SDE | SRI | SRE | WDI | WDE | WRI | WRE |
| Mean | 13.0 | 1.9 | 56.9 | 45.8 | 8.37 | 9.22 | 9.9 | 563.9 |
| Number of tests | 7 | 7 | 8 | 8 | 9 | 9 | 7 | 7 |
| Standard deviation | 1.24 | 0.25 | 27.16 | 2.53 | 0.81 | 1.2 | 0.32 | 33.08 |
| Standard error ± | 0.469 | 0.095 | 2.53 | 0.895 | 0.27 | 0.398 | 0.121 | 12.5 |
| Minimum value | 11.3 | 1.6 | 31.3 | 42.9 | 7.3 | 7.6 | 9.6 | 521.1 |
| Maximum value | 14.6 | 2.3 | 83.0 | 49.1 | 9.6 | 11.1 | 10.3 | 612.0 |

Table 5

Parameters of the variable variance analysis of TN with the use of ANOVA test at the significance level ($p<0.05$)

| ANOVA parameters | | | | | | | |
|---|---|---|---|---|---|---|---|
| *SS* effect | *df* effect | *MS* effect | *SS* error | *df* error | *MS* error | *F* | *p* |
| 129 146 | 7 | 18 449.4 | 300.43 | 54 | 5.5635 | 3316.152 | 0.000000 |

There were significant differences ($p<0.05$) found between average values of TN in the waters of the Służewiecki Stream (Table 6). In period when de-icers were not used, total nitrogen content in the waters of the Służewiecki Stream remained at a similar level, within the range of values from 2.4 to 5.5 mg TN · $dm^{-3}$ (Table 5). In the period when de-icing agents were used, a significantly higher ($p<0.05$) – Table 7 concentrations of TN were observed, both in rainless and rainy weather, in the effluent from the airport, in the range of 16 – 149.8 mg TN · $dm^{-3}$ in comparison with the influent – 7 to 5.7 mg

Table 6

Parameters of descriptive statistics of the values of TN concentration in the waters of the Służewiecki Stream

| Parameter | Variant of the experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SDI | SDE | SRI | SRE | WDI | WDE | WRI | WRE |
| Mean | 2.8 | 5.5 | 2.7 | 2.4 | 7.0 | 16.0 | 5.7 | 149.8 |
| Number of tests | 7 | 7 | 8 | 8 | 9 | 9 | 7 | 7 |
| Standard deviation | 0.37 | 0.28 | 1.18 | 0.06 | 0.7 | 0.76 | 0.17 | 6.84 |
| Standard error (±) | 0.14 | 0.107 | 0.416 | 0.02 | 0.234 | 0.254 | 0.06 | 2.585 |
| Minimum value | 2.4 | 5.1 | 1.5 | 2.3 | 5.9 | 15.3 | 5.45 | 139.7 |
| Maximum value | 3.4 | 5.8 | 3.9 | 2.5 | 8.1 | 17.1 | 5.9 | 161.3 |

TN · $dm^{-3}$, respectively (Table 5). This demonstrates the impact of de-icing agents used in the area of the airport, mostly of urea, on the quality of the waters in the stream, while this impact was significantly higher during rainfall.

Table 7
Calculated values of probability in post-hoc Tukey test at the significance level ($p$<0.05) of the diversification of average values of TN

| Variant | SDI | SDE | SRI | SRE | WDI | WDE | WRI |
|---|---|---|---|---|---|---|---|
| SDE | 0.402727 | | | | | | |
| SRI | 1.000000 | 0.356152 | | | | | |
| SRE | 0.999991 | 0.247899 | 0.999998 | | | | |
| WDI | **0.030681*** | 0.928923 | **0.012736** | **0.006578** | | | |
| WDE | **0.000134** | **0.000134** | **0.000134** | **0.000134** | **0.000134** | | |
| WRI | 0.312551 | 1.000000 | 0.272237 | 0.182347 | 0.965981 | **0.000134** | |

* **bold** – difference between means is significant

Significant differences ($p$<0.05) were observed between average values of $Cl^-$ in the waters of the Służewiecki Stream (Table 9). In period when de-icers were not used, the content of chlorides in the waters of the Służewiecki Stream was on a similar level, within the range of values from 8.7 to 14.0 mg $Cl^-$ · $dm^{-3}$ (Table 8). In the period when de-icing agents were used, a significantly increased ($p$<0.05) (Table 10) concentrations of chlorides were observed, both in rainless weather and during rain in the influent from the airport, in the range of 17.5 – 63 mg $Cl^-$ · $dm^{-3}$, higher than in the effluent – ranging from 7 to 31.5 mg $Cl^-$ · $dm^{-3}$, respectively (Table 8). This demonstrates the external origin of chloride pollution in the waters of the stream. The decrease in chloride concentration could be caused by their dilution by chloride-free water running off from the area of the airport.

Table 8
Parameters of the variable variance analysis of $Cl^-$ with the use of ANOVA test at the significance level of $p$<0.05

| ANOVA parameters | | | | | | | |
|---|---|---|---|---|---|---|---|
| *SS* effect | *df* effect | *MS* effect | *SS* error | *df* error | *MS* error | *F* | *p* |
| 18 317 | 7 | 2616.8 | 812.99 | 54 | 15.0553 | 173.810 | 0.000000 |

Table 9

Parameters of descriptive statistics of the values of $Cl^-$ concentration in the waters of the Służewiecki Stream

| Parameter | Variant of the experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SDI | SDE | SRI | SRE | WDI | WDE | WRI | WRE |
| Mean | 14.0 | 10.5 | 8.75 | 8.75 | 17.5 | 7.0 | 63.0 | 31.5 |
| Number of tests | 7 | 7 | 8 | 8 | 9 | 9 | 7 | 7 |
| Standard deviation | 2.86 | 3.5 | 2.64 | 4.18 | 2.73 | 0.68 | 7.0 | 5.19 |
| Standard error ± | 1.08 | 1.323 | 0.935 | 1.48 | 0.91 | 0.225 | 2.64 | 1.96 |
| Minimum value | 10.5 | 7.0 | 7.0 | 3.5 | 12.3 | 5.9 | 56.0 | 21.1 |
| Maximum value | 17.5 | 14.0 | 14.0 | 14.0 | 21.5 | 7.9 | 70.0 | 37.8 |

Table 10

Calculated values of probability in post-hoc Tukey test at the significance level f $p<0.05$ of the diversification of average values of $Cl^-$

| Variant | SDI | SDE | SRI | SRE | WDI | WDE | WRI |
|---|---|---|---|---|---|---|---|
| SDE | 0.695024 | | | | | | |
| SRI | 0.204851 | 0.989628 | | | | | |
| SRE | 0.204851 | 0.989628 | 1.000000 | | | | |
| WDI | 0.695024 | **0.027921*** | **0.000979** | **0.000979** | | | |
| WDE | **0.027921** | 0.695024 | 0.984679 | 0.984679 | **0.000142** | | |
| WRI | **0.000134** | **0.000134** | **0.000134** | **0.000134** | **0.000134** | **0.000134** | |

* **bold** – difference between means is significant

Table 11

Parameters of descriptive statistics of flow ($m^3 \cdot s^{-1}$)

| Type of exploitation of the airport area | Precipitation | Parameters of descriptive statistics of flow ($m^3 \cdot s^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *m* | *N* | *S* | *Se* ± | *Min* | *Max* |
| Using de-icers (S) | dry (D) | 0.033 | 7 | 0.023 | 0.009 | 0.017 | 0.076 |
| | rainfall (R) | 0.103 | 8 | 0.118 | 0.042 | 0.017 | 0.287 |
| Without using de-icers (W) | dry (D) | 0.105 | 9 | 0.074 | 0.025 | 0.017 | 0.240 |
| | rainfall (R) | 0.283 | 7 | 0.251 | 0.095 | 0.044 | 0.738 |

Explanations: *m* – medium, *N* – size, *S* – standard deviation, *Se* ± – standard error, *Min*/*Max* – minimum and maximum values

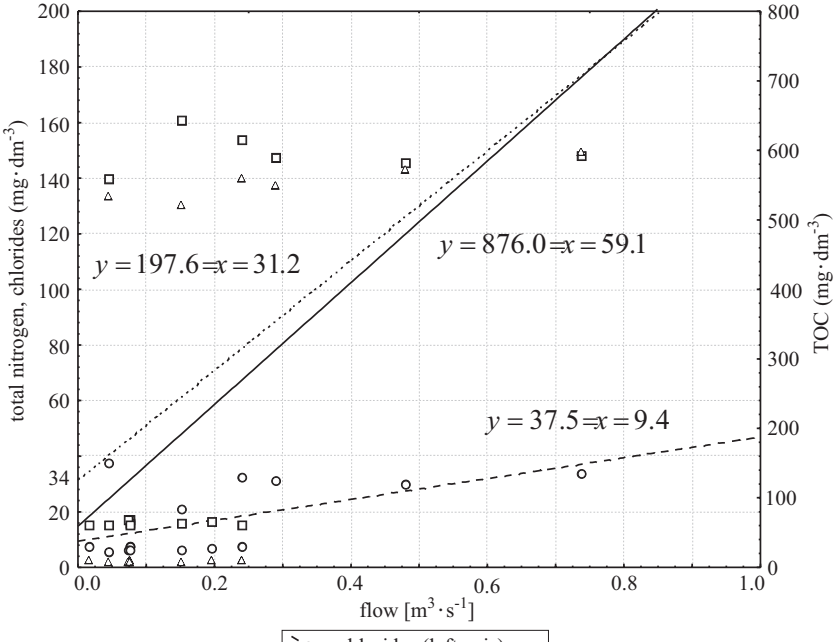
The obtained values of concentrations of individual impurities were compared with the flow rate on the days of water sampling in the Służewiecki Stream.

In the period when de-icing agents were not used, a significant linear correlation ($p<0.05$) between the flow rate and the values of concentration of

individual impurities was not found (Table 12). Such a correlation was observed in the period when de-icing agents were used, for all pollution indices under analysis (Figure 2).

Table 12
Parameters ($p$<0.05) of correlation between the flow and impurity indices in the period when de-icing agents were not used

| Specification | $r_{(XY)}$ | $r^2$ | $p$ | $N$ |
|---|---|---|---|---|
| Flow/TOC | 0.404983 | 0.164011 | 0.134275 | 15 |
| Flow/Chlorides | -0.014491 | 0.000210 | 0.959121 | 15 |
| Flow/Total Nitrogen | -0.376943 | 0.142086 | 0.166061 | 15 |




| | $r_{(X,Y)}$ | $r^2$ | $p$ | $N$ |
|---|---|---|---|---|
| Flow/TOC | 0.612001 | 0.374545 | 0.015316 | 15 |
| Flow/Chlorides | 0.572636 | 0.327912 | 0.025678 | 15 |
| Flow/Total Nitrogen | 0.565342 | 0.319612 | 0.028072 | 15 |

Fig. 2. Parameters of correlation and linear regression ($p$<0.05) between the flow and pollution indices in the winter period

It can be therefore expected that in the period when de-icing agents were used, the increase of flow rate caused by precipitation could cause an increase in the concentration, particularly of organic matter and nitrogen in the waters of the Służewiecki Stream flowing out of the area of the airport.

## Discussion

Large airports can, in various ways, affect the quality of rainwater runoff. Hydrocarbon-based fuel, oil and grease are particularly important. Besides these compounds, other extremely important substances include acetate glycol or urea derivatives, used in anti-icing and de-icing fluids for planes and airport surfaces.

Reports concerning the influence of using de-icers on the quality of rainwater runoff and the water environment of receiving bodies of sewage are well known. In Washington International Airport – WIA (FISHER at al. 1995), two de-icing fluids were used which contained 80% and 50% of glycol by weight, respectively. The content of organic compounds in rainwater flowing off from the area of WIA reached maximum values of COD even at the level of 270.000 mg $O_2 \cdot dm^{-3}$, and of BOD – 6.700 mg $O_2 \cdot dm^{-3}$, while nitrogen concentrations (Kiejdahl) reached 400 mgN-$NH_4$. Very high concentrations of organic compounds in rainwater running off from the airport are confirmed by VELTMAN et al. 1998 (follow SWITZENBAUM at al. 2001), who measured a value of $BOD_5$ at the level of 250.000 mg $\cdot dm^{-3}$. This research indicated that the amount of carbon compounds present in the rainwater running off from the airport increased 60 times in the period when de-icing agents are used, from 9.9 mg TOC $\cdot dm^{-3}$ at the inlet to the airport to 563.9 mg TOC $\cdot dm^{-3}$ at the outlet. The amount of organic compounds that reach the airport in the period when de-icing agents are used is constant and does not change with snowfall. The observed lower concentrations of organic compounds in comparisons with the values presented in the literature can result from a particular hydrological system in the region of Warsaw Okęcie. The whole body of melting water is discharged into the Służewiecki Stream, which flows through the airport, together with the water from the drainage basin situated above the airport. In this situation, the dilution of polluted water occurs.

As regards chlorides, almost twofold higher concentrations of these substances were observed in the water reaching the airport as compared to the effluent. This can result from the fact that streets and pavements in the municipal area are sprinkled only with the mixture of sand and calcium chloride or sodium, while in the area of the airport, the use of these substances is marginal.

Urea used for de-icing very negatively affects the condition of receiving bodies of rainwater from the area of the airport. The concentration of total nitrogen increased in the Służewiecki Stream up to 26 times in the period when de-icing agents were used. The issue of the influence of rainwater polluted with nitrogen compounds has been described in the literature. TURNBULL, BAVEN (1995) analysed the amount of ammonia nitrogen in the Tyne River in Newcastle before the airport, in the effluent from the airport and in the river out of the airport. Similar to the current research, periods of using chemical agents for de-icing and without using those agents were taken into consideration. The concentration of ammonia nitrogen in the river before the airport was 0.6 $mg \cdot dm^{-3}$ in the period when chemical agents were not used, and in the period when they were used, it reached the level of 1.02 $mg \cdot dm^{-3}$. Nitrogen concentrations observed in the canal carrying the water from the area of the airport varied from 2.52 $mg \cdot dm^{-3}$ in periods without de-icing to 173.4 $mg \cdot dm^{-3}$ in periods when chemical agents were used. Ammonia concentrations in the Tyne River out of the airport amounted from 1.60 $mg \cdot dm^{-3}$ in the period when urea was used to 36.79 $mg \cdot dm^{-3}$ in the period when urea was used. These results shows that the affluent of water from the airport increased the concentration of ammonia nitrogen in the waters of the Tyne River not only in the period when urea was used for de-icing airport surfaces, but also in times when airport apron was not frozen. This proves mainly the fact of urea accumulation in neighbouring areas. This is also supported by the presented research, as increased contents of nitrogen remained in the stream also when the usage urea was discontinued. TURNBULL, BAVEN (1995) indicate that urea obviously has no aquatic impact unless it is transported into streams and therefore its effects are not felt until rainfall or snowmelt carries the solution in into the airport tributary. This indicates that most of the ammonia load in the waters of the Służewiecki Stream could originate from snowmelt.

In the period when de-icing agents were not used, no significant differences in the quality of the waters of the Służewiecki Stream leaving the area of the airport were found. An increase in the concentration of organic compounds was observed during rainfall. In the region of the airport, as in each municipal drainage basin (VAZE, CHIEW 2002), impurities were accumulated on the surface of the area. They were subsequently washed away with the first flush of rain. In the season when de-icing agents were not used, in dry periods, an improvement of the quality of the waters of the Służewiecki Stream was observed, as concerns organic compounds in the water flowing through the area of the airport. This suggests a high influence of infiltration on the quality of water in dry periods. This is also supported by the increased concentration of nitrogen, which was accumulated in soil in the winter period and subsequently released in summer to underground water. In addition, TURNBULL,

BAVEN (1995) demonstrated the long term effect of using urea on the quality of water in the reception basin.

It should be emphasized that hazards to surface waters posed by the functioning of Warsaw Frederick Chopin Airport are treated by its user with due care. At the outlet of the Służewiecki Stream from the area of the airport, a pre-treatment plant has been built, the main task of which is to remove suspensions and petroleum derivative pollutants. Additionally, a system of water quality monitoring is used, which causes retention of heavy polluted water and directs it to the sewage system if allowable values of TOC are exceeded.

## Conclusions

1. Chemical de-icers used for in the area of the Warsaw Okęcie airport significantly affect the quality of water in the Służewiecki Stream.

2. The hydrological system, in which a small natural stream functions as a receiving body of rainwater from large hardened surfaces, especially when they are used in a specific way, is very disadvantageous.

3. In the winter period, the usage of mostly urea and glycols, compounds of proven toxic effect, caused an increase in their concentration in the waters running off from the area of the airport.

4. The research in the summer season revealed the long term effect of using urea on the condition of the waters of the Służewiecki Stream. In summer, in dry periods, there was an increase in the nitrogen content in the water of the stream and in the effluent from the airport.

5. In the summer season, the impact of the airport on the quality of water in the Służewiecki Stream is similar to that of the municipal drainage basin. The winter season – when it is necessary to use de-icing agents – poses a problem.

Translated by MARCIN ZIELIŃSKI

Accepted for print 8.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 389-402, Y. 2006

# THE EPIZOIC CILIATES INHABITING GILL LAMELLAE OF *Gammarus fossarum* KOCH. IN STREAM SŁUPSK CITY AGAINST A BACKGROUND OF FRESHWATER QUALITY

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K e y w o r d s: ciliated protozoa, epizoic ciliates, *Gammarus* sp.

A b s t r a c t

Occurrence and geographical distribution of *Gammarus* species – especially of *G. fossorum* in lotic water systems limits the value of its epizoic ciliates as indicators of water pollution. It lives in water with saprobic levels from oligosaprob to $\alpha$-mesosaprob (classes between I and III).

The studies on epizoic ciliates inhabiting gill lamellae of *Gammarus fossarum* Koch. were carried out from January until April 2001 and from October until December 2001 of the stream Słupsk city. In all, 60 specimens of amphipods were collected. The specimens revealed having epifauna organisms belonging to the groups Peritricha, Suctoria and Spirochona. A total of 5129 epizoic ciliates belonging to seven species were identified. The highest number of the epifauna belonged to the orders Peritricha (71.4%), Suctoria and Spirochona were represented by 14.3% of the identified ciliates each. The mean number of ciliates on the examined beach fleas was 160 per carrier; on average, 14 ciliates were found per 1 mm of the carrier's body length. The most numerous ciliates were: *Intrastylum steni* ($\bar{x}$ – 132 zooids carrier$^{-1}$), *Carchesium dipneumon* ($\bar{x}$ – 11.5 zooids carrier$^{-1}$) and *Dendrocometes paradoxus* ($\bar{x}$ – 7.7 zooids carrier$^{-1}$).

As a result of conducting *t*-Student and Cochran, Cox tests (essence of the differences between two averages for small tests with negligible and material differences of variation), it is proved that on the $\alpha$=0.05 level of essence such differences between tested positions do not occur.

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**ORZĘSKI EPIZOICZNE ZASIEDLAJĄCE SKRZELA KIEŁŻY *Gammarus fossarum* KOCH. W STRUMIENIACH MIASTA SŁUPSKA NA TLE JAKOŚCI WODY**

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S ł o w a k l u c z o w e: orzęski epizoiczne, *Gammarus* sp.

A b s t r a k t

Występowanie kiełży, w tym także *G. fossorum,* oraz orzęsków epizoicznych je zasiedlających jest związane z poziomem zanieczyszczenia wody. Szczególnie intensywnie żyją one w wodach, których żyzność mieści się w granicach wód od oligosaporobowych do $\alpha$-mezosaprobowych (klasa czystości między I a III).

Badania nad orzęskami epizoicznymi zasiedlającymi blaszki skrzelowe *Gammarus fossarum* Koch. prowadzono od stycznia do kwietnia oraz od października do grudnia 2001 r. Zebrano 60 osobników kiełży. Na zbadanych osobnikach stwierdzono występowanie symforionów należących do grup Peritricha, Suctoria i Holotricha. Łącznie wyznaczono 5129 orzęsków epizoicznych należących do 7 gatunków. Najwięcej epifauny należało do gromady Peritricha (71,4%). Do gromady Suctoria i Holotricha należało po 14,3% zbadanych orzęsków. Średnia liczba orzęsków na badanych kiełżach wynosiła 183,2 zooidy nosiciel$^{-1}$, średnio na 1mm długości nosiciela przypadało 14 orzęsków. Najliczniej występującymi orzęskami były: *Intrastylum steni* ($\bar{x}$ – 132 zooidy nosiciel$^{-1}$), *Carchesium dipneumon* ($\bar{x}$ – 11,5 zooidy nosiciel$^{-1}$) oraz *Dendrocometes paradoxus* ($\bar{x}$ – 7,7 zooidy nosiciel$^{-1}$).

W wyniku zastosowania testów *t*-Studenta oraz Cochrana i Coxa (istotności różnic między dwoma średnimi dla małych prób przy nieistotnej i istotnej różnicy wariancji) okazało się, że na poziomie istotności $\alpha$=0,05 takie różnice nie występują między badanymi stanowiskami.

# Introduction

The phenomenon of epizoism is one of the forms of interspecific life in animals, without a relationship of parasitic nature. Epizoism (symphony) is the use of entodermic integument or shell of the carrier by individuals of the same or, most often, alien animal species.

Several biological systems of indicating the quality of freshwater have been developed over the recent years. In addition to chemical and physical parameters, macrozoobenthic organisms and ciliated protozoa are widely used for the estimation of saprobic degrees of lotic and lenitic water systems. In contrast to the evaluation of the physical and chemical status of water systems, biological indicator systems are very important in determining the quality of water over longer periods. Although, most of the known indicator systems are well suited to improve water qualities, it is of great interest to have more knowledge about autochthonely freshwater organisms and the water quality. Also, it might be possible to create new indicator system with those.

The value of free-swimming and sessile ciliates as indicators of water quality has been investigated under different aspects (ALBRECHT 1983, 1986,

DORGELO 1977, JEAN, FRUGET 1994, MUSKÓ et al. 1990). Freshwater ciliates are distributed throughout a wide range of water qualities. In contrast to ciliates living in aquatic systems as free-swimming or sessile organisms on rock, plants or any other suitable surface, many epizoic ciliates (classes: Peritricha, Holotricha, Suctoria) are highly adapted to specific carrier organisms (Coelentarata, Insecta larvae), especially arthropods. Also, most epizoic species show preference for certain of the carrier animal (PIESIK, PIESIK 1978, RUSTIGE, FRIEDRICH 1994). The preference can be of high or rather low specifity (FENCHEL 1965, BIERHOF, ROOS 1977, KAHL 1935, MATTHES 1982, MUSIAŁKOWSKI 1965, PIESIK 1975, 1976a,b, RUSTINGE, FRIEDRICH 1994, RUSTINGE, MANNESMANN 1991, SCHÖDEL 1987, SZCZEPANOWSKI 1978). Normally, epizoic ciliates attach only to alive carriers. Also, their abundance depends on the developing cycle of the carrier (NENNINGER 1948, LUST 1950, SCHÖDEL 1987). The mutualistic system of gammarid species and their epizoic ciliates has benn investigated very intensely (BIERHOF, ROOS 1977, BUCHAR 1959, FENCHEL 1965, PIESIK 1975, 1976a,b, PIESIK, PIESIK 1978, SCHÖDEL 1987, STILLER 1957, SZCZEPANOWSKI 1978). However, little is known the question whether the quality of the water – the so called second environment – may influence the occurrence of certain epizoics on their carrier – the so called first environment of the stream Słupsk city.

The carrier *Gammarus fossarum* is widely distributed in lotic water system. Its distribution stretches between saprobic loves from oligosaprob to mesosaprob (DORGELO 1977, JEAN, FRUGET 1994, MUSKÓ et al. 1990). Within this range its epizoic ciliates differ in abundance and frequency. The presented data are part of a long-term study which has been carried out to investigate the distribution patterns of crustacean epizoa in comparison to the water quality, especially to the organic load.

## Material and Methods

Experiments were carried out at 3 sampling sites of the streams in Słupsk city area. This water system drains a large area Lasek Południowy in Słupsk and to flow into the River Słupia. The water level under normal weather conditions is relatively low. The epizoic ciliates was studied in winter (January), spring (April), autumn (October) and winter (December) 2001. The mean discharge within the investigation period from January 2001 until December 2001 ranged from 0.33 $m^3/s$ (site 1) to 0.44 $m^3/s$ (site 3). The water quality over the greater part of the stream ranges between the classes I and III (OBOLEWSKI 2003). Criteria for selecting the sampling sites were the different levels saprobity and a stable population of *Gammarus fossarum*.

Carrier individuals were collected at seasons except summer by using a small metal sieve and tweezers. From each site 5 individuals were selected for further laboratory investigations. On the same day, temperature, pH, dissolved organic matter content and the actual $O_2$-concent were measured and the water was taken to measure the $BOD_5$. All physico-chemical investigations were carried out according to the Polish Norms (*Ordinance...* 1991).

Following the dissection of the thorax appendages with gill lamellae, the number of ciliates inhabiting the gills as well as their specific or generic taxonomy was determined. The epizoites were examined under a microscope (magnification 300 x) in vivo on the day their carriers were captured. The identification followed descriptions and the identifications keys of BIERHOF, ROOS (1977), KAHL (1935), PIESIK (1975, 1976 a, b), WALKER, ROBERTS (1988). To obtain a quantitative analysis of the ciliate fauna the frequency (Fr) and the abundance (A) were calculated. The mean abundance was calculated by rating each species with a classification system. This system was developed in relation to the maximum amount of zooids, which single species are able to colonize a carrier.

To obtain a quantitative analysis of the ciliate fauna the domination ($D$) and statistics analysis (Shannon – Wiener) were calculated (GUHL 1987). Frequency was calculated and interpretation by using the equation of KASPRZAK, NIEDBAŁA (1981):

$$Fr = 100 \cdot b/a,$$

$a$ – amount of gammarids, the ciliate species were found;
$b$ – total amount of investigated gammarids)

The Shannon-Wiener diversity index was calculated according to the formula given by Margalef and Krebs:

$$H' = -\sum_{i=1}^{s} p_i \log_2 p_1 = \frac{n_i}{N},$$

where:
$n_i$ – abundance of ith species,
$N$ – total abundance community,
$s$ – number of species in the community studied.

The value of the index changes with changing taxon richness and evenness on individual species. Depending on environmental effects and biotic intentions, the Shannon-Wiener index for the total epiphytic ciliates varies usually within 0-4 (GUHL 1987).

Values of the Shannon-Wiener diversity index for the epiphytic ciliates of different part of sampling site were calculated from overall mean densities of the taxa identified.

The domination index was calculated according to the formula:

$$D = n_a/n,$$

$n_a$ – the amount of characters belonging to the same species and in all examined trials (tests),
$n$ – the amount of characters of the examined systematical group in all the trial – tests.

The test of *t*-Student and Cochran and Cox was used of nock (differences in meaning between two averages for small tests) to show prove significant arithmetic difference densities of the epiphytic ciliates of different part of sampling site. $C > C\alpha$ or $t > t\alpha$ points out on important arithmetic difference between examinated averages densities.

## Results

A total of 11 species of epizoic ciliates of *Gammarus fossarum* could be identified (Table 1). Of these, 3 species (marked with*) found occasionally (Table 1). For this reason, they were not considered as biological indicators and will not be discussed further. The research 60 carrier (*Gammarus*), where one ascertained 5129 the ciliates epizoic species, in this 288 *Dendrocometes paradoxus*. Table 1 shows differences in presence and abundance of all identified species. Gills (epipodit) researches gammarids (*Gammarus fossarum*) in streams of the Lasek Południowy in the Słupsk city inhabiting the ciliate epizoic (Ciliata) in classes: Peritricha (71.4%), Suctoria (14.3%) and Spirochona (14.3%). On all three sampling sites one ascertained 10 taxons epizoa, among of which dominated *Intranstylum steni*, mean density 132 zooids carrier$^{-1}$ ($D$ = 83%, Figure 1). In resoluteness to more weak density one ascertained *Carchesium dipneumon* $\bar{x}$ – 11.5 zooids carrier$^{-1}$ ($D$ = 7%) and *Dendrocometes paradoxus* – $\bar{x}$ – 7.7 zooids carrier$^{-1}$ ($D$ = 5%). Remaining fixed price epizoa on gill tin plates *Gammarus* stepped out sporadically ($D \leq 1$).

Compared with the chemical data (Table 2) a clear relation between abundance of certain epizoic ciliates can bee seen. As Table 1 shows, this species is *Intranstylum steini*. In particular *Intranstylum steini*, *Pseudocarchesium steni*, *Pseudocarchesium ovatum*, *Pseudocarchesium* sp., *Carchesium dipneumon*, *Carchesium polypinum*, *Vorticella* sp., *Spirochona gemmipara*, *Dendrocometes paradoxus* seem to tolerate water qualities in classes III, respectively to require a relevant nutrition. On the other hand *Carchesium dipneumon*, *Zoothamnium gammari*, prefer cleaner water (classes I/II). The streams in the Lasek Południowy of Słupsk have two sections with a higher organic load (site 1 and 2).

Table 1

Mean annual abundance (*A* – zooid carrier[-1]) and frequency (*Fr* – %) of epizoic ciliates of *Gammarus fossarum*

| Taxon | Sample site | | | | | |
|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | |
| | *A* | *Fr* | *A* | *Fr* | *A* | *Fr* |
| **Peritricha** | | | | | | |
| *Intranstylum steini* | 189.1 ± 200.2 | 94.4 | 177.6 ± 112.5 | 100.0 | 29.2 ± 30.6 | 85.3 |
| *Pseudocarchesium steni* | – | – | 0.1 ± 0.9 | 22.2 | 6.0 ± 11.4 | 59.0 |
| *Pseudocarchesium ovatum* | – | – | 0.2 ± 0.7 | 48.7 | 4.0 ± 4.7 | |
| *Pseudocarchesium* sp.* | 0.1 ± 0.2 | 11.1 | 0.1 ± 0.4 | 25.0 | – | – |
| *Carchesium dipneumon* | 5.3 ± 16.3 | 22.2 | 9.6 ± 7.9 | 100 | 12.3 ± 10.6 | 59.0 |
| *Carchensium polypinum* | – | – | 9.8 ± 7.9 | 87.5 | 7.8 ± 2.1 | 33.6 |
| *Vorticella* sp. a* | | | | | | |
| *Vorticella* sp. b* [1] | 1.7 ± 7.1 | 11.1 | – | – | 1.6 ± 3.5 | 46.7 |
| *Zoothamnium affine* | – | – | – | – | 0.1 ± 1.3 | 32.8 |
| *Zoothamnium gammari* | – | – | – | – | 0.3 ± 3.0 | 28.5 |
| **Spirochona** | | | | | | |
| *Spirochona gemmipara* | 0.5 ± 1.5 | 11.1 | 1.2 ± 1.3 | 68.7 | 0.6 ± 2.4 | 46.7 |
| **Suctoria** | | | | | | |
| *Dendrocometes paradoxus* | 13.3 ± 23.0 | 66.7 | 0.4 ± 0.9 | 28.5 | 9.4 ± 15.5 | 59.0 |
| Σ | **210.0** | | **199.0** | | **71.2** | |
| SD | ± 75.6 | | ± 61.9 | | ± 8.8 | |
| Shannon–Wiener index *H'* | 0.60 | | 0.55 | | 2.05 | |
| *t*–Student test<br>Cochran, Cox test | site 1 – site 2<br>$t < t_{\alpha(0.05)} = 0.005 < 2.131$ | | | | | |
| | | | site 2 – site 3<br>$C < C_{\alpha(0.05)} = 0.483 < 2.363$ | | | |
| | site 1 – site 3<br>$C < C_{\alpha(0.05)} = 0.373 < 2.568$ | | | | | |

* Species which were found occasionally

[1] – Species which could not be separated correctly

SD – standard deviation

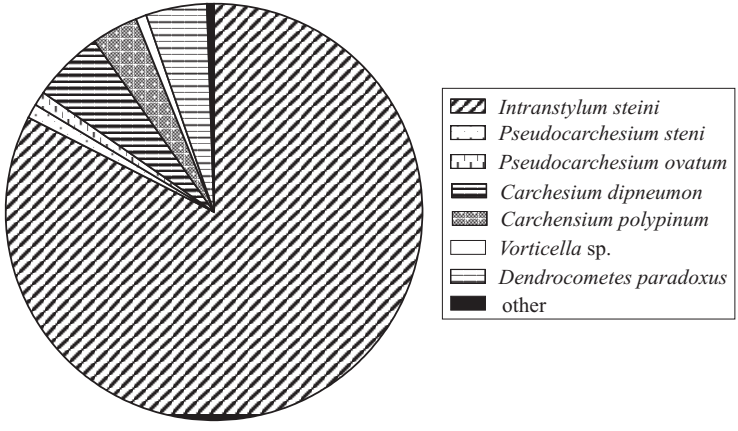


Fig. 1. Domination structure of epizoic ciliates inhabing gill lamellae of *Gammarus fossarum* Koch. on sampling sites in stream Słupsk city

Table 2

Chemical characteristic and water quality of the sample sites in 2001

| Parameter | | Sample site | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| $O_2$ – concent | ($mg \cdot dm^{-3}$) | 5.45 ± 4.12 | 5.07 ± 4.87 | 6.03 ± 5.55 |
| $BOD_5$ | ($mg\ O_2 \cdot dm^{-3}$) | 5.48 ± 6.02 | 4.55 ± 5.87 | 4.35 ± 6.31 |
| $N_{total}$ | ($mg\ N \cdot dm^{-3}$) | 2.94 ± 3.33 | 3.02 ± 3.98 | 2.81 ± 4.51 |
| $P_{total}$ | ($mg\ P \cdot dm^{-3}$) | 0.37 ± 0.44 | 0.42 ± 0.99 | 0.31 ± 0.45 |
| $P\text{-}PO_4$ | ($mg\ P \cdot dm^{-3}$) | 0.18 ± 0.22 | 0.16 ± 0.45 | 0.11 ± 0.25 |
| $N\text{-}NH_4$ | ($mg\ N \cdot dm^{-3}$) | 0.23 ± 0.35 | 0.16 ± 0.42 | 0.16 ± 0.30 |
| $N\text{-}NO_3$ | ($mg\ N \cdot dm^{-3}$) | 1.29 ± 2.36 | 1.37 ± 3.00 | 1.12 ± 2.11 |
| Water quality | | II-III | II-III | I-II |

The highest number of epizoic ciliates was found on site no. 1 – 3778 zooids ($\bar{x}$ = 209.9 zooids carrier$^{-1}$), where 6 taxa were found (Table 1). The highest number of epizoic ciliates was also found on the gills of beach fleas of 13 mm in body length. They appear on nearly all examined beach fleas. In the sampling site 1 in the *Gammarus* notable domination *Intranstylum steni* ($D$ = 90.1%). Its number establish to 23 from 712 zooids, and highest density gain in gammarids length 13-14 mm. Subdominant, though in notable low density in gills *Gammarus* was *Dendrocometes paradoxus* ($D$ = 6.3%). Range numbers that;s species carried out 1-82 zooids, and highest value abundance establish in carriers of length body 15 mm. Proportional participation remaining epizoa (*Pseudocarchesium, Carchesium, Vorticella* and *Spirochona*) in inhabiting gills gammarids of this sampling site, carried out only to 0.03 from 2.5%. The species inhabiting gills *Gammarus* max. from 13 mm length.

The value of Shannon-Wiener's diversity index for the epizoic ciliates inhabiting the amphipods' gills was $H'$ = 0.6 on site 1, which was the lowest of the studied sites (Table 1). Using the *t*-Student test on the $\alpha$ = 0.05 level, there are no statistically essential differences between the epizoic density of amphipods gills on sites 1 and 2. When comparing obtained average values on sites 1 and 3, the Cochran and Cox test was introduced (variations varied significantly), what also endorsed that they are not substantial on the $\alpha$ = 0.05 level (chart 1). It must be admitted that tests come from the same general population.

On site no. 2, a total of 995 zooids ($\bar{x}$ = 199 zooids carrier$^{-1}$) were found represented by 8 epizoic taxons. The most ciliates, both quantitatively and qualitatively, inhabited the gills of beach fleas of 12 mm in body length. These were again largely dominated by *I. steni* ($D$ = 89.2%); this is a typical species for the examined substrate, $F_r$ = 100%. The species achieves large numbers ranging between 40 and 286 zooids, with the highest densities of *I. steni* found on the gills of 12- and 14-mm-long beach fleas. *Carchesium polypinum* was

a subdominant species at this site ($D$ = 9.7%), which its number remaining within a narrow range 13 to 33 zooids and its maximum on the gills of 14-mm beach fleas. The percentage of each of the remaining taxons was very low, 0.2 to 0.6%.

Shannon-Wiener diversity index for the epizoic ciliates inhabiting *Gammarus* was $H'$ = 0.55 on site 2, which is the lowest value of the index recorded during the studies. Statistical analysis was executed (Cochran and Cox's test) on signification level $\alpha$ = 0.05 didn't prove any important differences of densities between of different part of sampling site 2 and 3 (Table 1).

Gill lamellae of *Gammarus fossarum* on site no. 3 were the poorest in terms of epizoic quantitative colonisation 356 zooids ($\bar{x}$ = 71.2 zooids carrier$^{-1}$). On the gills of the beach flea on this site, on the other hand, the highest number of taxa was found (10). The dominant also on this site *Intranstylum steni* achieved the lowest densities, which ranged between 5 – 73 zooids ($\bar{x}$ = 29.2 zooids carrier$^{-1}$) and its percentage in the epizoic fauna was as low as 41% (Table 1). The subdominants in this sampling site were three taxa: *Carchesium dipneumon* ($D$ = 28.1%), *Pseudocarchesium steni* ($D$ = 14%) and *D. paradoxus* ($D$ = 13.2%). The proportional participation others taxons epizoic ciliates are low and ranged from 0.8 to 2.2% (Table 1). Another sedentary ciliate species *Zoothamnium gammari* appeared also on site 3, which achieved low numbers and density ($\bar{x}$ = 0.4 zooids carrier$^{-1}$).

Shannon-Wiener's diversity index was $H'$ = 2.05, which is nearly 3-fold compared with that found on site 1 (Table 1).

The presence of a certain number of different species did not correspond to physico-chemical data. Although, in site 1 (with the highest load of $BOD_5$ and lowest $O_2$) only 6 species and in site 2 and 3 (with the lowest $BOD_5$ and highest $O_2$ load) 8 and 10 species could be identified, no relation can be concluded. The total abundance of epizoic ciliates, however, appears to be related to a significant degree of the water quality: The average amount of zooids per gammarid carrier (zooid gammarid$^{-1}$) was calculated as 71 (class I/II) and 205 (class II/III) (Table 1). Consequently, it can easily be related to the $O_2$ – supply and the biochemical $O_2$ requirement (Table 2). Thus the rate of individuals of epizoic ciliates species could be used as a rough indicator of organic pollution, whereas the amount of ciliate species itself would not be suitable as indicator sufficiently.

In addition to the dynamics of a population in water of a certain quality, the frequency of a species is a suitable parameter to describe. Table 3 shows the mean annual frequency of the *Gammarus fossarum* epizoa. In particular, *Pseudocarchesium steni*, *P. ovatum*, *P.* sp., *Spirochona gemmipara* and *Dendrocometes paradoxus* demonstrate a relationship with certain classes of the water quality. The frequence of domination species – *Intranstylum steini* – is

constant in all levels of saprobity. Frequencies of these ciliates are high even in water with low nutrition supply. So, their capacity to tolerate a certain organic load of the water can only be detected by calculating their abundances (Table 1).

Table 3
Comparison of mean density of ciliata epizoa inhabiting *Gammarus* in Polish and German stream

| Localization | Poznań<br>PIESIK 1975 | Szczecin<br>PIESIK, PIESIK 1978 | Bielefeld<br>MANNESMANN, RUSTIGE 1994 | Słupsk<br>Authors data |
|---|---|---|---|---|
| Carrier | *Rivulogammarus* Karaman | *Gammarus* Fabr. | *Gammarus pulex* L. | *Gammarus fossarum* Koch. |
| Range abundance (zooid carrier$^{-1}$) | 28 – 1333<br>$n = 5$ | 29 – 468<br>$n = 5$ | 255 – 1031<br>$n = 5$ | 71 – 210<br>$n = 3$ |
| Abundance min. – max. (zooid carrier$^{-1}$) | 1 – 629 | 1 – 139 | 1 – 667 | 1 – 712 |
| $\bar{x}$ – mean abundance (zooid carrier$^{-1}$) | 120.5<br>$n = 49$ | 184.2<br>$n = 23$ | 540.4<br>$n = 72$ | 160.0<br>$n = 60$ |
| SD | 275.1 | 333.7 | 611.1 | 805.4 |
| Median (Me) | 30.0 | 32.0 | 37.5 | 38.5 |
| Cochran, Cox test | Poznań – Szczecin<br>$C < C_{\alpha(0.05)} = 0.883 < 2.363$ | | | |
| | | | Bielefeld – Słupsk<br>$C > C_{\alpha(0.05)} = 3.253 > 2.363$ | |
| | | Szczecin – Bielefeld<br>$C > C_{\alpha(0.05)} = 2.483 > 2.363$ | | |
| | Poznań – Bielefeld<br>$C > C_{\alpha(0.05)} = 2.483 > 1.068$ | | | |
| | | Szczecin – Słupsk<br>$C < C_{\alpha(0.05)} = 1.104 < 1.770$ | | |
| | Poznań – Słupsk<br>$C < C_{\alpha(0.05)} = 0.987 < 2.555$ | | | |

## Discussion

Occurrence and geographical distribution of *Gammarus* sp. – especially of *G. fossorum* (which primarily analysed species by SCHÖDEL 1987) – in lotic water systems limits the value of its epizoic ciliates as indicators of water pollution. It lives in water of saprobic levels from oligosaprob to $\alpha$-mesosaprob (classes between I and III) (DORGELO 1977, JEAN, FRUGET 1994, MUSKÓ et al. 1990). The main physiological requirements are minimum $O_2$ – concent of 4.0 mg $\cdot$ dm$^{-3}$ and a pH-range from 7.6 to 5.1 (SCHLEITER et al. 1999).

Species, such as *Pseudocarachesium steni*, *Lagenophrys ampulla*, *Dendrocometes paradoxus* and *Spirochona gemmipora* can be isolated from different gammarid species which live in freshwater systems in many parts of Europe and even in the Bajkal Lake (MANNESMANN, RUSTIGE 1994). There is need for more information about the distribution of epizoic species on different carrier animal and also about their geographical distribution. Our investigation on *Gammarus fossarum* as carrier showed a similar situation of relationship between the frequency, abundance and biodiversity index of epizoic ciliates and water quality as was found with *Gammarus pulex* and *Asellus aquaticus* (MANNESMANN, RUSTIGE 1994, SCHÖDEL 1987). Furthermore, it can also be demonstrated than the carrier related of symphoriont settling show even carriertopographic specifica which could be used as parameters of water analysis. In particular, epizoics of the gills (*Pseudocarchesium steini*, *Spirochona gemmipara* and *Dendrocometes paradoxus*) show clear relation in presence frequency or abundance to certain saprobic loves.

On the gill lamellae of beach fleas living in the streams of Słupsk, 10 epizoic ciliate species were found. Those representing the class Peritricha were dominant. Suctoria or Holotricha ciliates were found in much lower numbers. This is typical for epiphytic ciliates inhabiting (PIESIK 1975, 1976 a, b). All examined beach fleas were colonised by epizoic ciliates. Comparing the epizoic colonisation of *Gammarus* in the vicinities of Poznań or Szczecin (PIESIK 1976a, PIESIK, PIESIK 1978), the density of epizoic ciliates colonisation on the examined beach fleas was similar. However, the density of epizoic ciliates in Słupsk streams are nearly 3-fold lower than the data reported in the Johannisbach River near Bilefeld (Table 3). It is confirmed by statistic analysis carried out with the help of the Cochran, Cox test. It showed that the epizoic density of beach fleas in the streams of Poland do not differ statistically (essence level of 0.05), but there are significant differences in comparison with streams of Bielefeld.

Little is known about abundances of gammarid epizoa. Also, we have little information about their levels of constancy. Colonisation of carriers by epifauna is determined by a number of factors, the most important of which are morphological structure of the carrier, biological factors, hydrodynamic conditions, temperature, and water physicochemical properties (BREHM, MEIJERING 1982, DORGELO 1977, JEAN, FRUGET 1994, MUSKÓ et al. 1990). Depending on the habitat of the carrier, epizoa may inhabit various parts of its body (THIENEMANN 1925). Also the structure of the carrier, particularly the character of its surface, provide various opportunities to settle. Epizoic ciliates seldom colonise oligochaetes or nematodes, which have smooth body integument, opposite to polychaetes, whose parapodia provide more protection (PRECHT 1935). The area and size of the carrier are also very important

(ALBRECHT 1983, 1986, PIESIK, PIESIK 1978, SLADECEK 1973). The largest numbers of epizoic organisms have been found on the appendages, gills, and coxal plates. The remaining parts of the carrier's body are colonised to a much lesser extent. However, first detailed data on frequency and abundance of symphorionts in comparison to different saprobic loves of water showed that they can be used as suitable criteria to describe organic loads of water (SCHÖDEL 1987, RUSTIGE 1990, RUSTIGE, MANNESMANN 1991). So, the frequencies of *Epistylis sommerae*, *Lagenophrys ampulla*, *Spirochona gemmipara* and *Dendrocometes paradoxus* is related to certain, degress of water pollution, whereas species such as *Carchesium dipneumon*, *Pseudocarchesium steini* and *Zoothamnium affine* do not correspond with different water qualities. As can be seen, the density of the ciliate population of *G. fossarum* depends on the saprobic level. The number of individuals increases with increasing organic load. Unfortunately, our data take account of only a range of water quality between classes I/II and III. However, it cannot be concluded yet, that this tendency also includes the classes I and IV. Conditions in these classes differ largely from II and III. Abundance of those species which could be identified (Table 1) shows rather different individual attributes of the relation zooid amount per carrier/water quality. Some species do not follow the above patterns. It could be concluded, that possible interactions between epizoic species on the same carrier animal influence the abundance of several species. Others are more or less eurypotent and do not show distinct preference for a certain quality level of water. In particular the species *Epistylis anastatica*, *Carchesium polypinum* and *Zoothamnium gammari* show a relation in presence, frequency and abundance to certain saprobic degrees. They can be used as indicator species to characterize the $\alpha$-$\beta$-mesosaprobic and h-mesosaprobic levels of lotic water systems.

Mobility and movements of the carrier influence the presence of epizoa and the shape of their zooids, as well as the length, thickness, and rigidity of their stalk (NENNINGER 1948). Constant water supply, rich in oxygen and nutrients, represents another key factor (RUSTIGE, MANNESMANN 1991, 1994, SCHÖDEL 1987). The specific environment of gill lamellae is favourable for the species that have strong oxygen demand and are sensitive to temperature changes. This results in the fact that such habitats of the beach fleas are inhabited by characteristic species (*Dendrocometes paradoxus*, *Spirochona gemmipora* and *Intrastylum steni*). Other epifauna are accidentally present. Zooids of the species occurring on gill lamellae are oval in shape, small in size, and rigid stalk of varied shape. If the carrier moves abruptly, the ciliate contracts a bit and shuts the peristome; thus, the shape of the zooid becomes more streamline and less susceptible to water action. The species of the class Suctoria (*Dendrocometes paradoxus*) represent another type of adaptation to such a specific

micro-environment. This ciliate has dorsally flattened body, tightly adhering to the substrate with a wide, flat "foot" – a typical form for external parasites (RAABE 1972). Due to this feature, the zooids are strongly and safely attached to the substrate.

Analysing species diversity indices, it appears that on site 3, located in the channel which is situated the furthest from strongly eutrophicated ponds; Shannon-Wiener's indices were the highest. This confirms the fact that numerous epizoic species of ciliates develop abundantly at constant nutrient supply; this also confirms that the most demanding species of this group require adequate physicochemical water regime for their existence. The sites where sewage come with a big amount of biogens, so exposed to the human impact, were characterised by lowest values of biodiversity indices – typical of heavy pollution water (GUHL 1987). Further studies may enlarge the set of species which could be used as indicators of water quality.

Despite the distinct drop of the epizoic density of amphipods gills on respective sites, the obtained averages are not significant on the $\alpha = 0.05$ level, what was confirmed by the implemented essence tests.

## Conclusions and Statements

1. Quantitative colonisation by epizoic ciliates on the beach fleas in streams near Poznań, Szczecin, and Słupsk is similar, unlike near Bielefeld, where it was much higher, what is proved by the statistic analysis**.**

2. The density of the ciliate population of *G. fossarum* depends on the saprobic level, but not significant for statistical meaning.

3. The frequencies of *Epistylis sommerae*, *Lagenophrys ampulla*, *Spirochona gemmipara* and *Dendrocometes paradoxus* is related to certain, degree of water pollution, whereas species such as *Carchesium dipneumon*, *Pseudocarchesium steini* and *Zoothamnium affine* do not correspond with different water qualities.

4. In particular the species *Epistylis anastatica*, *Carchesium polypinum* and *Zoothamnium gammari* show a relation in presence, frequency and abundance to certain saprobic degrees.

Translated by PIOTR BŁASZCZYK

Accepted for print 2.02.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 403-412, Y. 2006

# PHOSPHORUS COMPOUNDS REMOVAL IN A ROTATING ELECTRO-BIOLOGICAL CONTACTOR

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K e y w o r d s: waste waters, rotating biological contactors, phosphorus, electric current, steel electrode.

A b s t r a c t

The study was aimed at determining the impact of electric current passage on the removal efficiency of phosphorus compounds. Experiments were carried in a bench scale in a rotating biological contactor under conventional conditions (without electric current) and under conditions with the passage of electric current referred to as a rotating electro-biological contactor (REBC). The following current intensities were applied in the study: 0.1 A, 0.5 A and 0.9 A (current density: 1.5 $A \cdot m^{-2}$, 7.7 $A \cdot m^{-2}$ and 13.9 $A \cdot m^{-2}$, respectively). The experiment demonstrated the highest removal efficiency of phosphorus at the passage of electric current with the intensities of 0.5 A and 0.9 A (current density: 7.7 $A \cdot m^{-2}$ and 13.9 $A \cdot m^{-2}$, respectively), at which the efficiency of dephosphatation exceeded 99%.

## USUWANIE ZWIĄZKÓW FOSFORU NA ELEKTROBIOLOGICZNYM ZŁOŻU TARCZOWYM

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Abstrakt

Celem badań było określenie wpływu przepływu prądu elektrycznego na sprawność usuwania związków fosforu. Badania prowadzono w skali laboratoryjnej na złożu tarczowym (RBC) w warunkach konwencjonalnych, gdy nie przepływał prąd elektryczny, oraz w warunkach przepływu prądu elektrycznego, dla których przyjęto nazwę elektrobiologicznego złoża tarczowego (REBC). Natężenie prądu w doświadczeniu wynosiło: 0,1 A; 0,5 A; 0,9 A ( gęstość odpowiednio: 1,5 $A \cdot m^{-2}$; 7,7 $A \cdot m^{-2}$; 13,9 $A \cdot m^{-2}$). Badania wykazały, że najefektywniej proces usuwania fosforu przebiegał wówczas, gdy natężenie przepływającego prądu elektrycznego wynosiło 0,5 A i 0,9 A (gęstość odpowiednio: 7,7 $A \cdot m^{-2}$; 13,9 $A \cdot m^{-2}$). Osiągnięto ponad 99% sprawność defosfatacji.

## Introduction

The most common method of phosphorus removal is its biological treatment with the use of bacteria, referred to as phosphorus accumulating organisms (PAO), that are capable of absorbing and accumulating high amounts of phosphorus compounds (ARNZ et al. 2001, BRANDT et al. 2002, FALKENTOFT et al. 1999, HELNESS, ØDEGAARD 2001). Other extensively applied methods include those utilizing the processes of simultaneous removal of phosphorus that consist in introducing aluminum or iron salts and lime to activated sludge chambers (CLARK et al. 1997, MORSE et al. 1998). Compared to the biological methods, the chemical removal of phosphorus is a reliable process less susceptible to disturbances. AGUILAR et al. (2002) obtained nearly 100% efficiency of phosphorus removal in the coagulation – flocculation process. Slightly lower efficiency values were reported by CLARK et al. (1997) who used iron sulfate (III) as a coagulating agent. They observed 85.3% efficiency of phosphorus removal at 35.5% efficiency of simultaneous biological dephosphatation.

The chemical precipitation of phosphorus results in increased amounts of generated sludge. The implementation of more and more strict regulations limiting the amounts of phosphorus discharged to receiving water necessitates searching for more efficient methods of waste water treatment, including processes of crystallization, ionic exchange, magnetic attraction or adsorption (MORSE et al. 1998). Another example is the utilization of phenomena that occur during electric current passage in waste waters. Benefits of that process may be observed on the example of electrocoagulation which is characterized by a lower acidity of sewage compared to the chemical methods.

Under conditions of electric current passage between two electrodes, a coagulating agent is precipitated as a result of electrolytic oxidation of anode material. The best material for anode construction is iron or aluminum that transfer to a solution in the form of trivalent ions, whereas most of other cheap and easily available materials are observed to transfer to a solution in the form

of bivalent ions. The trivalent ions display a greater capacity for adsorption on molecules in treated waste waters than the bivalent ones, as they are characterized by a higher density of charges (KORE, SYVERSEN 1995). Still, aluminum is usually applied for the treatment of water, whereas iron – for the treatment of sewage (CHEN 2004).

In the case of an iron anode, Fe(OH)$n$ is produced at $n = 2$ or 3, which is consistent with the following mechanisms (LARUE et al. 2003):
(a) mechanism 1
anode:

$$Fe_{(s)} \rightarrow Fe^{2+}_{(aq)} + 2e^-$$
$$Fe^{2+}_{(aq)} + 2OH^-_{(aq)} \rightarrow Fe(OH)_{2(s)}$$

cathode:

$$2H_2O_{(l)} + 2e^- \rightarrow 2OH^-_{(aq)} + H_{2(s)}$$

total:

$$Fe_{(s)} + 2H_2O_{(l)} \rightarrow Fe(OH)_{2(s)} + H_{2(g)}$$

(b) mechanism 2:
anode:

$$4Fe_{(s)} \rightarrow 4Fe^{2+}_{(aq)} + 8e^-$$
$$4Fe^{2+}_{(aq)} + 10H_2O_{(l)} + O_{2(g)} \rightarrow 4Fe(OH)_{3(s)} + 8H^+$$

cathode:

$$8H^+_{(aq)} + 8e^- \rightarrow 4H_{2(g)}$$

total:

$$4Fe_{(s)} + 10H_2O_{(l)} + O_{2(g)} \rightarrow 4Fe(OH)_{3(s)} + 4H_{2(g)}$$

The formation of iron hydroxides results from the concentration of colloids (usually negatively charged) in the vicinity of anode. Molecules react with an iron hydroxide and are removed as a result of the surface formation of agglomerates or electrostatic activity. In the coagulation process, an electric field is created that, instead of mechanical mixing, sets into motion the smallest molecules, which in turn facilitates coagulation. A higher concentration of iron hydroxide in the vicinity of anode increases the probability of coagulation (LARUE et al. 2003).

The weight of a substance separated on one of the electrodes is determined by the electric charge of an electrolyte passed through a solution. Quantitative calculations of the separation processes of a substance on an electrode are based on the electrolysis equation which combines two laws of Faraday (MOLLAH et al. 2004):

$$m_e = \frac{I \cdot t \cdot M}{n \cdot \mathrm{F}},$$

where:
$m_e$ – weight of the separated substance (g),
$I$ – electric current intensity (A),
$M$ – molecular mass ($g \cdot mol^{-1}$),
$t$ – time of electrolysis (s),
$n$ – number of electrons in the redox reaction,
$F$ – Faraday's, (96 500 $C \cdot mol^{-1}$).

The theoretically-determined weight of the substance separated upon electric current passage is usually equal to that assayed experimentally. The quantity of iron released in the electrocoagulation process is estimated to reach 1041 $mg \cdot A^{-1} \cdot h^{-1}$. It should be emphasized, however, that increasing the electric charge of an electrolyte running through a solution may lead to losses of electrical energy consumed for heating the solution (CHEN 2004).

The electrocoagulation process enables the removal of over 98% of phosphorus (GRØTERUD, SMOCZYŃSKI 1986). In addition, increased elimination of organic compounds is observed (a decline in COD value), and the sludge generated is characterized by a lower content of water (JIANG et al. 2002). SAKAKIBARA, NAKAJIMA (2002) postulated the application of the electrolytic process for the removal and retrieval of phosphorus from waste waters. In their experiment, they used direct current to coagulate phosphates on an anode and to precipitate heavy metal ions on a cathode. This enabled obtaining relatively pure phosphates, less contaminated with heavy metals, which cannot be achieved upon chemical or biological methods of phosphorus removal. Apart from electrocoagulation, other beneficial phenomena are observed to occur at the passage of electric current, including electrofloatation with the use of gaseous hydrogen produced on the cathode, the bubbles of which hold and float pollutions suspended in waste waters (HOLT et al. 2002).

In spite of the fact that efficiencies of phosphorus compounds removal in the electrocoagulation process are comparable with those obtained in the processes of chemical dephosphatation, the electrocoagulation is more favorable as it results in lower amounts of sludge generated (LARUE et al. 2003). The sludge floccules formed are large and dense, and are characterized by higher settling velocities and are more readily dehydrated. In contrast, the chemical process of metal salts dosage is characterized by higher amounts of the sludge generated whose floccules demonstrate an open structure with a high water content and low density, which in turn determines a low velocity of their settling. Therefore, the chemical removal of phosphorus compounds often necessitates the dosage of organic polymers so as to improve the sedimentative properties of the sludge. What is more, the electrocoagulation process enables the removal and retrieval of relatively pure phosphorus compounds that

coagulate on the anode's surface, and cations of heavy metals potentially occurring in waste waters deposit on the cathode's surface (SAKAKIBARA, NAKAJIMA 2002). Apart from electrocoagulation, denitrification is likely to proceed in a reactor, which consists in the simultaneous removal of phosphorus and nitrogen compounds at a high efficiency (KRZEMIENIEWSKI, RODZIEWICZ 2005).

The efficiency of the electrobiological method in waste water treatment was determined in a study carried out on biological tape filter subjected to the activity of electric current (KRZEMIENIEWSKI 1989, JANCZUKOWICZ et al. 2001). In the systems analyzed, anode was made of steel or aluminum sheet located at the bottom of the tank, whereas cathode was constituted by a lower shaft made of stainless steel submerged in waste waters and enabling tape shift.

Research was, hence, undertaken to combine the electrochemical processes proceeding at electric current passage in the sewage medium with the biological processes occurring in a rotating contactor. In such a system, discs made of stainless steel with immobilized biological membrane serve as the cathode, whereas an electrode made of stainless steel and submerged in the overflow chamber of the contactor – as the anode. Technological design assumed that electric current passage will results in the electrocoagulation process of waste waters, namely the anode will be dissolved and iron ions generated upon hydrolysis will form precipitating hydroxides which, in turn, will adsorb phosphorus.

## Goal and scope of investigation

The study was aimed at determining the impact of electric current passage on the removal efficiency of phosphorus compounds from waste waters in a rotating biological contactor.

# Materials and Methods

The examinations were carried out with synthetic waste waters prepared according to Weinberger (PN-87/C – 04616/10) with the proximate composition as shown in Table 1 and values of typical pollution indicators as shown in Table 2.

Investigations were conducted in a rotating electro-biological contactor (REBC) – Figure 1. The contactor consisted of four sections. Each section was made of sets of discs mounted concentrically on a horizontal shaft, 0.42-m long. Each of four disc-sections consisted of 8 discs with 0.22-m diameter. Discs in

Table 1

Composition of synthetic waste waters

| Component | Weighed sample ($mg \cdot dm^{-3}$) |
|---|---|
| Enriched dry broth | 150 |
| Peptone | 50 |
| Urea ($NH_2CO \cdot NH_2$) pure | 30 |
| Anhydrous sodium acetate ($CH_3COONa$) pure | 10 |
| Soluble starch ($C_6HO_5$) pure | 50 |
| Green soap | 50 |
| Crystalline calcium chloride ($CaCl_2 \cdot 2H_2O$) analytically pure | 7 |
| Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) pure | 50 |
| Sodium chloride (NaCl) pure | 30 |
| Potassium chloride (KCl) analytically pure | 7 |

Table 2

Pollution indicators in the synthetic waste waters

| Pollution inidcators | Mean value |
|---|---|
| COD ($mg\ COD \cdot dm^{-3}$) | 487.6 |
| $BOD_5$ ($mg\ BOD_5 \cdot dm^{-3}$) | 332.0 |
| Total nitrogen ($mg\ N_{tot} \cdot dm^{-3}$) | 60.2 |
| Ammonia nitrogen ($mg\ NH_4\text{-}N \cdot dm^{-3}$) | 15.7 |
| Nitrate nitrogen ($mg\ NO^{3-}\text{-}N \cdot dm^{-3}$) | 10.4 |
| Total phosphorus ($mg\ P_{tot} \cdot dm^{-3}$) | 13.4 |
| Orthophosphates ($mg\ P \cdot dm^{-3}$) | 10.9 |

the sections were made of stainless steel. The total active surface of the contactor equaled to 0.6 $m^2$. Each section was placed in a half-round tank with 2 $dm^3$ volume. The discs rotating with a speed of 60 rpm were moved by an electric motor. In the flow-tanks, electrodes made of stainless steel were mounted and connected with insulated wires to a rectifier providing the required current intensity. The REBC worked at disc surface loading with organic wastes reaching 6.5 ($g\ COD \cdot m^2 \cdot d^{-1}$) and hydraulic loading of 0.01 ($m^3 \cdot m^{-2} \cdot d^{-1}$).

Experiments were carried in a rotating biological contactor under conventional conditions (without electric current) and with the passage of electric current at the following intensities: 0.1 A, 0.5 A and 0.9 A (which corresponded to current densities of: 1.5 $A \cdot m^{-2}$, 7.7 $A \cdot m^{-2}$ and 13.9 $A \cdot m^{-2}$).

The inflowing and treated waste waters were determined for the concentrations of organic compounds expressed as COD (bichromate method), orthophosphates (colorimetric method), and total phosphorus as well as pH, temperature and electrolytic conductivity.

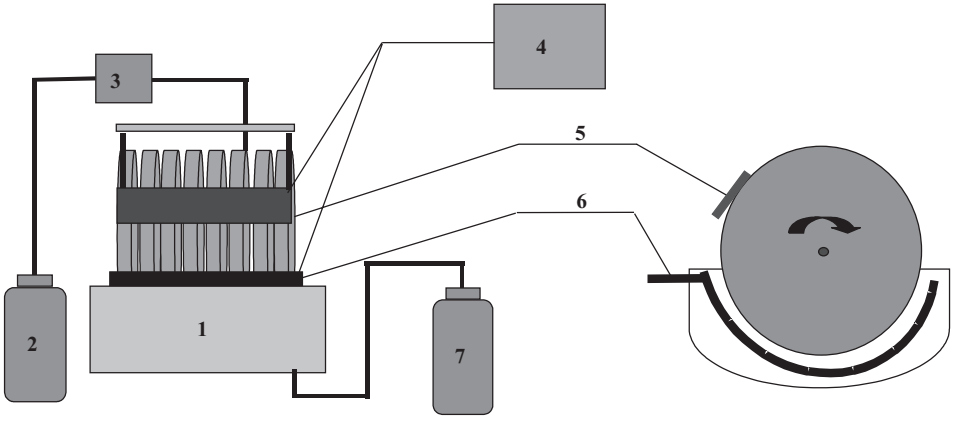


Fig. 1. The scheme of an experimental post: 1 – rotating electro-biological contactor, 2 – tank with untreated waste water, 3 – peristaltic pump, 4 – electric current source, 5 – cathode, 6 – anode, 7 – tank with treated waste water

## Results and Discussion

In each system analyzed, the efficiency of organic compounds removal was high and no effect of the electric current passage was observed on the size of organic loading removed (over 90% efficiency of removal was obtained in all treatment systems).

The removal efficiency of total phosphorus and orthophosphates in synthetic waste waters was found to depend on the density of electric current. In the systems with electric current passage at densities of 0.8 $A \cdot m^{-2}$ and 1.5 $A \cdot m^{-2}$ phosphorus compounds were removed with the highest efficiency. In those systems, neither orthophosphates nor total phosphorus were detected in the treated waste waters. The lowest removal efficiency of phosphorus compounds was reported for a system without the passage of electric current. Figure 2 presents the efficiency of phosphorus compounds removal. Over 99% efficiency was reached in systems with electric current passage at densities of 0.8 $A \cdot m^{-2}$ and 1.5 $A \cdot m^{-2}$. At electric current density of 0.2 $A \cdot m^{-2}$, the removal efficiency of total phosphorus was observed to reach over 96%, whereas that of orthophosphates – over 98%. In the conventional system, the removal efficiencies of phosphorus compounds were lower and accounted for 55.5% and 58.3% for total phosphorus and orthophosphates, respectively.

The results obtained in this study were similar to those reported by GRØTERUD, SMOCZYNSKI (1986) and higher than those described by JANCZUKOWICZ et al. (1993) who observed 87.5% efficiency of total phosphorus

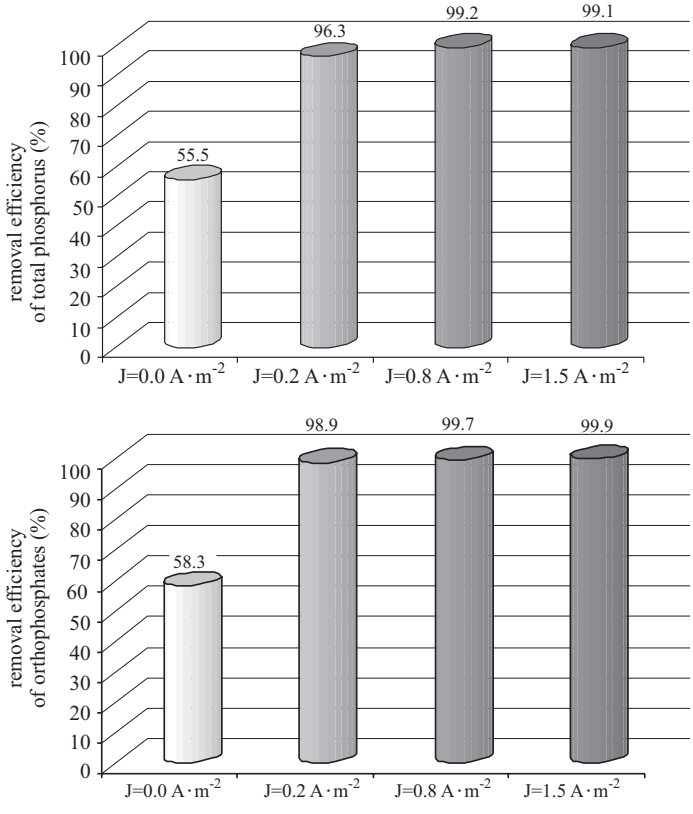


Fig. 2. The efficiency of phosphorus compounds removal in synthetic waste waters

removal in rotating contactors with simultaneous precipitation of phosphorus compounds with a 5% solution of aluminum sulfate.

The removal efficiency of phosphorus compounds in rotating contactors was higher than that on biological tape filters (JANCZUKOWICZ et al. 2001). Under conditions of electric current passage at the intensity of 0.15 A (density of 1.4 $A \cdot m^{-2}$), the removal efficiency was observed to reach 63% for orthophosphates and 47% for total phosphorus. Increasing the intensity of electric current to 0.3 A (density of 2.7 $A \cdot m^{-2}$) resulted in the augmentation of the removal efficiency to 72.4% in the case of orthophosphates and to 59% in the case of total phosphorus. In the system without electric current, the removal efficiency appeared to be the lowest, i.e. 35% for orthophosphates and 11% for total phosphorus.

Increasing the intensity of electric current led to shortening the time necessary for complete removal of pollutions, including phosphorus com-

pounds (HOLT et al. 2002, KRZEMIENIEWSKI 1989). In addition, it did not contribute to elevated consumption of electric energy. Based on results obtained by LI et al. (1996), who investigated the efficiency of electrochemical removal of nitrogen compounds from aqueous solution, it may be even supposed that the consumption of electric energy would be lower for higher intensities of electric current. They reported that increasing current intensity from 1A to 2A results in higher energy consumption, however the consumption of energy is observed to decline with increasing intensity of electric current due to a rapid shortening of time required for complete removal of nitrites.

## Conclusions

1. The experiment confirmed the usability of the electrobiological method for the removal of phosphorus compounds from waste waters.

2. Increased intensity of electric current results in a higher efficiency of phosphorus compounds removal.

3. Over 99% dephosphatation efficiency was reached with electric current passage at the intensity of 0.5 A and 0.9 A (density of 7.7 $A \cdot m^{-2}$ and 13.9 $A \cdot m^{-2}$, respectively).

4. Electrodes made of stainless steel were found useful in the removal of phosphorus compounds from waste waters.

Translated by JOANNA RODZIEWICZ

Accepted for print 17.03.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 413-422, Y. 2006

# EFFECT OF THE STRUCTURE OF PRODUCTS OF PARTIAL PROTEOLYSIS OF $\beta$-CASEIN ON THEIR EMULSIFYING PROPERTIES*

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K e y w o r d s: $\beta$-casein, emulsifying properties, peptides, proteolysis, structure.

A b s t r a c t

Peptides, as products of protein proteolysis, are characterized by different structural and functional properties, including emulsifying ones. The possibility to predict changes in the emulsifying properties of peptides and proteins based on their molecular characteristics helps to use these products in optimal manner for special-purpose foods, including hypoallergenic foods. The emulsifying properties of peptides determined by their structure and environmental factors were discussed on the example of $\beta$-casein. The factors enhancing the emulsifying properties of peptides are their minimum molecular weight, the amphiphilicity of their polypeptide structures, and the presence of $\alpha$-helix structure.

## WPŁYW STRUKTURY PRODUKTÓW CZĘŚCIOWEJ PROTEOLIZY KAZEINY-$\beta$ NA ICH WŁAŚCIWOŚCI EMULGUJĄCE

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S ł o w a k l u c z o w e: kazeina-$\beta$, peptydy, proteoliza, struktura, właściwości emulgujące.

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* This work was financially supported by own funds of UWM within the project: 522-0712-0203.

Abstrakt

Peptydy jako produkty proteolizy białek charakteryzują się odmiennymi cechami strukturalnymi i funkcjonalnymi, w tym emulgującymi. Możliwość przewidywania kształtowania się właściwości emulgujących peptydów i białek na podstawie znajomości ich charakterystyki molekularnej pomaga w optymalnym ich wykorzystaniu do wytwarzania żywności specjalnego przeznaczenia, w tym żywności hipoalergicznej. Na przykładzie kazeiny-$\beta$ omówiono właściwości emulgujące peptydów determinowane ich strukturą oraz czynnikami środowiskowymi. Czynnikami sprzyjającymi występowaniu korzystnych właściwości emulgujących peptydów są: ich minimalna masa cząsteczkowa, amfifilowość struktur polipeptydowych oraz obecność struktury $\alpha$-helikalnej.

## Emusifying properties of proteins

Due to their surface-active properties, proteins are enumerated amongst food emulsifiers next to such constituents of commercial surfactant preparations as monoacylglycerols and their derivatives, sorbitan derivatives, esters of polyglycerol and saccharose or lecithin (JOHANSSON, SVENSSON 2001, SÖDERMAN, JOHANSSON 1999, DALGLEISH 2006). For instance, myosin and purposefully added soybean proteins emulsify fat during meat chopping for sausages, whereas in mayonnaise the emulsifying agents are lipoproteins of the egg yolk. The capacity for the formation and stabilization of emulsions may be enhanced through increasing the acidity of the environment, e.g. through the addition of vinegar or lemon juice (DALGLEISH 2006). An improvement in the emulsifying properties of lipoproteins is linked to an increase in surface hydrophobicity and elasticity of their polypeptide chains (KIOSSEOGLOU 2003). The basic, though not only, difference between proteins and the other emulsifiers refers to their molecular weight that ranges from e.g. ten or so to several hundred kDa for proteins and several hundred Da for monoacylglycerols.

Proteins are likely to facilitate the formation of emulsion of non-mixing liquids owing to their capacity for: reducing surface tension, adsorption at oil/water phases interfaces, and formation of a cohesive layer around oil droplets (WALSTRA, DE ROSS 1993). An important issue to be discussed is differentiation of the formation and stabilization processes of emulsions (DALGLEISH 2006, DAREWICZ et at. 2000a,b, WALSTRA, DE ROSS 1993). The presence of an agent reducing surface tensions and certain energy input necessary for oil droplet formation are acknowledged as the basic factors in the processes of emulsion forming (MILLER et al. 2004). During mixing of oil and aqueous solutions of proteins their contact tends to be limited. Emulsions formed with the use of proteins provide minimal contact of hydrophobic oil droplets with the hydrophilic environment. An intentionally-supplied protein agent (emulsifier) acts not only during the formation, but also during the stabilization of a newly formed interface. Stability of the emulsion formed is

not an unchangeable phenomenon. Drainage, aggregation and coalescence are processes that may proceed while reducing emulsion stability (DALGLEISH 2006, DAREWICZ, DZIUBA 2005, VAN AKEN et al. 2003).

## Impact of limited proteolysis on the emulsifying properties of food protein hydrolyzates

Food proteins are not always characterized by such emulsifying properties that would fully satisfy the expectations of food producers and consumers. One of the methods used for modeling and improving those properties may be limited protein hydrolysis applied to obtain peptides with anenhanced capacity for forming and stabilizing emulsions (DAREWICZ et al. 2000a, PANYAM, KILARA 1996). Possibilities of applying peptides and proteins as emulsifiers are determined by the nature of their molecules (size, shape, elasticity, susceptibility to denaturation, hydrophobicity, hydrophilicity, interactions with other food constituents) as well as by environmental factors (pH, temperature, pressure, ionic strength) (DALGLEISH 2006, DAREWICZ et al. 2000a, DZIUBA, DAREWICZ 2000). Enzymatic hydrolysis of proteins, apart from decreasing their molecular weight, results in an increasing number of functional groups capable of ionization, and in the exposure of hydrophobic domains. This leads, among others, to a change in their surface properties. Recently much attention has been paid in scientific research to peptides displaying specific and predictable functional and biological properties (DZIUBA et al. 2004, DZIUBA, IWANIAK 2006). Conceptions of obtaining and applying such peptides gain significance in the aspect of intensively developing innovative strategies of production of special-purpose foods, including foods with hypoallergenic properties. In recent years, those studies have been supported by attractive tools – computer-aided methods offered by an intensively developing, new scientific domain – bioinformatics (ETTELAIE 2003). The database of proteins and biologically active peptides BIOPEP, developed at the Chair of Food Biochemistry, Faculty of Food Science, University of Warmia and Mazury in Olsztyn, has greatly contributed to that research area (DZIUBA, IWANIAK 2006).

A number of papers published in recent years have addressed investigations into the emulsifying properties of, above all, hydrolyzates and occasionally peptides obtained upon enzymatic hydrolysis of proteins (DAREWICZ 2001, PANYAM, KILARA 1996, TUNÇTÜRK, ZORBA 2006). However, based on results reported therein, it is difficult to reach some more general conclusions on correlations between structural and emulsifying properties mainly due to the fact that their analysis was usually carried out on peptide-protein mixtures, and with a variety of research methods. Still, it is worthy of notice that

all those papers pointed to the leading role of enzymes used for protein hydrolysis. As a consequence, it is its specificity that determines properties of the product obtained. In addition, possibilities of identifying peptides and carrying out their structural characteristics are helpful in the sound interpretation of results of surface properties assay. The ability to predict changes in the emulsifying properties of peptides and proteins based on the knowledge of their molecular characteristics constitutes a basic source of their optimal utilization in special-purpose foods (DAREWICZ et al. 2006, DZIUBA, DAREWICZ 2000, INNOCENTE et al. 2002). Molecular aspects of the functional, thus also emulsifying, properties of proteins and peptides are usually discussed based on experimental results in which use was made of proteins with clearly defined molecular characteristics. Such proteins include $\beta$-casein that constitutes one of the major protein fractions of milk (approx. 38% of casein content) consisting of 209 amino acid residues. $\beta$-casein is characterized by distinct differences in the distribution of amino acid residues (Figure 1). In its structure, the primary C-terminal domain is strongly hydrophobic, whereas the N-terminal one containing phosphate

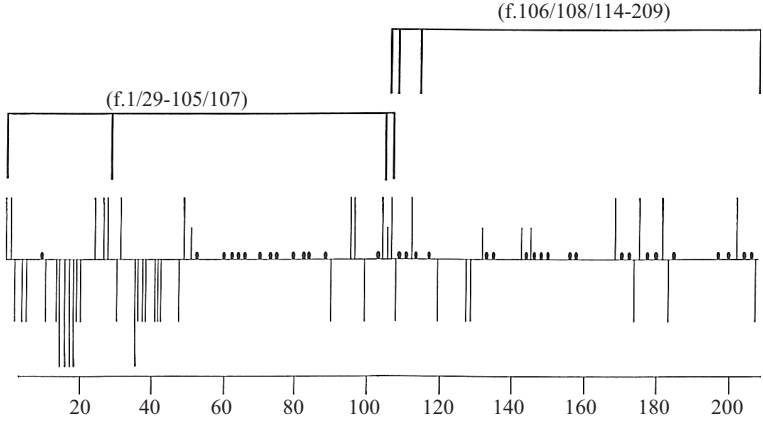


Fig. 1. Graphical representation of elements of the primary structure of $\beta$-casein and peptides released from it by plasmin (● – proline residues; | – charge at pH 6.7; | – phosphate residues)

residues – strongly hydrophilic (*The Swiss.*. 2004, DAREWICZ et al. 2006). Strongly amphiphilic nature of that protein facilitates $\beta$-casein concentration at the interface, which is a prerequisite for the occurrence of properties linked with the abilities to form and stabilize emulsions (DICKINSON 1999). Simultaneously, a negligible proportion of the ordered secondary structure and

a relatively high number of proline residues uniformly distributed along the polypeptide chain of $\beta$-casein imply elastic, amino acid residue exposure-enhancing, hydrophobic character of its molecule (DAREWICZ 2001, DAUPHAS et al. 2005, HOLT, SAWYER 1993, SWAISGOOD 2003). So far, nine genetic variants of $\beta$-casein have been identified, i.e. $A^1$, $A^2$, $A^3$, B, C, D, E (SWAISGOOD 2003), F (VISSER et al. 1995), and H (HAN et al. 2000), that differ in the amino acid sequence and in the number of phosphate residues attached (e.g. in variant $A^1$ of $\beta$-casein the residues occur at positions 15, 17, 18, 19, and 35).

Plasmin and alkaline phosphatase are enzymes that have been applied to prepare peptides with different molecular characteristics and emulsifying properties from $\beta$-casein. Plasmin, serine proteinase originated from blood plasma, is the major proteolytic enzyme occurring in milk (CRUDDEN et al. 2005, KELLY et al. 2006). Plasmin hydrolyses peptide bonds formed, primarily, in the presence of lysine (Lys-X) and arginine (Arg-X) with distinct preference for the first ones (BASTIAN, BROWN 1996). The main function of plasmin in blood consists in activating fibrin, a protein with fundamental significance in the process of blood coagulation. On the other hand, alkaline phosphatase may be applied to remove ester-bound phosphate residues in $\beta$-casein (DAREWICZ et al. 2000b, SHAKEEL-UR-REHMAN et al. 2006). The removal of the phosphate residues is likely to deprive $\beta$-casein of its allergenic properties (BERNARD et al. 2000). Enzymatic dephosphorylation of casein may be of significance to attempts of humanizing cow's milk (LI-CHAN, NAKAI 1989). In addition, attention is given to reduced precipitation and increased bioavailability of systems containing not completely phosphorylated $\beta$-casein or peptides derived from it as well as ions of zinc, copper, iron or magnesium as one of the elements of making cow's milk similar to human milk and of acquiring new dietetic agents (LI-CHAN, NAKAI 1989, PERES et al. 1997).

## Structural and functional relationships in partly-hydrolyzed $\beta$-casein

Plasmin-treated $\beta$-casein enables to obtain peptides with different molecular characteristics, i.e. peptides differing in molecular weight, hydrophobicity and charge distribution along their polypeptide chains (Figure 1). Those peptides can be classified as: hydrophilic – fragment 1-28 of $\beta$-casein; amphiphilic (with distinctively separated hydrophobic and hydrophilic domains) – fragments 1/29-105/107 of $\beta$-casein; and hydrophobic – fragments 106/108/114-209 of $\beta$-casein (DAREWICZ et al. 2000b, DAREWICZ 2001).

The characteristics of structural changes, following adsorption at the interface, of thus obtained peptides contribute to explaining the nature of

relationships occurring between molecular and emulsifying properties. Contrary to the low-molecular weight emulsifiers, the structure of peptides may be subject to changes as affected by the adsorption at the interface, which in turn is of fundamental significance to the specificity of development of their ability to form and stabilize emulsions (DAREWICZ et al. 2000b, NYLANDER, WAHLGREN 1999). Investigations onto peptides originated from $\beta$-casein have indicated that after adsorption, despite a relatively high number of proline residues, the contribution of the ordered $\alpha$-helical structure at the hydrophobic surface increased at the expense of the unordered structure (Table 1). Amphiphilic peptides are characterized by a higher susceptibility to $\alpha$-helical structure induction than the hydrophobic and hydrophilic ones. That phenomenon is more noticeable in the amphiphilic peptides, i.e. in the 1-105/107 fragment not devoid of phosphate residues, than in the fragment 29-105/107. Interestingly, the removal of phosphate residues from the amphiphilic fragments with the use of alkaline phosphatase implies further increase in the contribution of the

Table 1
Structural properties and emulsion forming/stabilizing capacity of peptides obtained upon $\beta$-casein hydrolysis by plasmin (DAREWICZ 2001)

| Peptide ($\beta$-casein fragment)[1] | Increase in $\alpha$-helical structure[5,6] (%) | Decrease in unordered structure[5,6] (%) | Sauter diameter $d_{32}$[7] | Emulsion stability[6,7] |
|---|---|---|---|---|
| 1-28[2] | 4 | – 21 | 3.13 | – |
| 29-105/107[2] | 17 | 26 | 1.60 | ++ |
| 1-105/107[2] | 41 | 24 | 1.51 | +++ |
| 1-105/107 BRF[2,4] | 55 | 42 | 1.72 | +++ |
| 106/108/114-209[3] | 12 | 9 | 2.01 | +++ |

Explanations:
[1] The peptides were produced by plasmin hydrolysis of intact $\beta$-casein with intermediate pellet separation, fractionation of the hydrolysate, and subsequent ion-exchange and hydrophobic-interaction chromatographic purification
[2] Results obtained at pH 6.7
[3] Results obtained at pH 9.0
[4] 1-105/107 Fragment of $\beta$-casein was incubated at 37°C for 24 h with alkaline phosphatase (2 U/mg of peptide) in 50 mM Tris-HCl buffer (pH 8.0)
[5] Far-UV CD wavelength-scan spectra of the 0.1 mg/mL peptide solutions (phosphate buffer pH 6.7 and 9.0) were recorded as averages of 10 spectra on spectropolarimetr. To calculate the peptide content of the secondary structure the spectrum analysis was performed using a non-linear regression procedure
[6] After adsorption at the hydrophobic surface
[7] Emulsions (tricaprylin oil/water = 1/9; 0.44% w/v protein solution) were made using a laboratory high-pressure homogeniser. The emulsion-forming capacity was determined by measuring particle-size distribution. The emulsion stability was determined by measuring the turbidity at 500 nm. Range of emulsion stability: (+++) maximal stability; (-) unstable emulsion
BRF- without phosphate residues

$\alpha$-helical structure in those peptides (DAREWICZ 2001). The same phenomenon was observed at increasing acidity of the environment. Values of isoelectric points of peptides obtained from $\beta$-casein are indicative of the acidic range of pH. With a decrease in pH values, their resultant charge is also observed to decrease. When the repulsion effect between charged amino acid residues is minimized, hydrogen bonds responsible for stabilizing the secondary helical structure are formed more readily.

A criterion used to classify peptides in terms of their capacity for forming emulsions is the value of Sauter diameter $d_{32}$ (DALGLEISH 2006, WALSTRA, DE ROOS 1993). The amphiphilic peptides form emulsions with the smallest droplets, as compared with the other peptides released from $\beta$-casein by plasmin (Table 1). Distinct separation of hydrophobic and hydrophilic domains in the amphiphilic peptides, at relatively high molecular weight provided, may be one of the reasons for their better ability to form emulsions. The presence of a hydrophilic "head", i.e. fragment 1-28, in a more amphiphilic peptide, i.e. 1-105/107, improves its capacity for forming and stabilizing emulsions as compared with the less amphiphilic fragment, i.e. 29-105/107. Simultaneously, the removal of phosphate groups practically did not change the stability of emulsion formed in the presence of fragment 1-105/107 of $\beta$-casein. The reduction in the force of electrostatic repulsion, related to the removal of phosphate groups, is compensated for by the induction of a more compact $\alpha$-helical structure and, consequently, results in an increased density of the resultant charge in dephosphorylated amphiphilic fragment 1-105/107 of $\beta$-casein.

In an acidic and neutral pH range, the hydrophobic peptides form emulsions that are subject to aggregation (Table 1). This phenomenon may be explained by the occurrence of strong hydrophobic interactions at a simultaneous lack of sufficient forces of electrostatic repulsion (DAREWICZ et al. 2000b, DAREWICZ 2001, DAUPHAS et al. 2005). In contrast, in the alkaline range of pH the improvement in their ability to stabilize emulsions might be connected with an increase in the resultant charge of hydrophobic peptides and forces of electrostatic repulsion.

The low-molecular, hydrophilic fragment 1-28 of $\beta$-casein is incapable of stabilizing emulsion formed in its presence (Table 1). Similarly unsatisfactory emulsifying properties of low-molecular weight peptides obtained from casein and whey proteins have been observed by other researchers (CHOBERT et al. 1988, TURGEON et al. 1991, 1992). The 1-28 fragment of $\beta$-casein, apart from a low molecular weight, was characterized by the lowest increase in the $\alpha$-helical structure following adsorption at the hydrophobic surface and by an increase, and not a decrease as in the case of the other peptides, in the contribution of the unordered structure. Figure 2 presents a hypothetical

conformation model of peptides released from $\alpha$-casein by plasmin, i.e. fragments 1/29-105/107 and 106/108/114-209, after adsorption at the interface.

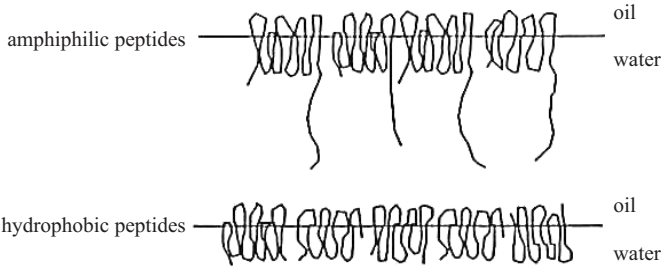


Fig. 2. Hypothetical conformation of peptides: amphiphilic (fragment 1/29-105/107) and hydrophobic (fragment 106/108/114-209), released from $\beta$-casein by plasmin and adsorbed at the oil/water interface

## Conclusion

Distinct separation of hydrophobic and hydrophilic fragments of proteins, as well as the exposure of amphiphilic structure, are the prerequisites for the occurrence of satisfactory emulsifying properties of peptides. An additional factor that limits the application of peptides as emulsifying agents is their molecular weight (that should be higher than 2 kDa). The emulsion-forming and stabilizing properties of low-molecular weight peptides are unsatisfactory. In addition, a relationship can be postulated between the contribution of amphiphilic $\alpha$-helix structure and the emulsifying properties of peptides.

Translated by authors

Accepted for print 11.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 423-431, Y. 2006

# EFFECT OF PRESSURIZATION ON THE MICROFLORA OF RIPENING CHEESE*

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K e y w o r d s: Edamski cheese, cheese microflora, high pressure treatment, cheese ripening.

A b s t r a c t

This experiment was aimed at determining the effect of pressurization on the microflora of ripening Edamski cheese. The pressure treatment applied at 50 and 100 MPa did not have significant impact on the total microbial count nor the number of lactococci, irrespective of the degree of cheese maturation. It was not unequivocally confirmed that high pressure treatment resulted in the inactivation of coliforms. A slight increase in the number of the resting spores of clostridia was observed after the pressurization.

## WPŁYW PRESURYZACJI NA MIKROFLORĘ SERA DOJRZEWAJĄCEGO

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S ł o w a k l u c z o w e: ser edamski, mikroflora sera, technika wysokich ciśnień, dojrzewanie sera.

A b s t r a k t

Celem doświadczenia było zbadanie wpływu presuryzacji na mikroflorę dojrzewającego sera edamskiego. Stwierdzono, że ciśnienie 50 i 100 MPa nie miało znaczącego wpływu na ogólną liczbę drobnoustrojów i liczbę paciorkowców mlekowych, niezależnie od stopnia dojrzałości sera. Nie potwierdzono jednoznacznie inaktywacji bakterii grupy coli pod wpływem ww. ciśnień. Po presuryzacji zaobserwowano nieznaczny wzrost liczby przetrwalników laseczek z rodzaju *Clostridium*.

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* This scientific study was financed from funds of the State Committe for Scientific Research in the years 2003-2006 as a research project.

## Introduction

In recent years, an increased interest has been observed in the application of alternative non-thermal methods for the preservation of raw materials and foodstuffs (MARTENS, KNORR 1992, TORRES, VELAZQUEZ 2005).

It has been demonstrated that pressures of 100-1000 MPa applied at room temperature reduced the number of microflora and extended the shelf life without causing significant changes in organoleptic quality or nutritive properties of food products (KOŁAKOWSKI et al. 1996).

Commercial-scale pressurization is applied to products with different chemical composition and consistency, e.g. fruit juices, jams, sauces, rice, desserts and ham (TRUJILLO et al. 2002).

Pressurization may also be applied in cheesemaking. The pressurization of milk to be used for cheese making can replace the pasteurization process. The microbiological quality of milk pressurized at 600-800 MPa was comparable to that of milk pasteurized at 72°C for 15 s (KOŁAKOWSKI et al. 1997). A considerable reduction in the total bacteria count in Gouda and Camembert cheeses was achieved upon the application of pressure of 400 MPa (REPS et al. 1998). Pressure treatment at 500 MPa for 15 min resulted in a diminution of the number of *Listeria monocytogenes* bacteria in sliced ripening cheese by 6 orders of magnitude (SZCZAWIŃSKI et al. 1997). A considerable resistance to the high pressure treatment has been demonstrated for resting spores (REPS et al. 1997).

In addition, the appropriate selection of the pressurization process parameters enables modifying technological properties of raw materials and certain organoleptic properties of food products. The pressurization of milk to be used for cheesemaking may increase cheese yield and shorten the time of rennet coagulation (HUPPERTZ et al. 2004), whereas cheese made from the pressurized milk is characterized by more favorable organoleptic properties, as compared to those produced from pasteurized milk (REPS et al. 1998). Cheese pressurization accelerates its ripening (KOŁAKOWSKI et al. 1998) and positively affects its sensory and physicochemical properties (MESSENS et al. 2000).

In Japan it was demonstrated that Cheddar cheese pressurized at 50 MPa for 3 days did not display any significant differences when compared with the cheese ripening under traditional conditions for 6 months (YOKOYAMA et al. 1992). Still, other publications do not confirm the considerable effect of pressures below 100 MPa on microflora (MESSENS et al. 1999), degree of maturation (O'REILLY et al. 2000b) or properties of the pressurized cheese (MESSENS et al. 2000).

The above discrepancy prompted an investigation of the effect of pressure treatment at 50 and 100 MPa on the microflora of ripening cheese.

## Material and Methods

The subject of investigation was commercially-produced Edamski cheese with different degrees of maturation.

Pressurization at 50 and 100 MPa for 30 min at room temperature was carried out at the Institute of High Pressures of the Polish Academy of Sciences in Warsaw. Cheese samples (6.5/5/6.5 cm in size) were paraffinated, packed prior to pressurization in additional barrier laminates and subjected to pressure treatment directly after salting as well as after 4, 6 and 8 weeks of ripening.

The microbiological analysis of pressurized and non-pressurized (control) cheese was carried out immediately after pressurization and periodically during ripening.

The samples were prepared according to the Polish Standard PN-93/ /A-86034/03.

The samples were subjected to the following microbiological assays:

– *Salmonella* and *Listeria* bacteria were determined with Tecra Unique *Salmonella* and Tecra Unique *Listeria* tests following producers' instructions;

– total microbial count was determined according to the Polish Standard PN-93 A-86034/04;

– lactococci were assayed on M17 agar according to Terzaghi;

– coliforms and *Escherichia coli* were assayed on Chromocult coliform agar;

– the most probable number (MPN) of resting spores of sulfite-reducing clostridia was determined according to the Polish Standard PN-93 A-86034/12;

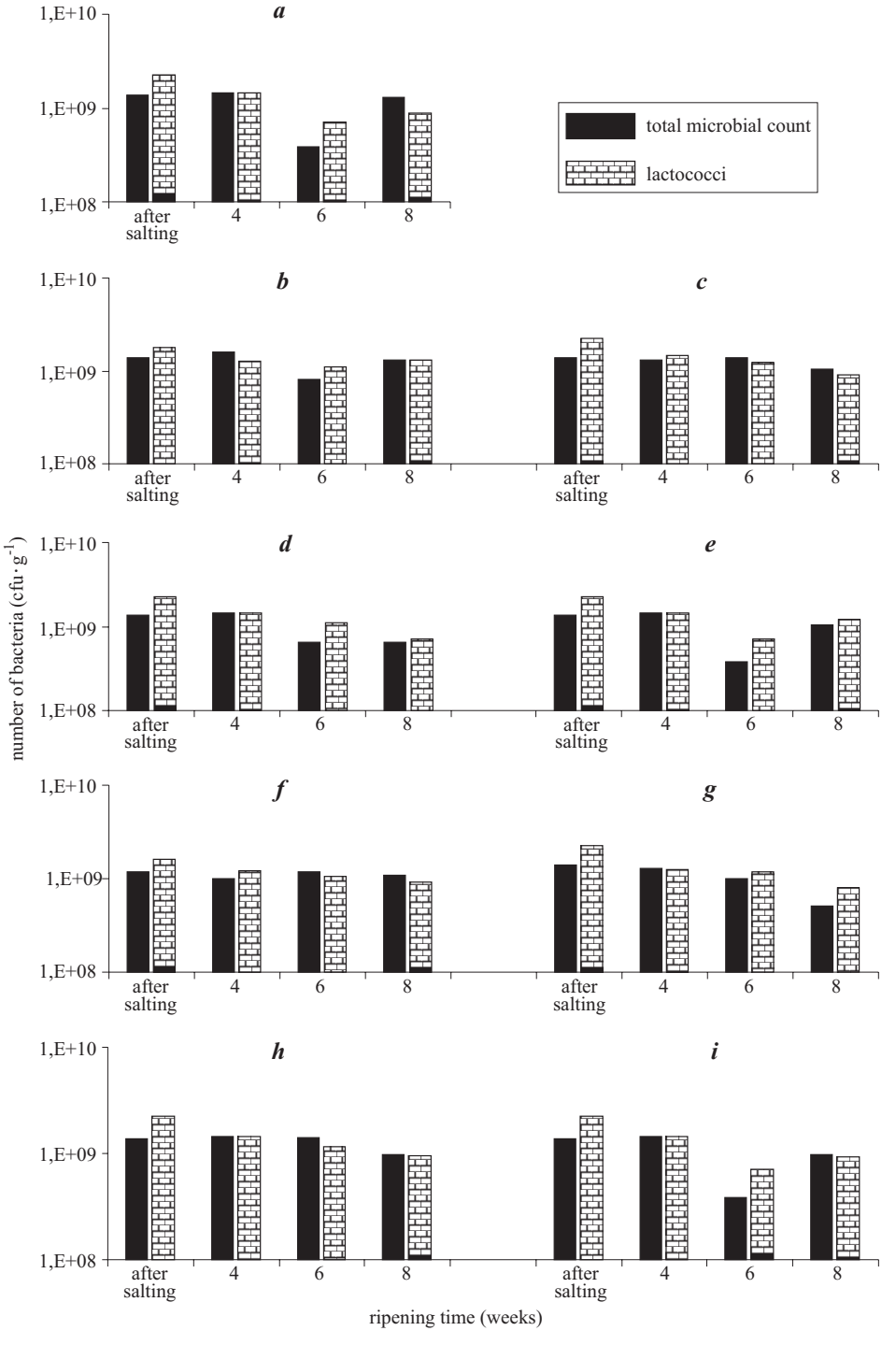
– the most probable number (MPN) of resting spores of gas-producing clostridia was assayed on Bryant Burkey broth with resazurin and lactate, and with Durham tube.

Determinations were carried out on media by Merck, in three replications.

## Results and Discussion

Microflora of the Edamski cheese examined is constituted mainly by lactococci. The most tangible discrepancies were observed for control cheese after salting and after 6 weeks of ripening (Figure 1*a*), for cheese pressurized at 50 MPa in the 6$^{th}$ week of ripening (Figure 1*d*), and for cheese pressurized at 100 MPa in the 4$^{th}$ week of ripening, determined after 4 subsequent weeks of ripening (Figure 1*i*).

In most cases the number of lactococci was higher than total microbial count, what might be caused by by richer medium, and therefore more favourable conditions for the growth of this group of bacteria.


a
b
c
d
e
f
g
h
i
1,E+10
1,E+09
1,E+08
after salting
4
6
8
total microbial count
lactococci
number of bacteria (cfu · g-1)
ripening time (weeks)

No definite effect of 50 and 100 MPa pressures on the total microbial count or the number of lactococci was observed, irrespectively of the degree of maturation (Figure 1).

It was confirmed that pressures as low as 100 MPa did not reduce microflora, which was consistent with results reported by other authors. Pressurising cheese at 50-400 MPa for 60 min, it was demonstrated that the statistically significant lowering of the number of *L. lactis* was achieved at 225 MPa and of total microbial count – at 312.5 MPa (MESSENS et al. 1999). The acceleration of cheese ripening as a result of pressurization is probably linked with cell lysis of starter cultures and release of hydrolytic enzymes from those cultures (KOŁAKOWSKI et al. 1998). A lack of a tangible effect of 50 MPa for 72 h at room temperature on cheese ripening indirectly implies a lack of the impact of that pressure on lactic fermentation bacteria (O'REILLY et al. 2000b).

The degree of cheese maturity did not exert any significant effect on either the number of lactococci or total microbial count.

The total microbial count in the control cheese ranged from $3.86 \cdot 10^8$ cfu $\cdot$ g$^{-1}$ to $1.39 \cdot 10^9$ cfu $\cdot$ g$^{-1}$, however, those fluctuations were irregular and after 8 weeks of ripening, the cheese was characterized by the same count of microflora as that immediately after salting.

The total microbial count in the cheese pressurized at 50 MPa ranged from $6.45 \cdot 10^8$ cfu $\cdot$ g$^{-1}$ to $1.61 \cdot 10^9$ cfu $\cdot$ g$^{-1}$, whereas that in the cheese pressurized at 100 MPa – from $5.10 \cdot 10^8$ cfu $\cdot$ g$^{-1}$ to $1.41 \cdot 10^9$ cfu $\cdot$ g$^{-1}$ (in pressurized cheese in the 6$^{th}$ week or ripening).

The number of lactococci ranged from $7.13 \cdot 10^8$ cfu $\cdot$ g$^{-1}$ to $2.23 \cdot 10^9$ cfu $\cdot$ g$^{-1}$ in the control cheese, from $7.19 \cdot 10^7$ cfu $\cdot$ g$^{-1}$ to $1.11 \cdot 10^9$ cfu $\cdot$ g$^{-1}$ in the cheese pressurized at 50 MPa, and from $7.94 \cdot 10^7$ cfu $\cdot$ g$^{-1}$ to $1.63 \cdot 10^9$ cfu $\cdot$ g$^{-1}$ in that pressurized at 100 MPa. The number of lactococci indicates a decline in their number along with extended time of cheese ripening.

Also other authors demonstrated the changes of the microflora of non-pressurised ripening cheeses. A reduction (by ca. 2.5 orders of magnitude) in total microbial count was observed during ripening of Cheddar cheese (LUES et al. 1999), and the decrease of total microbial count by over 1, and that of lactococci by nearly 2 orders of magnitude – during ripening of Valdeteja cheese (ALONSO-CALLEJA et al. 2002).

Fig. 1. The effect of high pressure on the number of selected bacteria in ripening Edamski cheese: *a* – control cheese (non-pressurized), *b* – cheese pressurized at 50 MPa after salting, *c* – cheese pressurized at 50 MPa after 4 weeks of ripening, *d* – cheese pressurized at 50 MPa after 6 weeks of ripening, *e* – cheese pressurized at 50 MPa after 8 weeks of ripening, *f* – cheese pressurized at 100 MPa after salting, *g* – cheese pressurized at 100 MPa after 4 weeks of ripening, *h* – cheese pressurized at 100 MPa after 6 weeks of ripening, *i* – cheese pressurized at 100 MPa after 8 weeks of ripening

At pressures higher than those applied in the reported study it is possible to reduce considerably, in cheese and milk, the number of bacteria posing risk to consumers; health, including *Salmonella* and *Listerii monocytogenes* (BOZOGLU et al. 2004, SZCZAWIŃSKI et al. 1997).

Over the experimental period, no bacteria of the genera *Salmonella* and *Listeria* were detected in cheese after salting.

Under the influence of technologically-detrimental microflora, numerous defects are likely to occur in cheese, including cheese blowing caused by coliforms and clostridia (WUYTACK et al. 2002, SU, INGHAM 2000).

The possibility of reducing coliforms in cheese as a result of pressurization was demonstrated at over 300 MPa (KOŁAKOWSKI et al. 1996, O'REILLY et al. 2000a).

No *E. coli* bacteria were detected in the cheeses examined, irrespective of the degree of their maturation.

Due to a negligible number of coliforms in the cheeses under scrutiny (the accuracy of the assay enabled detecting their presence in as little as 0.01 g of cheese), it is difficult to determine the actual effect of pressure on their presence in ripening cheese.

The coliforms were identified on average in 83% of samples of non-pressurized cheeses and in 50% of samples of cheeses pressurized at 50 and 100 MPa, including 58% of cheese samples immediately after the pressurization and 44% of samples that were subjected to further ripening after pressurization (Table 1).

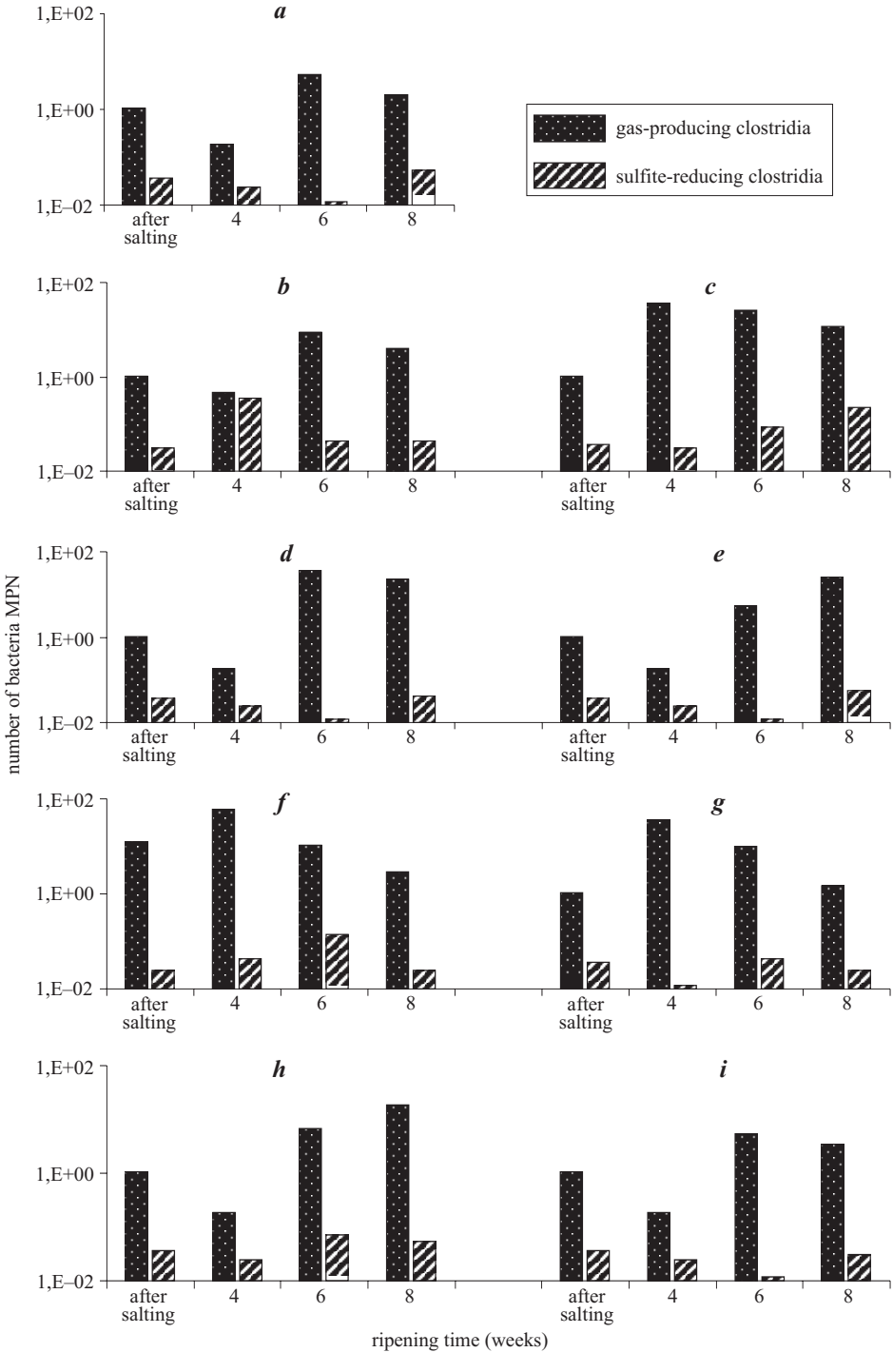
Table 1

The effect of high pressure on the survival of coliforms in ripening Edamski cheese

| Time of cheese ripening (weeks) | Control cheese | Time of cheese ripening before pressurization (weeks) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | after salting | | 4 | | 6 | | 8 | |
| | | pressure (MPa) | | | | | | | |
| | | 50 | 100 | 50 | 100 | 50 | 100 | 50 | 100 |
| | | samples with coliforms detected in 0.01 g of cheese (%) | | | | | | | |
| After salting | 100 | 100 | 100 | – | – | – | – | – | – |
| 4 | 100 | 67 | 33 | 33 | 33 | – | – | – | – |
| 6 | 67 | 0 | 33 | 67 | 100 | 100 | 100 | – | – |
| 8 | 67 | 33 | 33 | 100 | 0 | 0 | 67 | 0 | 0 |

(–) not analysed

Fig. 2. The effect of high pressure on the most probable number of the resting spores of clostridia in ripening Edamski cheese: *a* – control cheese (non-pressurized), *b* – cheese pressurized at 50 MPa after salting, *c* – cheese pressurized at 50 MPa after 4 weeks of ripening, *d* – cheese pressurized at 50 MPa after 6 weeks of ripening, *e* – cheese pressurized at 50 MPa after 8 weeks of ripening, *f* – cheese pressurized at 100 MPa after salting, *g* – cheese pressurized at 100 MPa after 4 weeks of ripening, *h* – cheese pressurized at 100 MPa after 6 weeks of ripening, *i* – cheese pressurized at 100 MPa after 8 weeks of ripening


a
b
c
d
e
f
g
h
i
1,E+02
1,E+00
1,E–02
gas-producing clostridia
sulfite-reducing clostridia
after salting
4
6
8
number of bacteria MPN
ripening time (weeks)

The content of the resting spores of saccharolytic (gas-producing) clostridiain the cheeses analyzed was negligible and their most probable number did not exceed $5.35 \cdot g^{-1}$ in the control cheese, $3.7 \cdot 10 \cdot g^{-1}$ in cheese pressurizedat 50 MPa, and $6.14 \cdot 10 \cdot g^{-1}$ in cheese pressurized at 100 MPa (Figure 2). A slight increase was observed in their number after pressurization, which was most likely to be due to the accelerated sprouting of resting spores upon pressure treatment, which was also observed by other authors (SALE et al. 1970).

The number of the resting spores of proteolytic (sulfite-reducing) clostridia in the cheeses examined was lower by ca. 2-3 orders of magnitude as compared to the number of the resting spores of gas-producing clostridia. Also in that case, the number of the resting spores of bacilli was observed to increase after pressurization. The most probable number of the resting spores of anaerobic proteolytic bacilli accounted for up to $5.47 \cdot 10^{-2} \cdot g^{-1}$ in control cheese, for $3.6 \cdot 10^{-1} \cdot g^{-1}$ in cheese pressurized at 50 MPa, and for $1.39 \cdot 10^{-1} \cdot g^{-1}$ in cheeses pressurized at 100 MPa (Figure 2).

During statistical analysis it was demonstrated, that the differences in the number of resting spores in pressurised and non-pressurised cheeses at different stages of maturation approximated standard deviation. In most cases the differences were not statistically significant.

The results obtained indicate that ripening time had no evident effect on the number of the resting spores of clostridia in the cheeses. In contrast, a substantial reduction of anaerobic bacilli over the ripening period of Cheddar cheese was observed (LUES et al. 1999).

The results compiled herein substantiate the advisability of further research into the effect of high pressures on the microflora of ripening cheese.

## Conclusions

1. The pressurization at 50 and 100 MPa for 30 min had no evident effect on the total microbial count nor on the number of lactococci.

2. The maturation degree of cheeses subjected to the pressurization also hardly had any effect on its microflora.

3. Pressure treatment at 50 and 100 MPa did not improve the microbiological quality of assayed cheese.

Translated by JOANNA JENSEN

Accepted for print 21.04.2006

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POLISH JOURNAL OF NATURAL SCIENCES
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# BOVINE $\alpha$-LACTALBUMIN IDENTIFICATION BASED ON A CHEMOMETRICAL ANALYSIS OF THE THIRD DERIVATIVES OF ULTRAVIOLET SPECTRA OBTAINED AFTER SEPARATION BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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K e y w o r d s: $\alpha$-lactalbumin, HPLC, UV-spectroscopy, chemometrics, denaturation, reduction.

A b s t r a c t

An experiment using bovine $\alpha$-lactalbumin showed that protein concentration (expressed as absorbance at the wavelength 280 nm – $A_{280}$) is a crucial factor affecting its identification based on a chemometrical interpretation of UV spectra third derivatives. If $A_{280}$ decreases below 0.02, the similarity index (SI) between third derivative of sample spectrum and third derivative of standard spectrum decreases. Standard deviation of SI increases with decrease of absorbance. Third derivatives of spectra of protein reduced in the presence of urea differ from the third derivatives of the spectra of non-reduced protein, although this difference does not create problems with identification. Difference between the spectra of non-reduced and reduced $\alpha$-lactalbumin is slight as compared with difference between its spectrum and spectra of other bovine milk proteins (e.g. $\beta$-lactoglobulin). The results indicate that a chemometrical interpretation of ultraviolet spectra may serve as a tool for the identification of $\alpha$-lactalbumin independently of disulphide bond reduction, while also discriminating between non-reduced and fully reduced protein if the analyte concentration is high enough ($A_{280} > 0.02$).

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* This work has been supported by own funds of UWM within the project: 522-0712-0809.

# IDENTYFIKACJA BYDLĘCEJ LAKTOALBUMINY-$\alpha$ NA PODSTAWIE CHEMOMETRYCZNEJ ANALIZY TRZECICH POCHODNYCH WIDM W NADFIOLECIE OTRZYMANYCH PO ROZDZIALE METODĄ WYSOKOSPRAWNEJ CHROMATOGRAFII CIECZOWEJ Z ODWRÓCONYMI FAZAMI

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S ł o w a k l u c z o w e: laktoalbumina-$\alpha$, HPLC, spektroskopia UV, chemometria, denaturacja, redukcja.

A b s t r a k t

W eksperymencie z użyciem bydlęcej laktoalbuminy-$\alpha$ wykazano, że stężenie białka (wyrażone jako absorbancja przy długości fali 280 nm – $A_{280}$) jest decydującym czynnikiem wpływającym na jego identyfikację z wykorzystaniem chemometrycznej interpretacji trzecich pochodnych widm UV. Jeśli $A_{280}$ maleje poniżej 0.02, maleje też wskaźnik podobieństwa (SI) trzeciej pochodnej widma badanego do trzeciej pochodnej widma UV wzorca. Odchylenie standardowe SI rośnie wraz ze zmniejszaniem się absorbancji. Trzecie pochodne widm UV białka redukowanego w obecności mocznika różnią się od trzecich pochodnych widm nie redukowanego białka, ale ta różnica nie stwarza problemów w identyfikacji. Różnica między widmami nie redukowanej i redukowanej laktoalbuminy-$\alpha$ jest niewielka w porównaniu z różnicą między widmem tego białka a widmami innych białek mleka krowiego (np. laktoglobuliny-$\beta$). Wykazano, że chemometryczna interpretacja widm w nadfiolecie może służyć jako narzędzie identyfikacji laktoalbuminy-$\alpha$ niezależnie od redukcji wiązań disiarczkowych, chociaż w przypadku wystarczająco wysokiego stężenia analitu ($A_{280} > 0.02$) możliwe jest także rozróżnianie między widmami białka nie redukowanego i całkowicie zredukowanego.

## Introduction

The action of denaturing agents, such as high temperature, donors of free thiol groups and chaotropic agents, such as urea, on bovine $\alpha$-lactalbumin ($\alpha$-LA) (Accession No P00711 in the Swiss-Prot database – http://www.expasy.org) causes the formation of many isomers with a different location of disulphide bonds. Such isomers and partially reduced forms of $\alpha$-LA are separable using reversed-phase high-performance liquid chromatography RP-HPLC (CHANG et al. 2000, 2005, CHANG, LI 2001, CHANG 2004). Other sources of $\alpha$-LA heterogeneity revealed during RP-HPLC separations are: presence of genetic variants (VISSER et al. 1991) and glycosylation (SLANGEN, VISSER 1999).

The chemometrical analysis of UV spectra derivatives has been recommended as a tool for identification of analytes separated using HPLC (MINKIEWICZ et al. 2003, 2004, 2006, MINKIEWICZ 2004). The third derivatives of UV spectra appear to be most efficient for such analysis among various orders of derivatives (MINKIEWICZ et al. 2004, 2006, MINKIEWICZ 2004).

In a previous experiment it was found that UV spectra obtained at low analyte concentration differ from these obtained at higher concentrations (DZIUBA et al. 2001, 2002, DAREWICZ et al. 2005). It has also been reported that long-time exposure to denaturing and reducing agents affects spectral properties of proteins separated using HPLC (BISHOP et al. 1996, BOBE et al. 1998).

The aim of present study was to evaluate influence of reduction of disulphide bonds, denaturation with urea and protein concentration expressed as absorbance at the 280 nm wavelength on the possibility of identification of bovine $\alpha$-LA.

## Materials and Methods

$\alpha$-Lactalbumin (Cat. No L-5385, lot 012K7048) and 2-mercaptoethanol were purchased form Sigma. Trifluoroacetic acid (TFA), acetonitrile (ACN) and 1,3-Bis[Tris(hydroxymethyl)-methylamine]propane (Bis-Tris) (HPLC grade) were purchased from Baker. All other reagents (analytical grade) were provided by the Polish Chemical Reagents. Deionised water was obtained using the MilliQsystem apparatus (Millipore). Sweet whey obtained during Gouda-type cheese production was purchased from the Dairy Co-operative in Działdowo (Poland). Whey was frozen and lyophilised.

$\alpha$-LA samples named "non-reduced $\alpha$-LA" were dissolved in 0.05 M phosphate buffer (pH = 6.6; protein concentration ca. 3.5 mg mL-1) or in 4 M urea-containing 0.1 M Bis-Tris – HCl buffer (pH = 6.6; protein concentration ca. 3.5 mg $mL^{-1}$) and subjected to further procedures as described in ref. (MINKIEWICZ et al. 2005) without reduction. Partially reduced $\alpha$-LA was obtained as follows: 100-200 μL of above-described protein solution in phosphate buffer was mixed with 200-300 μL of the urea-containing Bis-Tris – HCl buffer described above and 6 μL of 2-mercaptoethanol. To obtain fully reduced $\alpha$-LA 380 μL of protein solution in urea-containing Bis-Tris – HCl buffer was mixed with 20 μL of 2-mercaptoethanol. Reduction was carried out 1 h at room temperature. The further procedure of sample preparation has been described previously (MINKIEWICZ et al. 2005). Lyophilised whey was dissolved in a phosphate buffer (MINKIEWICZ et al. 2005). The concentration was 25 mg of lyophilisate per mL. Whey samples were not reduced.

RP-HPLC was carried out as described previously (DZIUBA et al. 2004, MINKIEWICZ et al. 2006). The following gradient was applied: Start: 25% of solvent B; 31% B after 10 min; 37% B after 17 min; 37% B after 25 min; 40% B after 50 min; 48% B after 60 min. The column was then washed and equilibrated using the following sequence: 80% B after 63 min; 80% B after 68 min; 25% B after 70 min; 25% B after 90 min. The data acquisition time was

80 min. The ratios of $H_2O$, ACN and TFA in solvents A and B were 900 : 100 : 1 and 100 : 900 : 0.7 v/v/v respectively (VISSER et al. 1991).

The UV spectra were collected at the tops of peaks. Spectra of non-reduced and fully-reduced $\alpha$-LA, used for further calculations, were collected before and after peak apex at the following values of absorbance at 280 nm: 0.002; 0.004; 0.006; 0.008; 0.010; 0.020 and 0.040 (Figure 1). The third derivatives of protein spectra were compared with the third derivatives of milk protein spectra from the library described by MINKIEWICZ et al. (2003) using similarity indices (SI) within the wavelength range 270-300 nm (MINKIEWICZ et al. 2003, 2004, MINKIEWICZ 2004).

The statistical significance of differences was checked using the Student *t*-test.

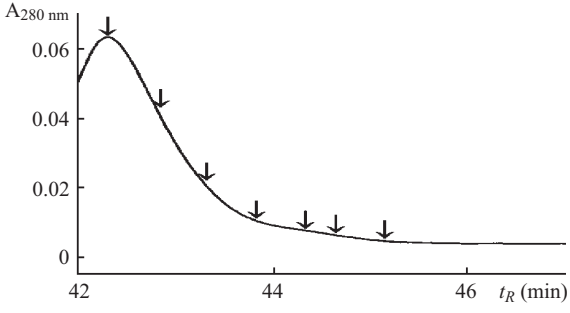


Fig. 1. Fragment of the chromatogram of non-reduced $\alpha$-lactalbumin. Arrows indicate points of collection of UV spectra used for further discussion. The whole chromatograms are presented in Fig. 2

## Results and Discussion

Chromatograms of non-reduced $\alpha$-lactalbumin, dissolved without and with urea, partially and fully reduced $\alpha$-LA are presented in Figure 2. There was no change in the chromatographic profile of non-reduced $\alpha$-LA due to exposure to urea at pH 6.6 (Figure 2a and b). $\alpha$-LA reduced with a low concentration of urea and 2-mercaptoethanol (Figure 2c) revealed heterogeneity, probably due to the presence of forms in which part of the disulphide bonds are reduced (CHANG 2004). The chromatographic pattern was different from those obtained by CHANG (2004), probably due to differences in reduction conditions, especially in pH. A chromatogram of sweet whey is presented in Figure 3. It is a typical chromatogram of whey proteins analysed without prior reduction (ELGAR et al. 2000, SICILIANO et al. 2000). A minor peak labeled as "1" probably correspon-

ded to glycosylated protein. It forms a peak with a retention time slightly shorter than that of sugar-free protein (SLANGEN, VISSER 1999, ELGAR et al. 2000). Another possibility is the presence of scrambled isomers which could be formed during heating (CHANG et al. 2005), e.g. pasteurisation of milk before cheesemaking. Third derivatives of UV spectra of $\alpha$-LA obtained at two concentrations are presented in Figure 4. The spectrum labeled *a* is an example of a spectrum of sufficient quality. The SI between the third derivative *a* presented in Figure 4 and the third derivative of the $\alpha$-LA standard spectrum was 0.997 (SI between two identical spectra is 1.000). The spectrum *b* presented in Figure 4 may serve as an example of a spectrum obtained at an insufficient concentration. The SI between the third derivative *b* presented in Figure 4 and the third derivative of the standard spectrum was 0.800. The spectrum was identified as a spectrum of bovine $\beta$-lactoglobulin ($\beta$-LG) (Access No in the SwisssProt database – P02754) (SI = 0.908). This change was similar to that corresponding to the decrease in the tryptophan to tyrosine

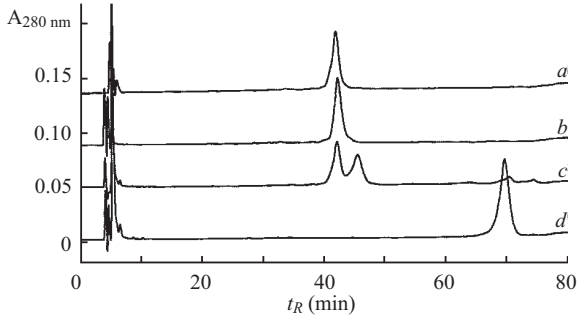


Fig. 2. Chromatograms of $\alpha$-lactalbumin: *a* – non-reduced, dissolved in phosphate buffer, *b* – non-reduced, dissolved in Bis-Tris buffer with urea, *c* – partially reduced, *d* – fully reduced

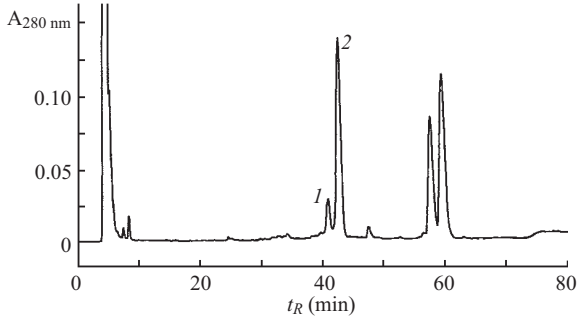


Fig. 3. Chromatogram of whey. Fractions identified as $\alpha$-lactalbumin are labeled as 1 and 2

molar ratio. The Trp/Tyr molar ratio is lower in $\beta$-lactoglobulin than in $\alpha$-lactalbumin (FARRELL et al. 2004). Quality deterioration of UV spectra derivatives due to protein concentration decrease has been described previously on the example of second derivatives (DZIUBA et al. 2001, 2002).

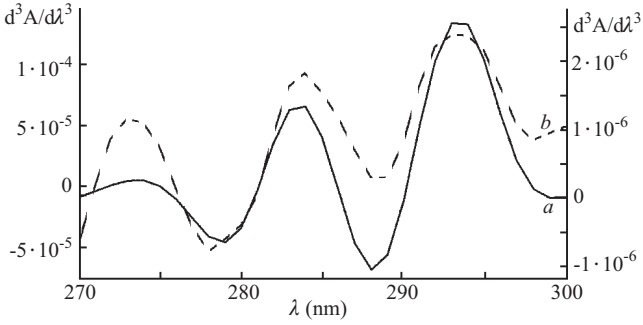


Fig. 4. Third derivatives of UV spectra of non-reduced $\alpha$-lactalbumin taken from the chromatogram *a* shown in Fig. 2: *a* – third derivative of a spectrum acquired from the peak apex ($t_R$ = 42.1 min, $A_{280}$ = 0.041) (solid line and left scale), *b* – third derivative of a spectrum acquired from the same chromatogram at $t_R$ = 45.9 min ($A_{280}$ = 0.003) (dashed line and right scale). Derivatives of spectra are normalised to show difference in shape as recommended by PAPADOYANNIS and GIKA (2004)

Table 1 presents the similarity indices between the third derivatives of spectra of individual protein samples and the third derivative of the standard spectrum of $\alpha$-LA. Results obtained for $A_{280}$ higher than 0.02 were reproducible. There was a slight, but statistically significant, difference between SI of the third derivatives of spectra of non-reduced and reduced proteins. The difference was lower, than that between the third derivatives spectra of anthocyanins containing the same aromatic chromophore, but different sugar residues (MINKIEWICZ et al. 2004). When $A_{280}$ decreases below 0.02, similarity indices of the third derivatives of spectra decrease, and the standard deviation increases. This is consistent with the conclusion that may be drawn from Figure 4, and with our previous results (DZIUBA et al. 2001, 2002). Thus, we can generally recommend $A_{280} > 0.02$ as an absorbance range in which proteins can be identified using the third derivatives of UV spectra. However, lower absorbance values may be also used to discriminate protein or peptide spectra if they differ significantly in the Trp/Tyr molar ratio. For instance, the SI between the third derivatives of $\alpha$-LA samples and the third derivative of $\beta$-lactoglobulin standard spectrum is usually below 0.95 (MINKIEWICZ 2004) and enables discrimination between these proteins even if absorbance is ca. 0.006 (Table 1). The $\beta$-LG spectra is more similar to these of $\alpha$-LA than spectra of other major milk proteins (MINKIEWICZ et al. 2004). The limit is expressed as

absorbance instead of concentration, due to the fact that peptides or proteins may significantly differ in aromatic amino acid content and hence in $A_{280}$, even if absorbance in the wavelength range characteristic of peptide bonds (210-220 nm) is the same (DZIUBA et al. 2002). The upper limit of concentration must generally satisfy the Lambert-Beer law over the entire wavelength range (DAREWICZ et al. 2005, MINKIEWICZ et al. 2006). The values of SI calculated for partially reduced α-LA and α-LA of sweet whey are presented in Table 2. SI of partially reduced protein depended on absorbance. SI at the tops of peaks with absorbance at 280 nm exceeding 0.02 did not differ from SI calculated using non-reduced protein. Peaks with absorbance below 0.02 revealed lower similarity indices. This finding is consistent with the data from Table 1. The SI value calculated for α-LA from whey may serve as proof of the method's robustness. There was no statistically significant difference between the similarity indices calculated for non-reduced protein from Sigma and SI calculated for α-LA from whey. The difference between similarity indices calculated for both peaks of α-LA from whey (Figure 3) cannot be explained by the differences in absorbance, due to the fact that absorbance at the tops of both peaks, "1" and "2", exceeded 0.02. It could be rather caused by some differences in protein structure.

Table 1

Influence of absorbance at a wavelength of 280 nm on the indices of similarity between third derivatives of α-lactalbumin spectra and the third derivative of a standard spectrum

| $A_{280}$ | Non-reduced α-LA dissolved in phosphate buffer SI ± SD[a] | Non-reduced α-LA dissolved in Bis-Tris buffer with urea SI ± SD[a] | Fully reduced α-LA SI ± SD[a] |
|---|---|---|---|
| 0.040 | 0.997[b] | 0.997[b] | 1.000[b] |
| 0.020 | 0.996[b] | 0.997[b] | 1.000[b] |
| 0.010 | 0.994[b] | 0.995[b] | 0.998[b] |
| 0.008 | 0.992 ± 0.002[d] | 0.994 ± 0.002[e] | 0.997 ± 0.002[d,e] |
| 0.006 | 0.989 ± 0.002[d] | 0.990 ± 0.002[e] | 0.995 ± 0.002[d,e] |
| 0.004 | 0.956 ± 0.004[d,f] | 0.983 ± 0.006[f] | 0.983 ± 0.006[d] |
| 0.002 | [c] | 0.844 ± 0.084 | 0.800 ± 0.170 |

Explanations:

*a* – mean of 4 measurements;

*b* – results of all measurements were identical with accuracy <0.001. SD not estimated;

*c* – similarity index not determined. $A_{280}$ value in the valley preceding and following the main α-LA peak was higher than 0.002;

*d* – statistically significant difference (at least 0.05) between the values obtained for non-reduced α-LA dissolved in phosphate buffer and for fully reduced α-LA;

*e* – statistically significant difference (at least 0.05) between the values obtained for non-reduced α-LA dissolved in Bis-Tris buffer with urea and for fully reduced α-LA;

*f* – statistically significant difference (at least 0.05) between the values obtained for non-reduced α-LA dissolved in phosphate buffer and for-non reduced α-LA dissolved in Bis-Tris buffer with urea.

Table 2
Indices of similarity between third derivatives of UV spectra of partially reduced $\alpha$-lactalbumin and $\alpha$-lactalbumin from sweet whey, and the third derivative of a standard spectrum

| Peak | SI | SD | $n$ |
|---|---|---|---|
| Partially reduced $\alpha$-LA, peaks with $A_{280} > 0.02$[a] | 0.997[b] | 0.003 | 9 |
| Partially reduced $\alpha$-LA, peaks with $A_{280} < 0.02$[a] | 0.995[b] | 0.003 | 17 |
| $\alpha$-LA from sweet whey, peak labeled as "1" (Fig. 3) | 0.998[c] | 0.001 | 5 |
| $\alpha$-LA from sweet whey, peak labeled as "2" (Fig. 3) | 0.995[c] | 0.002 | 5 |

*a* – there was no difference between spectra of two major peaks with different retention times (Fig. 2);
*b, c* – pairs of values which differ at a level of at least 0.05

Our results are in contrast with previous results (BISHOP et al. 1996, BOBE et al. 1998) suggesting that sample preparation may affect the spectral properties of proteins separated using RP-HPLC. On the other hand, proteins used in these experiments were exposed to a reducing and chaotropic agents for a longer period (8-24 h versus 1 h in the present experiment). The longer reduction time could lead to irreversible chemical modifications causing changes in structure. A statistically significant difference in the similarity index occurring at $A_{280} = 0.04$ (Table 1) may be interpreted as an incidental event caused by a low quality of spectra obtained at a low analyte concentration. Problems with identification of whey proteins reported previously (MINKIEWICZ et al. 2003) may be attributed to incomplete separation rather than changes in protein spectral properties caused by denaturation. Derivatives of UV spectra acquired on-line with RP-HPLC separations during the present experiment appear to be less sensitive to protein structure changes than those obtained via the off-line technique in the solvents without acetonitrile (LANGE, BALNY 2002). The changes in the structure of globular proteins during reduction in the presence of a chaotropic agent and chromatographic separations are not known in detail to date.

## Conclusion

Our results indicate that a chemometrical interpretation of the ultraviolet spectra may serve as a tool for the identification of $\alpha$-lactalbumin independently of reduction in disulphide bonds. The difference in spectra enables discrimination between non-reduced and fully reduced proteins.

Translated by authors

Accepted for print 22.05.2006

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 443-454, Y. 2006

# CHARACTERISTICS OF THE SELECTED QUALITY PARAMETERS OF EDIBLE CARROT VARIETIES

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K e y w o r d s: carrots, total carotenoids, $\beta$-carotene, sugars, ADF, TDF, colour.

A b s t r a c t

The objective of the present study was to determine selected properties of 12 hybrid and established carrot varieties and lineages grown in the year 2002. The experimental material were the following carrot varieties and cultural lineages: Allret, Bangor F1, Kazan F1, Kazan F1 (areola's seeds), Macon F1, Maestro F1, Maxima, Nandor F1, Nektarina, Simba F1, Tango F1, Tito, VCU – 30 F1 and one cultural lineage RX (94115). The results obtained were subjected to a one-factor analysis of variance. The significance of differences between treatments was determined by the Duncan multiple range test, at $p \leq 0.05$. It was found that the carrot lines and varieties examined differed in both contents of particular chemical components, and colour properties. Total carotenoid concentration varied from 16.69 to 40.59 mg · 100 g$^{-1}$ f.w., and total $\beta$-carotene concentration – from 9.67 to 23.00 mg · 100 g$^{-1}$ f.w.. Total sugars, reducing sugars and starch contents were as follows: 5.11 to 7.80 g · 100 g$^{-1}$ f.w., 1.04 to 4.48 g · 100 g$^{-1}$ f.w., and 0.40 to 0.90 g · 100 g$^{-1}$ f.w. respectively. Acid detergent fiber (ADF) content was 1.15 to 1.74 g · 100 g$^{-1}$ f.w., with cellulose as the dominant fraction. Total dietary fiber content was 2.65 to 4.41 g · 100 g$^{-1}$ f.w.. Colour lightness (L*) of carrots ranged from 60.1 to 72.8, redness (a*) – from 35.67 to 46.68, yellowness – from 45.07 to 56.76. Considerable differences between carrot varieties, concerning especially the concentrations of carotenoids, sugars and fiber, as well as colour properties, enable their appropriate selection for consumption and processing purposes.

## CHARAKTERYSTYKA WYBRANYCH WYRÓŻNIKÓW JAKOŚCI JADALNYCH ODMIAN MARCHWI

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S ł o w a k l u c z o w e: marchew, karotenoidy ogółem, $\beta$-karoten, cukrowce, ADF, TDF, barwa.

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Abstrakt

Celem badań była ocena 12 odmian marchwi mieszańcowych i ustalonych oraz 1 rodu hodowlanego, pochodzących z uprawy w 2002 r., pod względem wybranych cech fizykochemicznych. Materiał badawczy stanowiły mieszańcowe i ustalone odmiany: Allret, Bangor F1, Kazan F1, Kazan F1 (nasiona otoczkowane), Macon F1, Maestro F1, Maxima, Nandor F1, Nektarina, Simba F1, Tango F1, Tito, VCU – 30 F1 oraz ród hodowlany RX (94115). Wyniki doświadczenia poddano jednoczynnikowej analizie wariancji. W celu określenia istotności różnic między badanymi obiektami na poziomie istotności $p = 0.05$ przeprowadzono wielokrotny test rozstępu Duncana. Wykazano, że badane odmiany i rody marchwi różniły się pod względem zawartości oznaczanych składników chemicznych oraz barwy. Zawartość karotenoidów ogółem wahała się od 16.69 do 40.59 mg · 100 $g^{-1}$ św.m., natomiast $\beta$-karotenu od 9.67 do 23.00 mg · 100 $g^{-1}$ św.m.. Zawartość cukrów ogółem, cukrów redukujących oraz skrobi wynosiła odpowiednio: 5.11 – 7.80 g · 100 $g^{-1}$ św.m.; 1.04 – 4.48 g · 100 $g^{-1}$ św.m. i 0.40 – 0.90 g · 100 $g^{-1}$ św.m. Zawartość błonnika detergentowego kwaśnego (ADF) stanowiła od 1.15 do 1.74 g · 100 $g^{-1}$ św.m., przy czym frakcją dominującą była celuloza. Zawartość błonnika pokarmowego (TDF) wynosiła od 2.65 do 4.41 g · 100 $g^{-1}$ św.m. Jasność (L*) badanej marchwi wahała się od 60.1 do 72.8; czerwoność (a*) od 35.67 do 46.68; żółtość od 45.07 do 56.76. Duże różnice międzyodmianowe, zwłaszcza pod względem takich wyróżników, jak karotenoidy, cukry, błonnik, barwa, stwarzają duże możliwości wyboru odmian najbardziej przydatnych do konsumpcji lub przetwórstwa.

## Introduction

A systematic increase in the production of field-grown vegetables has been observed in Poland for almost twenty years. As estimated by the Central Statistical Office, in the year 2001 a total of 5.2 mln tons of field-grown vegetables were gathered (ANONYMOUS 2001). Vegetables are a rich source of vitamins, mineral components, microelements, terpenes, flavones, tannins, chinones and phytoncids. High vegetable consumption helps to prevent e.g. prostatic carcinoma (COHEN et al. 2000, WILLIS, WIANS 2003, STEINMETZ, POTTER 1996), so they should be present in our everyday diet, together with energy-providing products.

Due to their high nutritive value and health properties, carrots are among the most common and popular vegetables in Poland. They contain more $\beta$-carotene and vitamin B complex than other vegetables. Consumption of vegetables rich in $\beta$-carotene reduces the risk of breast cancer (ZHANG et al. 1999). Carrots contain also other valuable substances, such as organic acids, fiber, pectins, mineral compounds (ZADERNOWSKI, OSZMIAŃSKI 1994). They are light, easily digestible where 100 g provide only 40.32 kcal. Carrots are also known for their antiarteriosclerotic and anti-inflammatory effects (they prevent intestinal infections). They also have a positive influence on the pancreas and nervous system, and are helpful in mucous membrane and skin diseases. Carrots provide components indispensable for the development and proper functioning of the human body (GAWĘCKI 2001). The results of epidemiological investigations show that the quantity and type of carbohy-

drates affect our health state and the incidence of the so called civilization-related diseases (obesity, diabetes, antherosclerosis, colorectal carcinoma and other large intestine diseases, dental caries). Carrots are also widely used in the food industry.

The colour of carrots is determined first of all by the concentration of carotenoid dyes, mainly $\alpha$ – and $\beta$-carotene (MANGELS et al. 1993), which constitute over 90% of all carotenoids (SIMON, WOLFF 1987). The composition and concentration of carotenoids are important factors, affecting our evaluation of foodstuff quality, not only due to their attractive colour, but also valuable biological properties. All carotenoids, being polyene compounds, are antioxidants active both *in vitro* and *in vivo*. Some carotenoids contain in their molecules fragments whose structure is identical with that of retinol, so they show the same activity as vitamin A. $\beta$-carotene shows the highest activity of provitamin A. It is believed that carotenoids are present in plant tissues in the form of complexes with proteins or polysaccharides, so mild heat processes result in disintegration of these complexes and increase bioavailability of carotenoids (RODRIGUEZ-AMAYA 1993, HARTMANN et al. 1996). The carotenoids content of carrots is determined genetically to a high extent, so appropriate selection of carrot varieties allows to obtain the product fully satisfying consumer expectations, both as regards its nutritive value and colour.

One of the colour description models applied most often to identify the colour of food products is the CIELab system. The colour is described by three coordinates in the colour space, which may be measured directly with a spectrophotometer. The L* coordinate describes lightness, the a* coordinate describes colour on the scale from green to red, and the b* coordinate – from blue to yellow (HUTCHINGS 1994). The CIELab system has been employed by many authors to control the quality of vegetables and fruits, and to evaluate undesirable changes in colour, taking place during technological processes e.g. in peaches (AVILA, SILVA 1999), apples (FENG, TANG 1998), fruit pulp concentrates (LOZANO, IBARZ 1997), tomato concentrates (BARREIRO et al. 1997), pears (IBARZ et al. 1999) and bananas (MASKAN 2000).

An increase in carrot production and a market demand for carrot – based products make it necessary to constantly improve carrot varieties used for processing purposes. Further research on the existing varieties and new breeding forms is indispensable, taking into account various directions of processing. Therefore, the objective of the present study was to determine selected physicochemical properties and colour ob new carrot lines and varieties.

# Materials and Methods

## Materials

The chemical composition of 12 hybrid and established carrot varieties and lineages grown in the year 2002 was determined. The experimental materials were grown under the same agricultural conditions. The carrot roots came from experimental plots of the Agricultural Research Institute in Skierniewice in Poland. The sampling were carried out in October 2002. The following 12 hybrid and established carrot varieties and cultural lineages were used in this study: Allret, Bangor F1, Kazan F1, Kazan F1 (areola's seeds), Macon F1, Maestro F1, Maxima, Nandor F1, Nektarina, Simba F1, Tango F1, Tito, VCU – 30 F1. The contents of dry matter, total carotenoids, $\beta$-carotene, total sugars, reducing sugars, starch, acid detergent fiber (ADF), dietary fiber (TDF) and carrot colour were determined. Samples for chemical analyses were prepared by carrot pureeing in a Fryma colloidal mill, type MZ – 80/R.

## Methods

### Dry matter

Dry matter content was determined according to the Polish Standard PN-90/A-75101.

### Carotenoids content

Carotenoids content was determined according to the Polish Standard PN-90/A-75101. Carotenoids were extracted from carrot samples with a mixture of alcohol and ether. After filtration the alcohol-water fraction was rinsed with petroleum benzin until discolouring. The ether extracts obtained were mixed, washed with water and dried with odor-free sodium sulfate. The total carotenoids content of this extract was measured directly with a spectrophotometer, and their concentration was determined on the basis of an analytical curve plotted for $\beta$-carotene. At the next stage $\beta$-carotene was separated from other dyes by column chromatography. The absorbance of a $\beta$-carotene solution was measured with a spectrophotometer at a wavelength of 467, and $\beta$-carotene concentration was determined using an analytical curve.

## Sugars content

Sugars content was determined by the Lane-Eynon method (PN-90/A--75101). In order to determine reducing sugars content, an alkaline solution of copper salt was subjected to hot reduction by direct titration of a protein-free solution, in the presence of methylene blue, without inversion. Total sugars content was determined after inversion with concentrated hydrochloric acid. Further procedures were the same as in the case of reducing sugars.

## Acid detergent fiber (ADF) content

Acid detergent fiber was determined according to the procedure recommended by AOAC (1990). The method consists in gravimetric ADF determination, after elimination of minor constituents by heating in 1N $H_2SO_4$ containing 5% of detergent. After separation in a fritted disc funnel (G3), the precipitate was washed with hot water and acetone, dried to constant mass at 105°C, and weighted. In order to determine the lignin and cellulose fractions, the precipitate was treated with 72% $H_2SO_4$. The filtration residue, washed with water and dried, constituted lignin fraction. Cellulose content was the difference between total fiber content and lignin content.

## Dietary fiber (TDF) content

Total dietary fiber content was determined by the enzymatic-gravimetric method described by LEE et al. (1992). The procedure includes the use of heat-stable alpha-amylase, protease, and amyloglucosidase. Total dietary fiber was calculated as the sum of the amounts of soluble dietary fiber and insoluble dietary fiber.

## Starch content

Starch content was determined by the polarimetric method (AOAC, 1975). Starch hydrolysis was carried out using concentrated hydrochloric acid, and the solution was clarified with acid sodium phosphotungstate. Sugar content was determined on the basis of angle of polarization.

### Colour properties

Instrumental measurement of colour was performed using a MiniScan XE Plus spectrophotometer (HunterLab), at illumination D65, observer 10° and light diffusion 8°. The colour was described on the CIELab scale. The colour of particular carrot varieties was measured on 30 cubes selected at random. The colour of carrots was described by three coordinates in the colour space: L*(lightness), a*(redness), b*(yellowness), measured directly with a spectrophotometer.

### Statistical analysis

The results obtained were subjected to a one-factor analysis of variance. The significance of differences between treatments was determined by the Duncan multiple range test, at $p = 0.05$. The calculations were done using the computer program STATISTICA 6.0 (StatSoft Inc.).

## Results and Discussion

### Dry matter

Dry matter is one of the most important factors, deciding about the processing value and storage quality of carrots (KLUZ 1997). In the carrot lines and varieties analyzed, water content varied from 85.20% (Macon F1) to 90.00% (VCU – 30 F1) – Table 1. There were statistically significant differences ($p \leq 0.05$) between most of the breeding forms examined. Similar values (86 – 90%) were obtained by MICHALIK (1993) and KLUZ (1997).

### Carotenoids content

The statistical analysis showed significant differences ($p \leq 0.05$) in the concentrations of total carotenoids and $\beta$-carotene. Total carotenoids content ranged from 16.69 to 40.59 mg · 100 $g^{-1}$ f.w., and $\beta$-carotene content – from 9.67 to 23.00 mg · 100 $g^{-1}$ f.w. The highest total carotenoids content (29.14 to 40.59 mg · 100 $g^{-1}$ f.w.) was recorded for the following varieties: Nektarina, Allret, Kazan F1 (areola's seeds), Macon F1, VCU – 30 F1. The other varieties contained 16.69 to 24.08% carotenoids. Nektarina, Kazan F1 (areola's seeds), Allret were characterized by the highest $\beta$-carotene content, i.e. 20.45 to

Table 1
Moisture content (wt %), total carotenoids and $\beta$-carotene content (mg · 100 g$^{-1}$ f.w.) of edible carrot varieties – the multiple Duncan test[1]

| Variety | Moisture (%) | Total carotenoids content | $\beta$-carotene |
|---|---|---|---|
| Allret | $87.10 \pm 0.45^{f}$ | $35.05 \pm 0.50^{b}$ | $20.45 \pm 0.15^{c}$ |
| Bangor F1 | $88.20 \pm 0.60^{d,e}$ | $24.08 \pm 0.63^{f}$ | $12.67 \pm 0.50^{g}$ |
| Kazan F1 | $86.90 \pm 0.20^{f}$ | $17.19 \pm 0.45^{j}$ | $10.02 \pm 0.40^{i}$ |
| Kazan F1* | $85.75 \pm 0.30^{g}$ | $32.50 \pm 0.40^{c}$ | $22.02 \pm 0.22^{b}$ |
| Macon F1 | $85.20 \pm 0.28^{g}$ | $30.05 \pm 0.57^{d}$ | $15.38 \pm 0.51^{e}$ |
| Maestro F1 | $89.18 \pm 0.54^{b,c}$ | $15.65 \pm 0.76^{k}$ | $9.67 \pm 0.52^{i}$ |
| Maxima | $88.00 \pm 0.25^{e}$ | $18.15 \pm 0.60^{i}$ | $11.41 \pm 0.60^{h}$ |
| Nandor F1 | $89.60 \pm 0.56^{a,b}$ | $17.11 \pm 0.64^{j}$ | $11.11 \pm 0.52^{h}$ |
| Nektarina | $88.20 \pm 0.17^{d,e}$ | $40.59 \pm 0.48^{a}$ | $23.00 \pm 0.50^{a}$ |
| RX 94115 | $88.10 \pm 0.60^{d,e}$ | $21.30 \pm 0.40^{h}$ | $13.64 \pm 0.37^{f}$ |
| Simba F1 | $87.20 \pm 0.18^{f}$ | $22.63 \pm 0.50^{g}$ | $12.78 \pm 0.40^{g}$ |
| Tango F1 | $88.60 \pm 0.65^{c,d,e}$ | $21.78 \pm 0.50^{h,g}$ | $13.06 \pm 0.45^{g,f}$ |
| Tito | $88.87 \pm 0.45^{b,c,d}$ | $16.69 \pm 0.53^{j}$ | $9.68 \pm 0.13^{i}$ |
| VCU – 30 F1 | $90.00 \pm 0.30^{a}$ | $29.14 \pm 0.52^{e}$ | $17.75 \pm 0.53^{d}$ |

* areola's seeds
[1] mean of $n = 4 \pm$ standard error
*a,b,c* – homogenous groups; the letters correspond to the descending values of successive variables

23.00 mg · 100 g$^{-1}$ f.w. The lowest $\beta$-carotene concentration (9.67 to 11.41 mg · · 100 g$^{-1}$ f.w.) was noted for Maestro F1, Tito, Kazan F1, Maxima, Nandor F1 (Table 1). The $\beta$-carotene content was at a similar level (12.67 – 17.75 mg · · 100 g$^{-1}$ f.w.) in other carrot varieties and lineages. The $\beta$-carotene concentration in the varieties analyzed ranged from 51.2% for Macon F1 to 67.7% for Kazan F1 (areola's seeds). The results of the present experiment are consistent with those obtained by other authors, who also consider $\beta$-carotene to be the dominant carotenoid in carrots (SIMON, WOLF 1987).

## Sugars content

The sugar content of carrots affects, to a high extent, their organoleptic properties. It follows that it is one of the key criteria while evaluating their processing suitability.

In the carrot varieties and lines examined, the concentrations of total and reducing sugars were 5.11 to 7.80 g · 100 g$^{-1}$ f.w., and 1.04 to 4.48 g · 100 g$^{-1}$ f.w. respectively. The Duncan multiple range test was employed to separate 10 groups considered homogenous in terms of total sugar content, which was the highest (7.61 to 7.80 g · 100 g$^{-1}$ f.w.) in the varieties: RX 94115, Macon F1 and Simba F1. The varieties: Tito, Maestro F1 and VCU – 30 F1 were characterised

by the lowest total sugar concentration (5.11 to 5.45 g · 100 $g^{-1}$ f.w.) – Table 2. The highest reducing sugar content (4.03 to 4.48 g · 100 $g^{-1}$ f.w.) was noted for Macon F1, Tito, Maestro F1 and VCU – 30 F1, whereas in Allret, Maxima, RX 94115, Nandor F1 and Nektarina it was by approx. fourfold lower (1.04 to 1.82 g · 100 $g^{-1}$ f.w.).

These results confirm the findings of SELJASEN et al. (2001). In their studies the total sugar content of carrots varied between 5.94 and 7.32 g · 100 $g^{-1}$ f.w.

Table 2

Content of sugars and starch (g · 100 $g^{-1}$ f.w.) of edible carrot varieties – the multiple Duncan test[1]

| Variety | Total sugars content | Reducing sugars | Starch |
|---|---|---|---|
| Allret | 6.49 + 0.09$^{g}$ | 1.04 + 0.08$^{i}$ | 0.50 + 0.06$^{e}$ |
| Bangor F1 | 7.06 + 0.08$^{d,e}$ | 3.57 + 0.02$^{c}$ | 0.60 + 0.03$^{d}$ |
| Kazan F1 | 7.17 + 0.05$^{d}$ | 2.47 + 0.04$^{f}$ | 0.80 + 0.04$^{b}$ |
| Kazan F1* | 7.38 + 0.07$^{c}$ | 2.58 + 0.05$^{e}$ | 0.80 + 0.03$^{b}$ |
| Macon F1 | 7.64 + 0.05$^{b}$ | 4.48 + 0.07$^{a}$ | 0.60 + 0.03$^{d}$ |
| Maestro F1 | 5.37 + 0.07$^{i}$ | 4.38 + 0.07$^{a}$ | 0.50 + 0.06$^{e}$ |
| Maxima | 6.35 + 0.05$^{h}$ | 1.31 + 0.07$^{k}$ | 0.60 + 0.07$^{d}$ |
| Nandor F1 | 6.79 + 0.08$^{f}$ | 1.68 + 0.06$^{i}$ | 0.40 + 0.02$^{f}$ |
| Nektarina | 7.03 + 0.06$^{e}$ | 1.82 + 0.09$^{h}$ | 0.70 + 0.03$^{c}$ |
| RX 94115 | 7.80 + 0.06$^{a}$ | 1.50 + 0.03$^{j}$ | 0.40 + 0.02$^{f}$ |
| Simba F1 | 7.61 + 0.04$^{b}$ | 2.10 + 0.04$^{g}$ | 0.70 + 0.03$^{c}$ |
| Tango F1 | 6.54 + 0.07$^{g}$ | 2.90 + 0.01$^{d}$ | 0.70 + 0.01$^{c}$ |
| Tito | 5.11 + 0.08$^{j}$ | 4.41 + 0.07$^{a}$ | 0.50 + 0.05$^{e}$ |
| VCU – 30 F1 | 5.45 + 0.08$^{i}$ | 4.03 + 0.05$^{b}$ | 0.90 + 0.06$^{a}$ |

* areola's seeds

[1] mean of $n$ = 4 + standard error

*a,b,c* – homogenous groups; the letters correspond to the descending values of successive variables

## Starch content

The starch content of carrots is relatively low. In the varieties analyzed it ranged from 0.40 to 0.90 g · 100 $g^{-1}$ f.w.. The results of the Duncan multiple range test show that 6 homogenous groups can be distinguished in terms of starch concentration. The variety VCU – 30 F1 was characterized by the highest starch content (0.9 g · 100 $g^{-1}$ f.w.). Its concentration was also high (0.8 g · 100 $g^{-1}$ f.w.) in Kazan F1 and Kazan F1 (areola's seeds). The lowest starch content (0. g · 100 $g^{-1}$ f.w.) was noted in the variety Nandor F1 and lineage RX 94115 (Table 2).

## Acid detergent fiber (ADF) and total dietary fiber (TDF)

The statistical analysis indicated significant differences ($p \leq 0.05$) in ADF content (including the lignin and cellulose fractions) and TDF content (Table 3). Total acid detergent fiber varied from 1.15 to 1.74 g · 100 $g^{-1}$ f.w., whereas the contents of the lignin and cellulose fractions – from 0.5 to 0.26 g · 100 $g^{-1}$ f.w., and from 1.11 to 1.63 g · 100 $g^{-1}$ f.w. respectively. The varieties Maestro F1, Tito, Macon F1 and Kazan F1 (areola's seeds) were characterized by the highest total fiber content (1.68 to 1.74 g · 100 $g^{-1}$ f.w.), and Nektarina – by the lowest (1.15 g · 100 $g^{-1}$ f.w.). The dominant fraction was cellulose. The percentage of lignin, considered a less desirable fiber fraction, both for its rheological properties (fibrous structure) and lower processing value, was relatively low, compared with total ADF content, and constituted from 3.36% (Kazan F1) to 15.3% (Tito). In relation to fresh weight, lignin concentration was the highest in the variety Tito, whereas Allret, Kazan F1, Nandor F1 and Nektarina were characterized by a fivefold lower content of this fraction. In the variety Macon the most cellulose contain were found (1.63 g · 100 $g^{-1}$ f.w.), and in the variety Nektarina – the least (1.11 g · 100 $g^{-1}$ f.w.).

Table 3
Acid detergent fiber (ADF) and total dietary fiber (TDF) contents (g · 100 $g^{-1}$ f.w.) in edible carrot varieties – the multiple Duncan test[1]

| Variety | Total | ADF | Celulose | TDF |
|---|---|---|---|---|
| Allret | $1.42 \pm 0.03^{f}$ | $0.05 \pm 0.01^{g,h}$ | $1.37 \pm 0.03^{e}$ | $3.62 \pm 0.29^{b}$ |
| Bangor F1 | $1.42 \pm 0.01^{f}$ | $0.07 \pm 0.01^{f,g}$ | $1.35 \pm 0.02^{e}$ | $3.60 \pm 0.25^{b}$ |
| Kazan F1 | $1.49 \pm 0.02^{e}$ | $0.05 \pm 0.00^{g,h}$ | $1.44 \pm 0.02^{c,d}$ | $3.69 \pm 0.28^{b}$ |
| Kazan F1* | $1.68 \pm 0.01^{b}$ | $0.19 \pm 0.01^{b}$ | $1.49 \pm 0.01^{c}$ | $2.65 \pm 0.01^{f}$ |
| Macon F1 | $1.70 \pm 0.02^{b}$ | $0.07 \pm 0.01^{f,g}$ | $1.63 \pm 0.05^{a}$ | $3.24 \pm 0.29^{c,d}$ |
| Maestro F1 | $1.74 \pm 0.03^{a}$ | $0.16 \pm 0.03^{c}$ | $1.58 \pm 0.02^{b}$ | $3.59 \pm 0.22^{b,c}$ |
| Maxima | $1.39 \pm 0.02^{f}$ | $0.10 \pm 0.01^{d,e}$ | $1.29 \pm 0.02^{f}$ | $3.70 \pm 0.24^{b}$ |
| Nandor F1 | $1.22 \pm 0.02^{h}$ | $0.05 \pm 0.01^{g,h}$ | $1.17 \pm 0.02^{g}$ | $4.38 \pm 0.18^{a}$ |
| Nektarina | $1.15 \pm 0.02^{i}$ | $0.05 \pm 0.02^{h}$ | $1.11 \pm 0.04^{h}$ | $3.09 \pm 0.06^{d,e}$ |
| RX 94115 | $1.53 \pm 0.02^{d}$ | $0.10 \pm 0.01^{d,e}$ | $1.43 \pm 0.04^{d}$ | $4.41 \pm 0.05^{a}$ |
| Simba F1 | $1.35 \pm 0.01^{g}$ | $0.09 \pm 0.01^{e,f}$ | $1.26 \pm 0.03^{f}$ | $3.48 \pm 0.23^{b,c}$ |
| Tango F1 | $1.48 \pm 0.02^{e}$ | $0.12 \pm 0.01^{d}$ | $1.37 \pm 0.03^{e}$ | $4.26 \pm 0.02^{a}$ |
| Tito | $1.70 \pm 0.02^{a,b}$ | $0.26 \pm 0.01^{a}$ | $1.44 \pm 0.03^{c,d}$ | $2.83 \pm 0.07^{e,f}$ |
| VCU – 30 F1 | $1.62 \pm 0.02^{c}$ | $0.20 \pm 0.02^{b}$ | $1.42 \pm 0.03^{d}$ | $3.43 \pm 0.10^{b,c}$ |

* areola's seeds
[1] mean of $n = 4 \pm$ standard error
*a,b,c* – homogenous groups; the letters correspond to the descending values of successive variables

Differences between carrot varieties concerning total fiber content and percentages of its fractions were also observed by other authors (MATHEE, APPLEDORF 1978, CHOBOT 1991). The results of the present experiment correspond with the findings of the above authors.

Total dietary fiber content was higher than ADF content, and varied from 2.65 to 4.41 g · 100 $g^{-1}$ f.w. It was the highest in the variety RX 94115 (4.41 g · 100 $g^{-1}$ f.w.), and approx. threefold lower in Kazan F1 (areola's seeds) and Tito (2.65 – 2.83 g · 100 $g^{-1}$ f.w.) – Table 3. Similar values were reported by PENNER and KIM (1991), and lower – on average by 2.03% – by REDONDO et al. (1997). The results obtained confirm the fact that carrots are rich in dietary fiber, which contributes to the proper functioning of the alimentary tract.

## Colour properties

The colour of raw carrots affects consumer preferences to a great degree. The colour of carrot products is also highly determined by the colour of raw material. The colour of the carrot varieties examined was described by the parameters: L*, a*, b*. Colour lightness (L*) ranged from 60.1 to 72.8, redness (a*) – from 35.67 to 46.68, and yellowness – from 45.07 to 56.76. Four homogenous groups were distinguished for colour lightness L* at $p \leq 0.05$ (Table 4). Its value was the highest in the varieties Maestro F1 (72.8), Tito (68.6) and VCU – 30 F1 (68.2), and the lowest Kazan F1 – areola's seeds (60.1). The varieties Tango F1 and Nandor F1 were characterized by the highest values of a* (46.68 and 46.58 respectively), and Tito – by the lowest (35.67). Significant differences between carrot varieties were also noted for the b* coordinate, whose value was the highest in Macon F1 (56.76), and the lowest in VCU – 30 F1 (45.07).

Table 4

Colour indexes (L*, a*/b*) of edible carrot varieties – the multiple Duncan test[1]

| Variety | L* | a* | b* |
|---|---|---|---|
| Allret | $65.6 \pm 2.9^{b,c}$ | $45.70 \pm 2.8^{a,b}$ | $51.37 \pm 2.9^{b,c,d}$ |
| Bangor F1 | $65.3 \pm 2.8^{b,c}$ | $42.22 \pm 2.7^{a,b,c,d}$ | $56.38 \pm 2.7^{a,b}$ |
| Kazan F1 | $66.4 \pm 2.5^{b,c}$ | $43.16 \pm 2.6^{a,b,c}$ | $51.38 \pm 2.7^{b,c,d}$ |
| Kazan F1* | $60.1 \pm 2.7^{d}$ | $40.39 \pm 2.7^{b,c,d,e}$ | $47.42 \pm 2.6^{d,e}$ |
| Macon F1 | $64.8 \pm 2.9^{b,c,d}$ | $45.19 \pm 2.5^{a,b}$ | $56.76 \pm 2.5^{a}$ |
| Maestro F1 | $72.8 \pm 2.7^{a}$ | $37.58 \pm 3.0^{d,e}$ | $54.38 \pm 2.8^{a,b,c}$ |
| Maxima | $66.9 \pm 2.5^{b}$ | $43.06 \pm 2.9^{a,b,c}$ | $51.11 \pm 2.9^{b,c,d}$ |
| Nandor F1 | $63.9 \pm 2.8^{b,c,d}$ | $46.58 \pm 2.8^{a}$ | $56.46 \pm 2.6^{a,b}$ |
| Nektarina | $64.3 \pm 2.6^{b,c,d}$ | $42.49 \pm 2.7^{a,b,c,d}$ | $54.29 \pm 2.9^{a,b,c}$ |
| RX 94115 | $66.6 \pm 2.6^{b}$ | $42.01 \pm 2.9^{a,b,c,d}$ | $49.90 \pm 2.8^{c,d,e}$ |
| Simba F1 | $66.8 \pm 2.4^{b}$ | $44.11 \pm 2.6^{a,b}$ | $54.26 \pm 3.0^{a,b,c}$ |
| Tango F1 | $61.3 \pm 2.7^{c,d}$ | $46.68 \pm 2.9^{a}$ | $55.51 \pm 2.9^{a,b}$ |
| Tito | $68.6 \pm 2.8^{a,b}$ | $35.67 \pm 2.8^{e}$ | $48.93 \pm 2.7^{d,e}$ |
| VCU – 30 F1 | $68.2 \pm 2.8^{a,b}$ | $38.72 \pm 3.0^{c,d,e}$ | $45.07 \pm 2.7^{e}$ |

* areola's seeds

[1] mean of $n = 4 \pm$ standard error

*a,b,c* – homogenous groups; the letters correspond to the descending values of successive variables

## Summary

The 12 hybrid and established carrot varieties and lineages analyzed in the present experiment differed in the contents of dry matter, total carotenoids, $\beta$-carotene, total sugars, reducing sugars, starch, acid detergent fiber, total dietary fiber, and colour. In most cases these differences were statistically significant ($p \leq 0.05$).

Among the varieties and lineages examined, Nektarina, Kazan (areola's seeds) and Allret were characterized by high concentration of carotenoids (including $\beta$-carotene), RX 94115, Macon F1 and Simba F1 – by a high sugar content, Maestro F1, Macon F1 and Tito – by a high ADF content, and RX 94115, Nandor F1 and Tango F1 – by a high TDF content. Considerable differences between carrot varieties, concerning especially the concentrations of carotenoids (including $\beta$-carotene), sugars and fiber, as well as colour properties, enable their appropriate selection for consumption and processing (e.g. pomace, deep – frozen and dried products).

Accepted for print 04.11.2005

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., No 20(1): 455-464, Y. 2006

# EFFECT OF THE HERBICIDE AVANS 330 SL AND AZOPRIM 50 WP ON SKIN PATHOMORPHOLOGY OF HEALTHY AND PATIENT CARP WITH *ICHTHYOPHTHIRIASIS*

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K e y w o r d s: carp (*Cyprinus carpio* L.), skin, *Ichthyophthirius multifiliis*, glyphosate, atrazine, pathomorphology.

A b s t r a c t

Clinically healthy and naturally infected by ciliates (*Ichthyophthirius multifiliis*) invasion fingerlings of carp (*Cyprinus carpio* L.) were studied. Fish were affected with Avans 330 SL and Azoprim 50 WP in a concentration of 2 mg · $dm^{-3}$ of active substance: glyphosate and atrazine, respectively. The aim of the study was to define influence of the herbicides, both separately and in combination, on skin morphology of healthy and infected carp. The most frequently appeared changes were skin mucous cells proliferation and epidermis desquamation, occasionally necrosis. Parasitic invasion and combined herbicide application strengthened, both degree of intensification and range, of the morphological lesions in carp skin during the experiment.

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# WPŁYW HERBICYDÓW AVANS 330 SL ORAZ AZOPRIM 50 WP NA PATOMORFOLOGIĘ SKÓRY KARPI ZDROWYCH I CHORYCH NA ICHTIOFTIRIOZĘ

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S ł o w a k l u c z o w e: karp (*Cyprinus carpio* L.), skóra, *Ichthyophthirius multifiliis*, glifosat, atrazyna, patomorfologia.

A b s t r a k t

Badaniom poddano narybek karpia (*Cyprinus carpio* L.) klinicznie zdrowego i zarażonego naturalną inwazją orzęska (*Ichthyophthirius multifiliis*). Ryby kąpano w roztworze preparatu herbicydowego Avans 330 SL lub/i Azoprim 50 WP o stężeniu 2 mg · $dm^{-3}$ aktywnej substancji (odpowiednio glifosatu i atrazyny). Celem pracy było określenie wpływu herbicydów, stosowanych oddzielnie lub razem, na morfologię skóry zdrowych i zainfekowanych ryb. Najczęściej występującymi zmianami był rozplem komórek śluzowych i złuszczanie naskórka, rzadziej martwica. Inwazja pasożytnicza i łączne zastosowanie preparatów nasilały zarówno stopień intensywności zmian morfologicznych, jak i ich rozległość w skórze karpia w czasie trwania doświadczenia.

## Introduction

The generally used in agriculture herbicides are the main source of non punctual water pollution. Their harmful influence is strengthened by accumulation, bioaccumulation and concentration in food chain what is dangerous for environment and alive organisms, including fish (GLUTH et al. 1985).

Gills are the most exposed to pollutants organs; pesticides penetrate them most easily and quickly. Also kidneys and liver are the organs reacting with morphological lesions to accumulation and transformation of xenobiotics. Skin and gastrointestinal tract show usually small changes in the specific morphology. Harmful influence of pesticides was also revealed with immune suppressive effect (SOPIŃSKA et al. 1995, NEŠKOVIĆ et al. 1996, KOLMAN et al. 2003). Recently, increase of water and soil pollution is noted but there is lack of morphological studies of organs of fish being under influence of low pesticide doses.

There are few experiments demonstrating moderately harmful influence

of glyphosate, an active substance of Avans 330 SL (AV), on mammals and fish (FRANZ et al. 1997, RASZKA et al. 1998). Microscopic lesions in hepatocytes and cells of kidney ducts and ultrastructural changes in the liver and pancreas were observed (STUDNICKA, SIWICKI 1997, SZAREK et al. 1997, 2000a,b).

Atrazine, the active substance of Azoprim 50 WP (AZ), is in a small part only cleared away from the environment through biodegradation processes, and became persistent contaminant of surface and ground-water (SOLOMON et al. 1996, SAGLIO, TRIJASSE 1998). This phenomenon promotes chronic toxicity inducing morphological, biochemical and physiological changes in fish (FISHER-SCHERL et al. 1991, SAGLIO, TRIJASSE 1998). FISHER-SCHERL et al. (1991) proved that 28 days exposition to atrazine at a concentration of 5 $\mu g \cdot dm^{-3}$ caused morphological lesions in rainbow trout, and ALAZEMI et al. (1996) and NEŠOVIĆ et al. (1993) observed histological changes of gills. It has been found (SZAREK et al. 2000a) that atrazine in a concentration of 2 $mg \cdot dm^{-3}$ caused pathological lesions in carp hepatopancreas (blood circulation disturbance, seldom regressive and sporadic – progressive lesions).

Homeostasis distemper noted in various (viral, bacterial, parasitical) diseases can contribute to increased sensitivity of fish to xenobiotic activity and thus, magnify their hazardous influence. Increase of ciliates *Ichthyophthirius multifiliis* (ICH) invasion is noted as a result of pond conservation limitation.

Morphological patterns of skin of carp fingerlings, healthy or naturally infected with ICH, treated separately or simultaneously with glyphosate and atrazine at low concentrations, were studied at the experiment. The study also included an observation of carp behaviour.

The above mentioned active substance of herbicides were taken into consideration to fix the preparations concentration (2 $mg \cdot dm^{-3}$): the presence of the examined active substances in fish organisms (BANASZKIEWICZ 2003), the results of own studies indicating that the so-called toxically safe herbicide level causes changes in fish organisms (SZAREK et al. 2000a,b), data signalled by other authors (GHOSH, KONAR 1983, DEMAEL et al. 1990, DUNIER, SIWICKI 1993).

## Materials and Methods

Carp fingerlings (*Cyprinus carpio* L.) of 81 – 98 g in body mass were used in the experiment. Fish were obtained from two breeding centres: Fish Farm Wąsosze and Experimental Fish Farm of the Freshwater Fishery Institute in Żabieniec (Poland). Animals were fed with granulated feed (Aller 37/12) produced by Aller Aqua. Feed contained 35% protein, 31% carbohydrate, 12% fat, 7% ash and 4% fibre and its energetic value was 19.5 MJ/kg. Before the experiment, carp were acclimated to laboratory conditions for two weeks.

The study was conducted in two separate experiments: A – clinically healthy carp, free from ICH and B – infected with this protozoa (permission of Local Ethical Commission No 31/N). In each experiment, fish were divided into four equal groups ($n = 10$). Fish of group A1 and B1 serve as control ones and A2 and B2 carps were treated with Avans 330 SL (AV), A3 and B3 – Azoprim 50 WP (AZ) and A4 and B4 – mixture of the preparations. Carps were bathed in water with preparation additive in an active substance concentration equal to 2 $mg \cdot dm^{-3}$; in case of mixture concentration of each active substance was 1 $mg \cdot dm^{-3}$.

Fish stayed in 200 $dm^3$ tanks in similar environmental conditions: water temperature 18 – 19°C, oxygen level 7.5 – 8.0 $mg \cdot dm^{-3}$, pH 7.5 – 8.5. The amount of total ammonia nitrogen did not exceed 0.2 $mg \cdot dm^{-3}$. Fish were not fed during the experiment, i.e. for 96 hours.

The AV preparation used in the experiments was produced by Zeneca Agrochemicals, Great Britain. Its active substance is trimethylosulfonium glyphosate with a 3 days period of half-life, 360 g per 1 $dm^3$ of the preparation. AZ was produced by Zakłady Chemiczne „Organika-Azot" S.A. in Jaworzno, Poland and contained 50% of atrazine at half-time of degradation equal to 8 – 14 days.

After 96 hours of bathing the carps in water with the addition of AV and AZ, fish were recovered from the tanks. They were moved into 10 $dm^3$ tanks containing water with the addition of Propiscin preparation (0.2% etomidate solution), produced by the Inland Fisheries Institute in Żabieniec (Poland) and anesthetized.

Macroscopic examinations were conducted directly after sacrificing of fish. Skin samples were taken for microscopic analysis, fixed in 5% neutralized formalin and after dehydration in a series of alcohols and acetone, embedded in paraffin blocks. Microscopic fragments were stained with haematoxylin and eosin (BANCROFT, COOK 2000).

## Results and Discussion

It was clinically stated that carps of experiment A behaved properly and clearly reacted to the external impulses. In the experiment B only control fish (B1) behaved properly throughout the time course of the experiment; in the remaining B groups only during the first day. Since the second day of experiment increased excitability and activity have been observed in fish bathed with pesticide addition. Violent motions without clear stimulation from outside were noted but on the third and fourth day ponderousness was noticeable. Fingerlings from both experiments were checked macroscopically and proper morphological structure was found.

Skin of control carps (A1) was characterized by proper structure (Figure 1). In two cases proliferation of mucous cells (Figure 1) was found in epidermis of fish exposed to AV (A2 group). Sporadic morphological changes were observed in skin of carps bathed in AZ (A3) and AV and AZ simultaneously (A4). In A3 group one case of congestion and two cases of mucous cells proliferation were stated. The same morphological changes were found in fish from A4 group (2 cases of congestion and three cases of mucous cells proliferation) – Figure 2.

Two control carps of experiment B revealed congestion of skin capillary vessels, desquamation of epidermis and proliferation of mucous cells and in one case – defect of epidermis (Figure 3). Desquamation of epidermis and proliferation of mucous cells were observed in 2 fish infected with *Ichthyophthirius multifiliis* and simultaneously bathed with addition of AV (Figure 4). In B3 group epidermis desquamation was observed in 3 fish, congestion of capillary vessels in 2 carps, proliferation of mucous cells in one fingerling and their hypertrophy in 2 individuals (Figure 5).

Desquamation of epidermis was noted in 4 fish of group B4. Two carps infected with protozoa and exposed simultaneously at AV and AZ (group B4) revealed necrosis of epidermal cells and in 1 case congestion was detected (Figure 6).

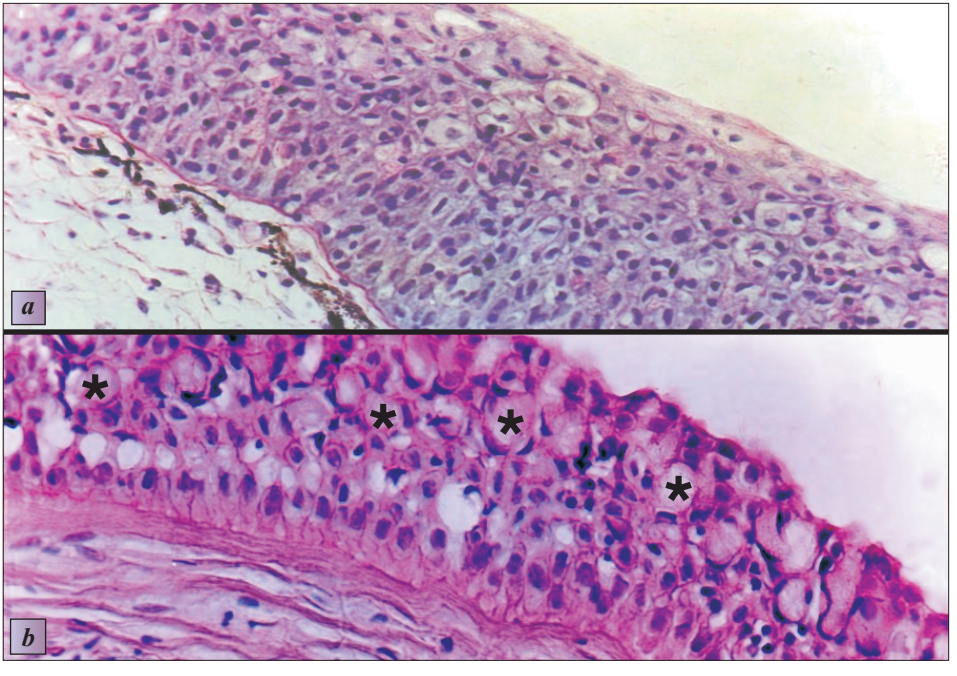


Fig. 1. Proper morphology of skin of carps from experiment A: *a* – epidermis with surface, glandular and generative layers – carp from group A1, *b* – proliferation of mucous cells – carp from group A2. HE stain., magn. X 500

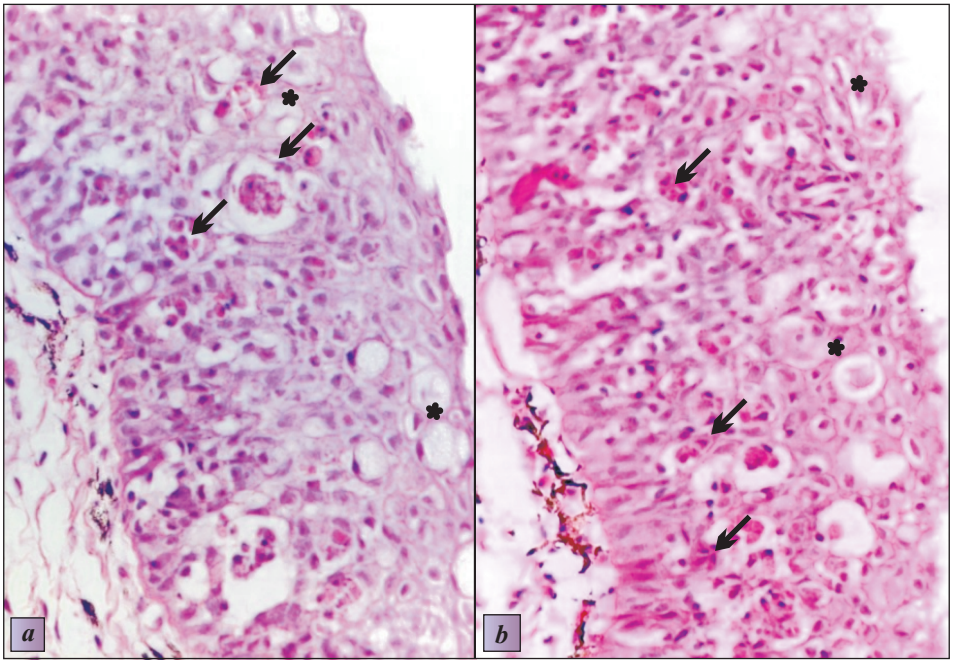


Fig. 2. The microscopic pattern – the skin of carps from experiment A: *a* – congestion (arrows) and proliferation of mucous cells (stars) – carp from group A3, *b* – congestion (arrows), proliferation of mucous cells (stars), epidermis desquamation – carp from group A4. HE stain., magn. X 500

The observed lesions in behaviour of fish infected with ICH and exposed to glyphosate and/or atrazine proved that ICH had an influence on skin morphology in AV and AZ intoxication. Parasite infection, even in the initial stage, strengthened pathogenic effect of the preparations on the fingerling organism.

Considering influence of pesticides on fish organism one must turn over both their direct action and indirect effect resulting from changes in water quality and feed accessibility and from contact with agents creating fish health, such as parasites, bacteria or viruses. Xenobiotic presence in the environment can be understand as stress, which impact results in changes of biochemical and physiological parameters, particularly at lower biological levels.

Analysis of morphological lesions is one of three evaluation criteria proposed to evaluate influence of sublethal concentrations of xenobiotics on fish (MITROVIĆ 1972, NEŠKOVIĆ et al. 1996, SZAREK et al. 1997, 2000b). Histological changes are the final effect of unfavourable biochemical and physiological changes appearing in fish organism under the influence of herbicides, so, they can show mechanism of toxicity and point out the most imperilled organs. This kind of experiment can also be applied to evaluate the impact of xenobiotics in very low concentration.

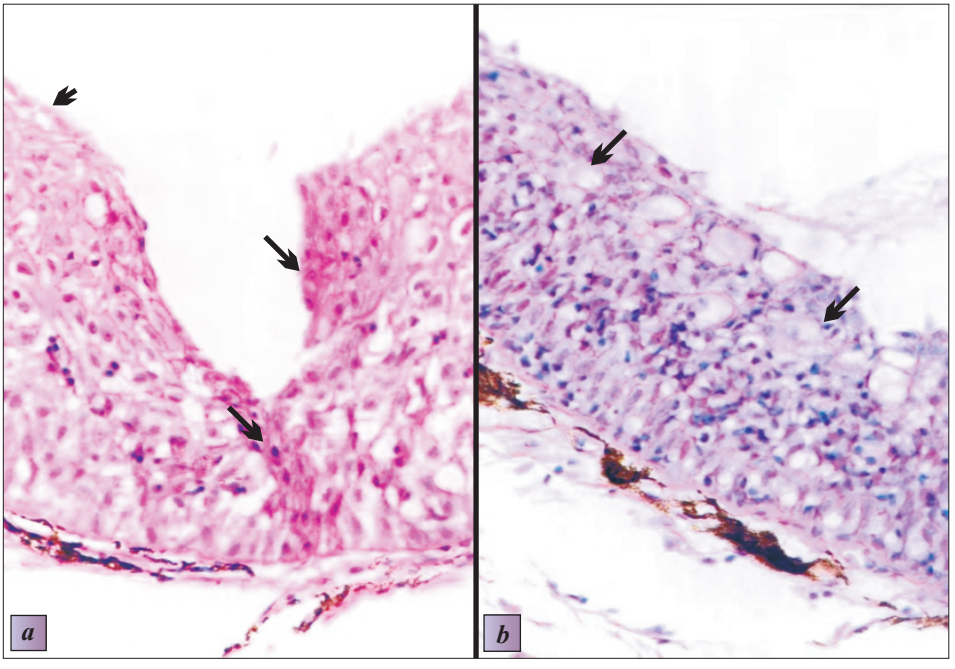


Fig. 3. The microscopic pattern – the skin of carps from group B1: *a* – defect in epidermis with marked regeneration (long arrows), epidermis desquamation (short arrow), *b* – epidermis desquamation and proliferation of mucous cells (arrows). HE stain., magn. X 500

The available literature shows only the morphological patterns of organs of clinically healthy fish influenced with herbicide preparations or their active substances in various concentrations (FISHER-SCHERL et al. 1991, NEŠKOVIĆ et al. 1996, SZAREK et al. 1997, 2000a,b). There is no information on fish affected with infections. For that reason the study on carp infected by ICH contributes new pathological data.

It is worth to stress that concentrations of the active substances of the pesticide preparations were 2 $mg \cdot dm^{-3}$ of glyphosate or atrazine and 1 $mg \cdot dm^{-3}$ of each one when used in mixture. This allowed to have a new look at the results, and to observe the morphological changes on microscopic level in carp organisms influenced by the xenobiotics.

Summarizing we should emphasize that the herbicides: Avans 330 SL and Azoprim 50 WP, at a concentration of 2 $mg \cdot dm^{-3}$ of one active substance or of 1 $mg \cdot dm^{-3}$ of them in mixture, caused relatively few lesions in skin of clinically healthy and *Ichthyophthirius multifiliis* infected carps after 96 h of bath. The most frequent lesions were progressive changes (proliferation and hypertrophy of mucous cells, regeneration of epidermis),

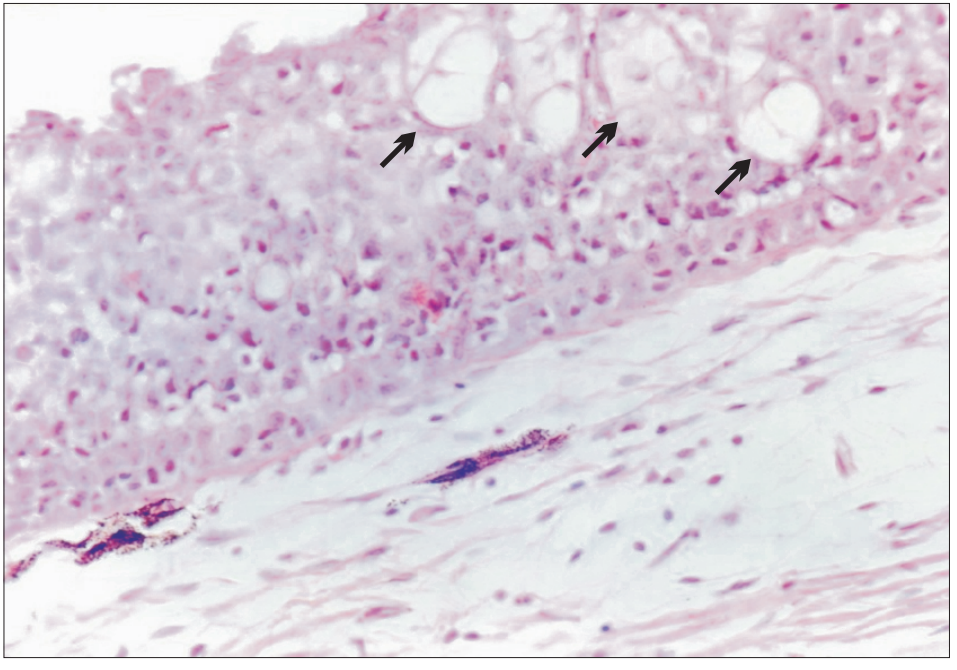

Fig. 4. Fragment of skin of carp from group B2: – hypertrophy of mucous cells (arrows), epidermis desquamation. HE stain., magn. X 500

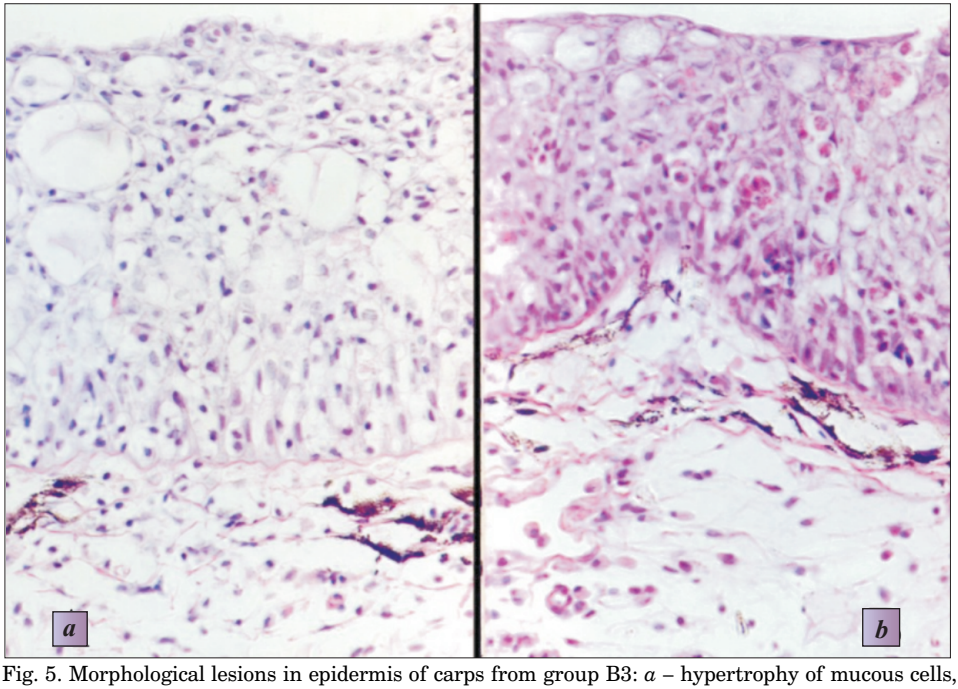


Fig. 5. Morphological lesions in epidermis of carps from group B3: *a* – hypertrophy of mucous cells, *b* – congestion. HE stain., magn. X 500

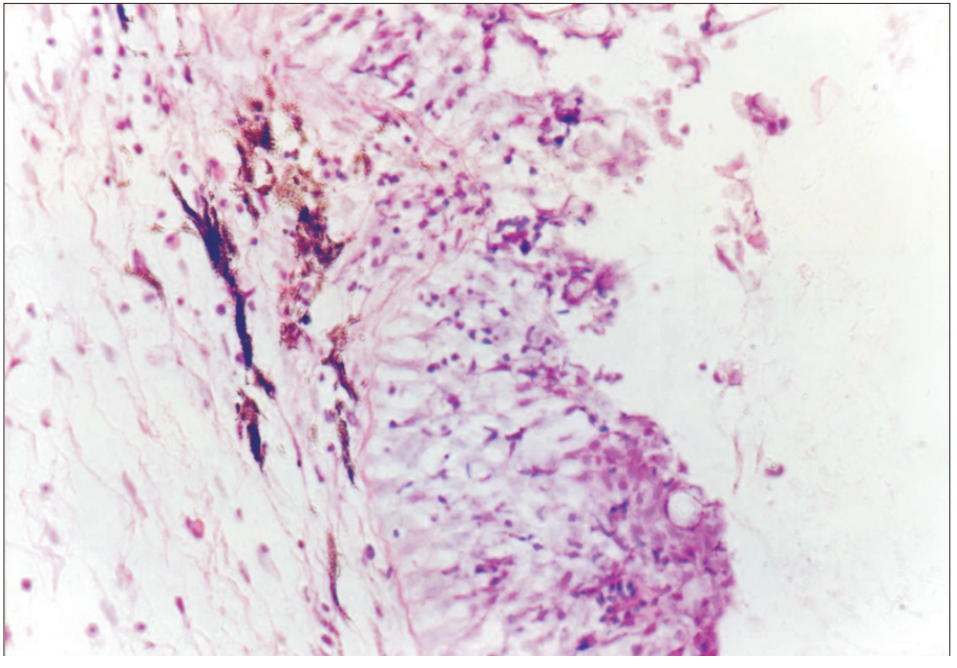

Fig. 6. The microscopic pattern – the skin of carp from group B4 – necrosis and epidermis desquamation. HE stain., magn. X 500

more scarce – regressive ones (epidermis desquamation and necrosis) and disturbances in circulation (congestion). It has been also found that AV and AZ caused morphological changes in skin of ICH infected carps with frequency of 20% higher than in healthy ones.

We can conclude that glyphosate and atrazine contribute to morphological lesions in carp skin, especially infected with *Ichthyophthirius multifiliis*.

## Acknowledgements

The research was carried out within the framework of grant KBN No 3 P04G 019 22.

* Financial support from the funds of the European Social Fund and national budget in terms of the Integrated Operation Programme of the Regional Development 2004-2006.

Translated by KRYSTYNA A. SKIBNIEWSKA

Accepted for print 2006

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