UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

Polish Journal of Natural Sciences

(1/2007) 22

PUBLISHER UWM
OLSZTYN 2007

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

The Journal is also available (from volume 22) in electronic form. The online edition
is hosted by MetaPress (www.metapress.com) in partnership with Versita
(www.versita.com)

PL ISSN 1643-9953

PUBLISHER UWM OLSZTYN

Address
ul. Jana Heweliusza 14
10-718 Olsztyn-Kortowo, Poland
tel.: (48) (089) 523-36-61
fax: (48) (089) 523-34-38
e-mail: wydawca@uwm.edu.pl

Ark. wyd. 12,3 ark. druk. 10,00
Druk – Zakład Poligraficzny UWM w Olsztynie
zam. nr 219

TABLE OF CONTENTS

Agriculture

Animal Breeding and Husbandry

Environmental Protection

Fishery

Food and Nutrition Sciences

Veterinary Medicine

SPIS TREŚCI

Rolnictwo

Chów i Hodowla Zwierząt

Ochrona Środowiska

Rybactwo

Nauka o Żywności i Żywieniu

Medycyna Weterynaryjna

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 1-14, Y. 2007

DOI 10.2478/v10020-07-0001-z

THE EVALUATION OF AFTER-WINTER WATER RESERVES IN THE ARABLE LAYER OF LIGHT SOIL

***Czesław Koźmiński*[1], *Bożena Michalska*[2], *Małgorzata Czarnecka*[2]**

[1] Department of Climatology and Marine Meteorology
Szczecin University
[2] Chair of Meteorology and Climatology
University of Agriculture

K e y w o r d s: after-winter reserves of water, regression models, evaluation, verification.

A b s t r a c t

The present study was based on the results of the measurements of soil moisture taken by means of the dryer-weight method at the end of March and also the results of the measurements of ten day and monthly meteorological measurements taken in the region of Szczecin in the years 1961-1992 and 2001-2004. An equation of multiple regression was determined for evaluating after-winter water reserves in bare light soil at the end of March in the layers of 0-10, 0-20 and 0-30 cm. The variables explaining the quantity of after-winter reserves of water were precipitation, the number of days with precipitation, the temperature of soil, the temperature of air and the sunshine from different autumn and winter months. Prognostic usefulness of regression models were assessed by means of two methods: the method of relative errors and the Cross-Validation test of the LOO (Leave-One-Out) version. The created regression models make it possible to form, on the basis of meteorological data gathered in autumn and winter, good estimates of after-winter water reserves for the end of March in the 0-20 cm and 0-30 cm layers of light soil and satisfactory estimates in the 0-10 cm layer.

SZACOWANIE POZIMOWYCH ZAPASÓW WODY UŻYTECZNEJ W ORNEJ WARSTWIE GLEBY LEKKIEJ

***Czesław Koźmiński*[1], *Bożena Michalska*[2], *Małgorzata Czarnecka*[2]**

[1] Zakład Klimatologii i Meteorologii Morskiej
Uniwersytet Szczeciński
[2] Katedra Meteorologii i Klimatologii
Akademia Rolnicza w Szczecinie

S ł o w a k l u c z o w e: pozimowe zapasy wody w glebie; modele regresyjne; szacowanie; weryfikacja.

Address: Czesław Koźmiński, Szczecin University, Wąska str. 13, 71-415 Szczecin, Poland, phone: 48 (091) 444 16 43, e-mail: klimet@univ.szczecin.pl

Abstrakt

W pracy wykorzystano wyniki pomiarów wilgotności gleby metodą suszarkowo-wagową wykonane w końcu marca oraz dekadowe i miesięczne pomiary meteorologiczne w rejonie Szczecina w latach 1961-1992 i 2001-2004. Opracowano równania regresji wielokrotnej do szacowania pozimowych zapasów wody użytecznej w nieporośniętej glebie lekkiej w końcu marca, w warstwach: 0-10, 0-20 i 0-30 cm. Zmiennymi objaśniającymi wielkość pozimowych zapasów wody były: opady, liczba dni z opadem, temperatura gleby i powietrza oraz nasłonecznienie w miesiącach jesienno-zimowych. Prognostyczną przydatność modeli regresyjnych oceniono dwoma metodami: względnych błędów relatywnych oraz testem Cross-Validation w wersji LOO (Leave-One-Out).

Utworzone modele regresyjne umożliwiają (na podstawie danych meteorologicznych z jesieni i zimy) tworzenie dobrych szacunków pozimowych zapasów wody użytecznej w warstwach gleby lekkiej 0-20 i 0-30 cm w końcu marca, a szacunków dostatecznych w warstwie 0-10 cm.

Introduction

In Poland climatic balances of water are characterized by large variability during a year, particularly in the second half of spring and during summer. A significant component of these balances are the after-winter reserves of water in the layer up to 100 cm, which are used by cultivated plants, usually up to the middle of May, and in the wet months even up to the beginning of June (KOŹMIŃSKI, MICHALSKA, 1995). The quantity of water reserves in soil depends mainly on the meteorological conditions in autumn and winter (KOŹMIŃSKI, MICHALSKA 2005a,b, YOO et al. 1998). In winter the quantity of infiltration and accumulation of water results from the mutual relation of precipitation and soil temperature, what was indicated by CZARNECKA (2004), SHOOP and BIGL (1997). Statistically significant tendencies of an increase in sunshine and temperature in February and March observed in Poland are favourable for enlarging water retention on the one hand and for faster vaporization of the surface layer of soil on the other hand. As a result, large fluctuation in after-winter water reserves in soil is observed year after year (KOŹMIŃSKI, MICHALSKA 2004). Hence the appropriate use of water in early spring by means of, among other things, development and control of the irrigation system becomes more and more important for agriculture and water management (ŁABĘDZKI, BĄK 2004).

So far the authors have worked out equations of multiple regression for forecasting after-winter reserves of water in the soil layers up to the depth of 50, 70 and 100 cm on the basis of meteorological data (KOŹMIŃSKI, MICHALSKA 2005a). Whereas in the present paper, regression models for evaluating after-winter reserves of water in the light soil layer up to the depths of 10, 20 and 30 cm have been worked out, using many year measurements of soil moisture taken by means of the dryer-weight method carried out at the agrometeorological station in the region of Szczecin and also using parallel

meteorological measurements. Variability of water reserves in these layers of soil is twice as high in comparison with variability of the water reserves in deeper layers.

Materials and Methods

In the paper the results of the measurements of soil moisture at the depths of 5, 10, 20 and 30 cm taken at the end of March at the agrometeorological station in Lipki near Szczecin (53°21'N,14°28'E) in the years 1962-1991 and 2001-2004 were used.

In the area of the station there is a brown sour soil formed from the boulder clay sand with clay inserts at the depth of 60-80 cm. The ground water is at the depth of about 6 m. Field water capacity for the soil layer up to the depth of 30 cm is 53.3 mm and the withering coefficient pF = 2.42-2.52 of dry mass of soil. The measurements of moisture on bare soil were made by means of the dryer-weight method at the end of March. The reserves of useful water (RUW) were calculated according to the following equation:

$$\mathrm{RUW} = 0.1 {}^{b}_{fs} (m - MP)\, z$$

where:
${}^{b}_{fs}$ – soil density (kg · m^{-3});
m – soil moisture (% of dry mass);
MP – moisture of the withering point (%);
z – thickness of the layer (m).

Statistical characteristics of after-winter useful water reserves in soil at the end of March are shown in Table 1, and their variability according to 11 quantile intervals – in Table 2.

For forecasting after-winter reserves of water in soil for the 31st of March, multiple regression equations were worked out, in which several meteorological factors in the ten day and monthly periods system from autumn and winter were taken into consideration. As a result of successive approximations (a method of stepwise ridge regression) the regression equations which described the after-winter reserves of useful water in soil in the best way were eventually chosen and the explaining variables were significant at the level of at least p<0.05.

The usefulness of multiple regression equations for evaluation purposes was assessed by means of relative errors b_i:

$$b_i = \frac{(y_i - \hat{y}_i)}{y_i} \cdot 100\%$$

and absolute errors of forecasts b.

$$b = \frac{1}{n} \sum_{i=1}^{n} |b_i|$$

where:

y_i – real yield,

$\hat{y}_i$ – field calculated according to the prognostic model.

Table 1

Statistical characteristics of after-winter reserves of useful water at the end of March in the layers of soil up to the depth of 10, 20 and 30 cm (1962-1991)

Layer cm	Average mm	Maximum mm/year	Minimum mm/year	Standard deviation mm	Coefficient of variation	Coefficient of correlation
0-10	11.5	19.6 ('70)	5.4 ('74)	3.9	34.2	-0.303
0-20	22.8	38.1('70)	10.1 ('90)	6.8	29.8	-0.382*
0-30	35.6	55.0 ('70)	19.8 ('90)	8.7	24.5	-0.456**

* significant at $p = 0.05$
** significant at $p = 0.01$

Table 2

Criteria of classification of after-winter reserves of useful water

Order of quantiles 1962-1991 (%)	Characteristics of after--winter reserves of useful water	Class	Values of reserves of water in the layers of soil (mm)		
			0-10 cm	0-20 cm	0-30 cm
>95	extremely large	11	> 17.8	> 34.0	> 49.8
90.01-95	anomalously large	10	16.5 – 17.8	31.6 – 34.0	46.7 – 49.8
80.01-90	very large	9	14.8 – 16.4	28.6 – 31.5	42.9 – 46.6
70.01-80	large	8	13.5 – 14.7	26.5 – 28.5	40.2 – 42.8
60.01-70	moderately large	7	12.5 – 13.4	24.6 – 26.4	37.8 – 40.1
40.01-60	average	6	10.5 – 12.4	21.2 – 24.5	33.4 – 37.7
30.01-40	moderately small	5	9.5 – 10.4	19.3 – 21.1	31.0 – 33.3
20.01-30	small	4	8.2 – 9.4	17.2 – 19.2	28.3 – 30.9
10.01-20	very small	3	6.5 – 8.1	14.2 – 17.1	24.4 – 28.2
5.01-10	anomalously small	2	5.0 – 6.4	11.6 – 14.1	21.1 – 24.3
<5	extremely small	1	< 5.0	< 11.6	< 21.1

It was assumed according to FABER et al. (1996) that the results of modelling are considered to be good if the difference between real values and those assessed by means of regression equations does not exceed 10%. In the course of the verification of the equations shown in tables concerning the years 1962-1991, each time the data from the examined year were eliminated ($n - 1$) and the quantity of after-winter reserves of useful water in soil was calculated.

The second method that was used for the verification of equations is the Cross-Validation test (KUCHAR 2001) in the LOO (‚Leave-One-Out') version. The test consisted in multiple division of the data into two subsets. One of them served for the estimation of the parameters of the model whereas the other – for the verification of the model. The subset serving for the estimation consisted, each time, of $n - 1$ elements and the remaining element was used for the verification. According to this rule each observation was subject to the verification in turns. The basic measure of adjusting a testing function by this method is a coefficient of multiply correlation R^2 (%) in the form of:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y})^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \qquad R^2 \in \langle 0.1 \rangle$$

and during the elimination of the i^{th} observation in the estimation and using it for the verification of the model, the value of the determination coefficient of prediction RR_{pred} (%) was calculated by means of the Cross-Validation method in the form of:

$$RR_{\text{pred}} = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_{i-1})^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$$

where:

y_i – empiric observations,
$\hat{y}$ – values assessed by means of the model,
$\hat{y}_{i-1}$ – values assessed by means of the model using the Cross-Validation test,
$\bar{y}$ – an average value of the sample,
n – the number of observations.

If the RR_{pred} value differed slightly from R^2 and was significant, it was assumed that the model was built properly and can constitute the basis for forecasting.

In the next stage of the studies the equations were assessed on the grounds of soil moisture measurements for the successive years 2001-2004, not included in the regression models constructed earlier.

After-winter reserves of useful water were divided according to 11 quantile intervals – from the extremely small ones (<5%) to the extremely large ones (>95%).

Results and Discussion

In the 0-10 cm layer of soil average reserves of useful water amounted to 11.5 mm in 1962-1991, varying from 5.4 mm in 1974 to 19.6 mm in 1970 (Table 1). In the 0-20 cm layer of soil average reserves of useful water amounted to 22.8 mm with extreme values varying from 10.1 mm (1990) to 38.1 mm (1970). Whereas in the layer of 0-30 cm the above reserves were 35.6 mm, 19.8 mm (1990), 55.0 mm (1970) respectively. The standard deviation of after-winter reserves of water increases with an increase in depth from 3.9 mm to 8.7 mm, while values of variation coefficient varies from 34.2 to 24.5% (Table 1). In the analyzed period of 30 years a negative tendency of the values of after-winter reserves of useful water was observed (Figure 1) and this might indicate the worsening of moisture of the surface layer of soil in early spring and, at the same time, more frequent occurrence of soil droughts (KOŹMIŃSKI, MICHALSKA 1995, ŁABĘDZKI, BĄK 2004).

The values of quantiles limiting the interval of after-winter reserves of water in soil (the values of quantiles – 40.01 and 60%) amount, in the 0-10 cm layer, to 10.5 and 12.4 mm respectively, in the 0-20 cm layer – 21.2 and 24.5 mm and in the 0-30 cm layer – 33.4 and 37.7 mm. Anomalously small reserves of water, not exceeding 10% of quantile values amount to 6.4 mm in the 0-10 cm layer, 14.1 mm in the 0-20 cm layer and 24.3 mm in the layer of 0-30 cm. Whereas anomalously large water reserves – exceeding 90% of quantile values amount, in particular layers of soil to 16.5, 31.6 and 46.7 mm, respectively. On the basis of 11 determined quantile intervals assessment of each year from the periods of 1962-1991 and 2001-2004 was made (Table 3). In the three analyzed layers, in the 60's dominating were the reserves of useful water the values of which were higher than the average values and in the 70's the values lower than the average ones were dominating in these analyzed layers. It is noteworthy that in the region of Szczecin the discussed reserves of water, both those of the values above the average and those of the values below the average often occurred in two or three successive years. A decreasing tendency of after-winter reserves of water in the surface layer of light soil is confirmed by the results of measurements made in the years 2001-2004 in which small and very small reserves of water were observed (Table 3).

Large variability of weather conditions in winter and autumn is the main cause of fluctuations in after-winter reserves of water in soil measured at the end of March (KOŹMIŃSKI, MICHALSKA 2005 a,b, ŻYROMSKI 2001, YOO et al. 1998, MEIXUE et al. 2003). Hence weather services in various countries commonly use weather information from these two seasons in statistical models for the assessment and forecast of the soil moisture conditions and the amount of water in soil during plant vegetation. (YANG et al. 2003). Variability

of water reserves in soil, according to GUSEV et al. (1998), ŻYROMSKI (2001), KOŹMIŃSKI, MICHALSKA (2001), depend more on current fluctuations in precipitation, evaporation and a plant growing phase than on the initial amount of water in soil.

In the present paper out of 15 variables, explaining the values of after-winter reserves of water, used in the regression equations, the largest influence on their quantity have air temperature in March (x_{15}), soil temperature in

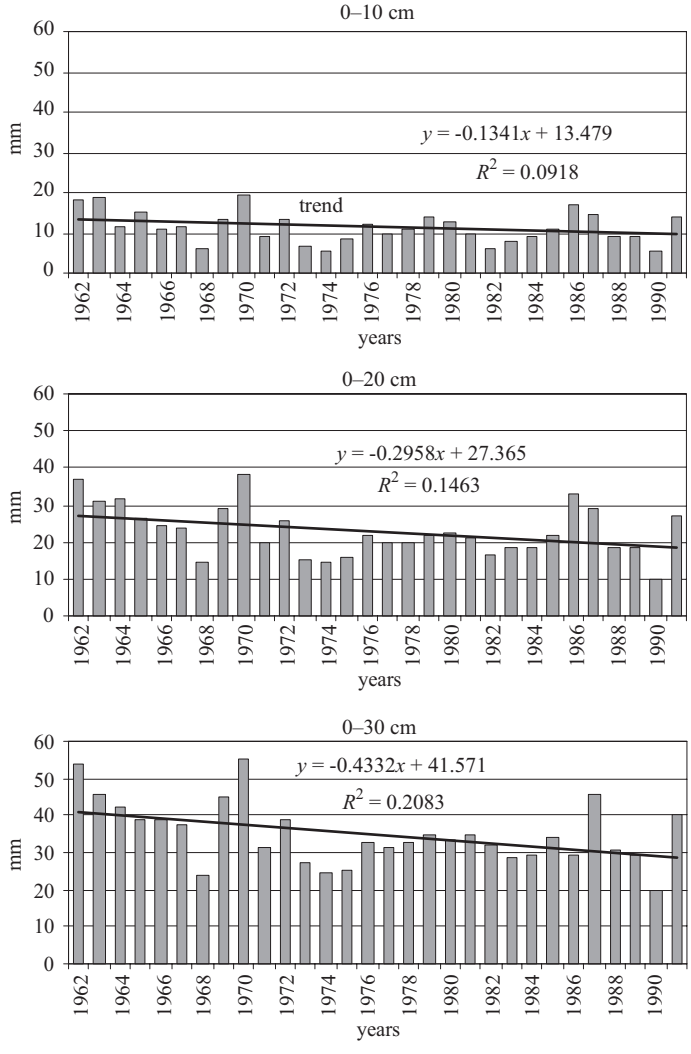


Fig. 1. After-winter reserves of useful water on 31st March in the soil layers: 10, 20 and 30 cm; in successive years of the period 1962-1991

Table 3
Evaluation of useful water reserves in the soil layers of 0-10, 0-20, 0-30 cm at the end of March according to 11 quantile intervals: 11 – extremely large, 10 – anomalousy large, 9 – very large, 8 – large, 7 – moderately large, 6 – average, 5 – moderately small, 4 – small, 3 – very small, 2 – anomalousy small, 1 – extremely small

Year	0-10 cm	0-20 cm	0-30 cm
1962	11	11	11
1963	11	9	9
1964	8	9	8
1965	9	7	7
1966	6	6	7
1967	6	6	6
1968	2	3	2
1969	8	9	9
1970	11	11	11
1971	4	5	5
1972	7	7	7
1973	3	3	3
1974	2	3	3
1975	4	3	3
1976	6	6	5
1977	5	5	5
1978	6	5	5
1979	8	6	5
1980	7	6	6
1981	5	6	6
1982	2	3	5
1983	3	4	4
1984	4	4	4
1985	6	6	6
1986	10	10	10
1987	9	9	9
1988	4	4	4
1989	4	4	4
1990	2	1	1
1991	8	8	7
2001	4	6	6
2002	2	3	3
2003	5	4	4
2004	4	4	4

March (x_{13}), precipitation in March (x_5) and in the third 10 day period of this month (x_6), and the precipitation in January (x_3) and the number of days with precipitation in January (x_8) and the number of days with precipitation above 5 mm in October (x_{10}). The above mentioned meteorological factors included in the regression equations are significant at least at the level of $p^{**} < 0.01$. It should be emphasized that the soil temperature and the temperature of air in March show a statistically significant increase ($p^* < 0.05$) in the analyzed period of 30 years, whereas the total of hours with the sun shows a decrease ($p = 0.09$). In the case of the remaining explaining variables no statistically significant positive or negative tendencies were observed the variation coefficient for the sunshine in September is 20.7%, for the monthly precipitation totals it varies from 51.6% in November to 62.2% in February and for the number of days with precipitation – from 33.6% in January to 47.5% in October. The standard deviation of soil temperature in February is 1.6, in March – 2.0 and that of air temperature in the same month – 2.4°C.

The regression equations included in Table 4, worked out for the three layers of soil, make it possible to determine with large accuracy, on the basis of the meteorological data from autumn and winter, after-winter reserves of useful water in light soil. This is indicated by the values of determination coefficient R^2 which is above 92%, the values of the test F – *Snedecor* which vary from 37.30 to 42.55 and the low error values of regression equations *Sy* – three times as small as the standard deviations of after-winter reserves of water from the three analyzed layers of soil.

The quantity of after-winter reserves of useful water in the soil layers of 0-10 cm and 0-20 cm is described by almost the same meteorological factors, whereas there are other factors in the 0-30 cm layer, except soil temperature in February (x_{12}). According to the studies by YANG and et al. (2003), ŻYROMSKI (2001) and KOŹMIŃSKI and MICHALSKA (2005a,b) with an increase in the depth of soil up to 70 or 100 cm the number of meteorological factors describing the amount of water in soil decreases. The largest influence on the amount of after-winter reserves of water in the 0-10 cm layer have the soil temperature from March – the coefficient of partial correlation – 58.1%, precipitation from January – 53.3% and precipitation from the third 10 day period of March – 40.8%; in the 0-20 cm layer- precipitation from the third ten day period – 51.8%, the soil temperature from March – 45.4% and precipitation from January – 46.4% and in the 0-30 cm layer – the air temperature from March – 80,6 %, precipitation from March – 76.3% and the number of days with precipitation in January – 58.2%

A close relation between water retention in soil and meteorological factors from autumn and winter are the basis for the formation of satisfying forecasts of after-winter reserves of water for the 31st of March already at the

end of February. Then the following equation of multiple regression can be used:

$$y = 22.0444 + 0.1389x_1 - 0.1909x_3 + 0.2602x_4 - 5.3383x_{12} - 0.9449x_{14}$$

in which the value of the determination coefficient $R^2 = 82.1\%$, the values of the test F – *Snedecor* = 22.40 and the error values of the regression equation Sy = 4.04 mm. The average relative error of the equation, as a difference between the measured and calculated reserves of useful water for the analyzed years (1962-1991) amounted to 11.1%. It might indicate that there is a possibility of forecasting reserves of water for the end of March, a month in advance.

Table 4

Multiple regression equations for evaluating after-winter reserves of useful water at the end of March in the layers of soil up to the depth of 10, 20 and 30 cm on the basis of meteorological data

Depth of the layer (cm)	Regression equation	R^2	F	Sy
0-10	$y = 13.0046 + 0.0357x_1 - 0.0657x_3 + 0.1244x_6 - 0.1363x_7 - 0.3593x_{11} - 0.6435x_{12} - 0.9899x_{13}$	92.5	38.91	1.23
0-20	$y = 18.1608 + 0.0718x_1 - 0.1039x_3 + 0.0809x_4 + 0.2807x_6 - 0.1902x_7 - 1.6274x_{12} - 1.4371x_{13}$	92.2	37.30	2.18
0-30	$y = 56.5718 - 0.0735x_2 + 0.3372x_5 - 0.7459x_8 - 0.7385x_9 - 0.6499x_{10} - 0.9225x_{12} - 2.8218x_{15}$	93.1	42.55	2.64

Explanations:

y – reserves of useful water, mm
x_1 – sunshine in September
x_2 – precipitation in November,
x_3 – precipitation in January,
x_4 – precipitation in February
x_5 – precipitation in March,
x_6 – precipitation in third ten day period of March,
x_7 – number of days with precipitation in October,
x_8 – number of days with precipitation in January,
x_9 – number of days with precipitation in March,
x_{10} – number of days with precipitation > 5 mm in October,
x_{11} – number of days with precipitation > 5 mm in November
x_{12} – soil temperature at the depth of 5 cm in February,
x_{13} – soil temperature at the depth of 5 cm in March,
x_{14} – air temperature in November,
x_{15} – air temperature in March
R^2 – coefficient of determination, %
F – Snedecor test
Sy – error of regression equation, mm

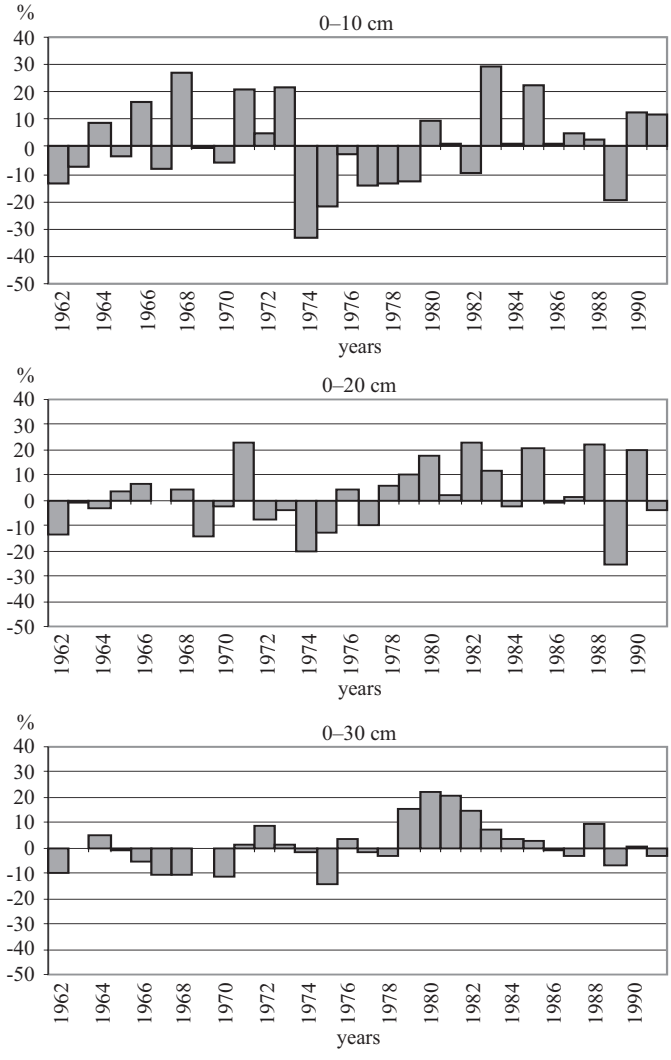


Fig. 2. Relative errors of evaluations estimated from real values of reserves of useful water at the end of March in the soil layer: 0-10, 0-20 and 0-30 cm and calculated from the models using meteorological data

Table 5 and Figure 2 and 3 show the results of verification of regression models carried out by means of the method of a relative error of forecasts and the determination coefficient of prediction RR_{pred} the Cross-Validation method in 1962-1991 and also in 2001-2004, not included in the regression equations. The average relative error of forecast for the 0-10 cm layer of soil in the years 1962-1991 was 12.1%, ranging from 0.7% in 1969 to 33.5%

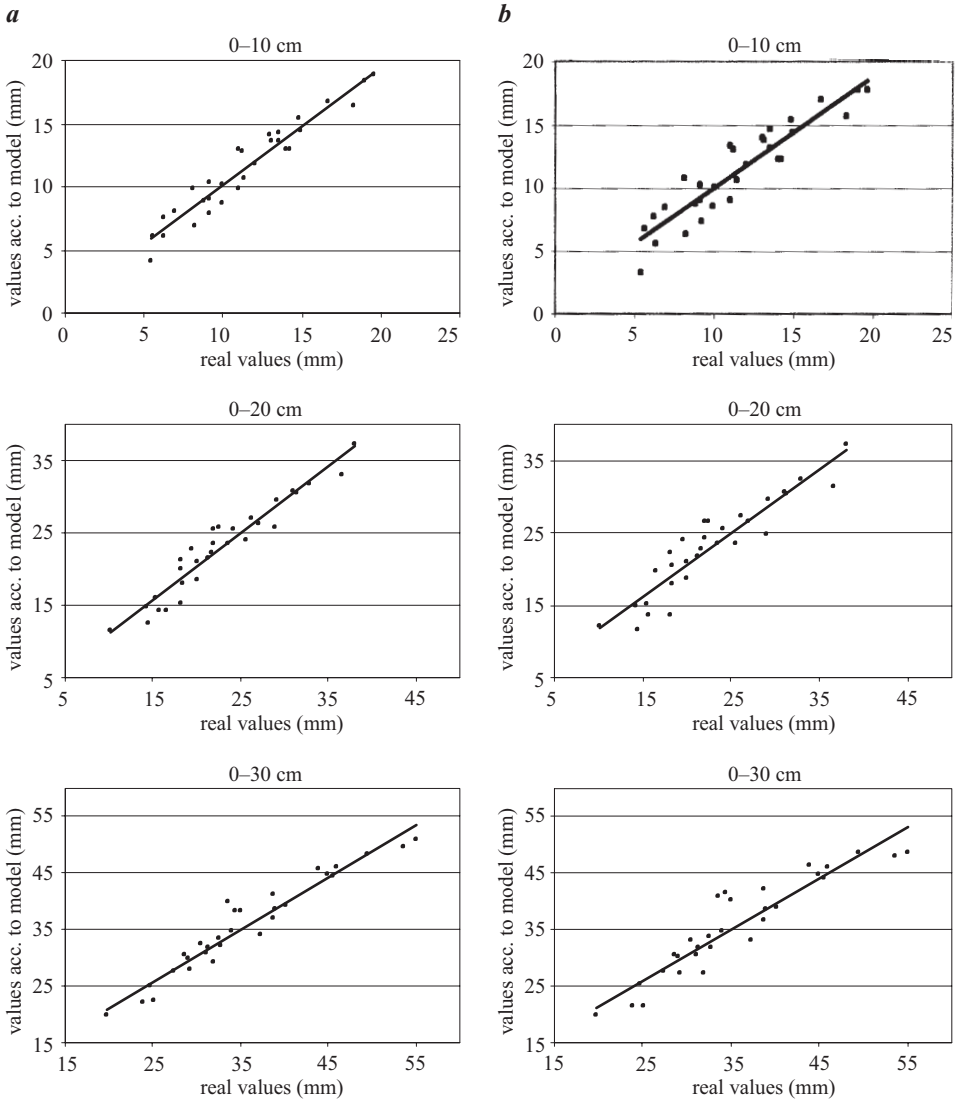


Fig. 3. After-winter reserves of useful water at the end of March in the soil layer to the depth of 10, 20 and 30 cm estimated by means of the basic model (*a*) and model using the Cross-Validation (*b*)

in 1974. RR_{pred} was at the level of 86.7% and was lower than the determination coefficient R^2 in the basic model by 5.8%. In the 0-20 cm soil layer the average relative error of forecast was lower in comparison with the shallower layer and it was 9.9%, varying from 0.4% in 1967 to 25.8% in 1980. RR_{pred} equalled 85.5% and it was lower than R^2 by 6.7%. In the 0-30 cm soil layer the

described average relative error of forecast was 6.9% with the values from 0.0 in 1963 to 21.2% in 1980. RR_{pred} was 86.1%, differing from R^2 by 7.0%. Considerably smaller differences of the average relative error of forecast of after-winter reserves of water at the end of March were observed for the deeper layers of soil as it was 5.9% up to the depth of 50 cm and 4.2% up to the depth of 100 cm (KOŹMIŃSKI, MICHALSKA 2005 a). While the largest influence on the after-winter reserves of water in the soil layer up to 30 cm have weather conditions in March, in the soil layer up to the depth of 100 cm the largest effect is observed in January, February and September.

Table 5
Evaluation of regression equations for estimating after-winter reserves of useful water at the end of March 1962-1991 (*a*) and 2001-2004 (*b*)

Layer (cm)	R^2	RR_{pred}	Relative error (%)	
			a	*b*
0-10	92.5	86.7	12.1	16.7
0-20	92.2	85.5	9.9	8.4
0-30	93.1	86.1	6.9	4.7

Good results of the assessment of worked out models for determining after-winter reserves of useful water at the end of March were obtained for the years 2001-2004. For these years the average relative error between the measured and calculated values varied from 4.7% for the 0-30 cm layer to 16.7% in the 0-10 cm layer.

Conclusion

– In the analysed years 1962-1991 there is a negative trend of after-winter reserves of water in a light soil without a rise in ground waters. This trend will get larger with an increase in air temperature in early spring.

– The largest contribution to the formation of quantity of after-winter reserves of water in the arable layer of soil at the end of March have mainly air temperature, soil temperature and precipitation in March and in January.

– The formed regression models make it possible to create, on the basis of meteorological data from autumn and winter, good forecasts of after-winter reserves of water in light soil for the end of March in the 0-20 and 0-30 cm layers and satisfactory forecasts for the 0-10 cm layer. The worked out models can be used in practice by weather services.

Acknowledgements

This research was financed by the Ministry of Education and Science – Ministry of Scientific Research – project nr 3.PO.6S04024

Accepted for print 6.01.2007

References

Czarnecka M. 2004. *Atmospheric thaw and soil thaw*. In: *Atlas of climatic resources and Hazards in Pomerania.* Eds. Michalska B., Koźmiński C., Agric. Univ. Szczecin, pp. 53-54.

Faber A., Błoch Z., Czajkowski M. 1996. *Wstępne wyniki prób zastosowania modelu wzrostu i plonowania roślin w warunkach Polski*. Zesz. Probl. Post. Nauk Rol., 447: 43-66.

Gusev Y. M., Busarowa, O.Y., Nosonowa O. N. 1998. *Modelling soil water dynamics and evapotranspiration for heterogenous surfaces of the steppe and forest-steppe zones on a regional scale*. J. Hydrol., 206: 281-297.

Koźmiński C., Michalska B. 2001. *Extreme soil moisture*. Agric. Univ. Szczecin, pp. 70-74.

Koźmiński C., Michalska B. 1995. *Soil moisture under crop cultivation*. In: *Atlas of soil moisture in Poland.* Agric. Univ. Szczecin, pp. 7-10.

Koźmiński C., Michalska B. 2004. *After-winter water reserves in the light soil*. In: *Atlas of climatic resources and hazards in Pomerania*. Agric. Univ. Szczecin, pp. 44.

Koźmiński C., Michalska B. 2005a. *Evaluation of the models for forecasting after-winter water reserves in light soils*. Water-Environment-Rural Areas, 5, 2(15): 93-110.

Koźmiński C., Michalska B. 2005b. *Variability of meteorological factors responsible for after-winter resources of water in light soil in Poland*. Water-Environment – Rural Areas, 5, 2(15): 67-92.

Kuchar L. 2001. *Ocena modeli matematycznych na podstawie testu Cross Validation*. Prz. Nauk. SGGW, 21: 165-169.

Łabędzki L., Bąk B. 2004. *Zróżnicowanie wskaźnika suszy atmosferycznej SPI w sezonie wegetacyjnym w Polsce*. Water-Environment Rual Areas, 4, 2a(11): 111-122.

Meixue Yang, Tandong Yao, Xiaohua Gou, Toshio Koike, Yuanqing He 2003. *The soil moisture distribution, thawing-freezing processes and their effect on the seasonal transition on the Qinghai – Xizang (Tibetan) plateau*. J. Asian Earth Sci., 21: 457-465.

Shoop S.A., Bigl S.R. 1997. *Moisture migration during freeze and thaw of unsaturated soils: modeling and large scale experiments*. Cold Reg. Sci. Techn. 25: 33-45.

Yang Qiu, Bojiie Fu, Jun Wang, Liding Chen 2003. *Spatiotemporal prediction of soil moisture content using multiple-linear regression in a small catchment of the Loess Plateau*. China, Catena., 54: 173-195.

Yoo C., Valdes J., B., North G. R. 1998. *Evaluation of the impact of rainfall on soil moisture variability*. Adv. Water Res., 21: 375-384.

Żyromski A. 2001. *The effect of agrometeorological conditions on water supplies in light soil characterized by spring ascent*. Agric. Univ. Wrocław, Monogr. 404.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 15-22, Y. 2007

DOI 10.2478/v10020-007-0002-y

EFFECTS OF VARIOUS FERTILIZATION SYSTEMS ON THE DYNAMICS OF NITRATE (V) CONCENTRATIONS IN POTATO TUBERS AFTER HARVEST AND DURING STORAGE

Wiera Sądej, Andrzej C. Żołnowski, Katarzyna Przekwas

Chair of Environmental Chemistry
University of Warmia and Mazury in Olsztyn

K e y w o r d s: nitrates (V), mineral fertilization, organic fertilization, storage, *Solanum tuberosum* L., potato.

A b s t r a c t

The aim of the present study was to determine the effects of three systems of long-term fertilization (only organic, only mineral and organic-mineral) on nitrate (V) concentrations in potato tubers, as well as to analyze changes in the levels of this form of nitrogen during storage. It was found that all fertilization systems contributed to an increase in nitrate (V) concentrations in potato tubers. The application of mineral fertilizers and farmyard manure (FYM) resulted in a slight exceedance of the maximum permissible NO_3^- concentrations in potato tubers. In potatoes fertilized with swine slurry, applied at a rate equivalent to the (FYM) rate in terms of the amount of organic carbon introduced into the soil, nitrate levels exceeded 1.6 to 2.1-fold the maximum permissible concentration – 200 mg $NO_3^- \cdot kg^{-1}$ of fresh mass. During storage the concentration of NO_3^- in potatoes increased in all treatments, on average by 13.5 mg $NO_3^- \cdot kg^{-1}$ in comparison to the concentration determined after harvest. This increase was higher in potato tubers fertilized with slurry, as compared with mineral fertilizers and (FYM).

WPŁYW RÓŻNYCH SYSTEMÓW NAWOŻENIA NA KSZTAŁTOWANIE SIĘ ZAWARTOŚCI AZOTANÓW(V) W BULWACH ZIEMNIAKA PO ZBIORZE ORAZ W CZASIE PRZECHOWYWANIA

Wiera Sądej, Andrzej C. Żołnowski, Katarzyna Przekwas

Katedra Chemii Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: azotany (V), nawożenie mineralne, nawożenie organiczne, przechowywanie, *Solanum tuberosum* L., ziemniak.

Address: Wiera Sądej, University of Warmia and Mazury in Olsztyn, pl. Łódzki 4, 10-726 Olsztyn, Poland, phone 48 (089) 523 39 64, e-mail: wiera.sadej@uwm.edu.pl

Abstrakt

Celem badań była ocena wpływu wieloletniego stosowania trzech systemów nawożenia (organicznego, mineralnego oraz organiczno-mineralnego) na zawartość azotanów (V) w bulwach ziemniaka oraz prześledzenie kierunku zmian tej formy azotu w czasie ich przechowywania. Każdy zastosowany rodzaj nawożenia przyczynił się do wzrostu koncentracji azotanów (V) w bulwach ziemniaka w stosunku do bulw zebranych z poletek, gdzie nie stosowano nawożenia. Niewielkie przekroczenie dopuszczalnej zawartości NO_3^- stwierdzono po zastosowaniu nawozów mineralnych i obornika. Zawartość azotanów (V) w bulwach ziemniaków pochodzących z obiektów nawożonych gnojowicą, aplikowaną w dawce równoważnej z obornikiem pod względem ilości wprowadzanego do gleby węgla organicznego, przekraczała od 1,6 do 2,1-krotnie dopuszczalną ich normę, tj. 200 mg $NO_3^- \cdot kg^{-1}$ świeżej masy bulw. Podczas przechowywania ziemniaków, w bulwach ze wszystkich obiektów nastąpił wzrost koncentracji NO_3^-. Wynosił on 13,5 mg $NO_3^- \cdot kg^{-1}$ świeżej masy bulw w stosunku do zawartości odnotowanej po zbiorze. Największy przyrost tej formy azotu wystąpił w bulwach ziemniaków nawożonych gnojowicą.

Introduction

Plants contain various substances that may be dangerous to our health, such as e.g. nitrates (III) and (V), whose concentrations in crops vary greatly. Nitrates (V) are not very toxic to humans, but in the human body they can be easily reduced to nitrates (III) which are responsible for anemia, methemoglobinemia, breakdown of vitamin A and β-carotenes. Moreover, nitrates are precursors to toxic N-nitroso compounds found in foods. One of such compounds is 1.2-dimethylnitrosomine, causing hepatic damage and inducing neoplastic diseases (LIN 1990). According to MÖHLER (1982) and KARŁOWSKI (1990), potato tubers accumulate low amounts of nitrates. However, field trials and studies on potatoes available on the market revealed that nitrate concentrations in potato tubers may vary from several dozen to several thousand milligrams per kg^{-1} of fresh matter (GISLASON et al. 1984, NEUBAUER and PIENZ 1993, ROGOZIŃSKA 1995, LIS 1996). The main factor affecting nitrate levels in potatoes is nitrogen fertilization, both mineral and organic (LIS 1996, FRYDECKA-MAZURCZYK and ZGÓRSKA 1996). Soil and weather conditions (LIS 1996, CIEŚLIK 1995, MIĘDZOBRODZKA et al. 1992) and the genotype of a potato variety (CIEŚLIK and SIKORA 1998) are also important. Due to certain biochemical changes, the time and conditions of storage can modify the morphological structure and chemical composition of potato tubers, thus affecting their quality (i.e. an increase in the amount of reducing sugars, a decrease in the concentration of vitamin C).

The aim of the present study was to determine the effects of three long-term fertilization systems (only organic, only mineral and organic-mineral) on nitrate (V) concentrations in potato tubers, as well as to analyze changes in the levels of this form of nitrogen during storage.

Materials and Methods

The study was based on the results of a long-term field experiment established in 1973 at the Experimental Station in Tomaszkowo near Olsztyn, on brown soil developed from slightly loamy sand, of quality class IV b and of good rye complex, classified by FAO (1990) as *Calcaric Cambisols*. Swine organic fertilizers were used in the experiments. Slurry was applied at two rates. Slurry rate I and slurry rate II were equivalent to the farmyard manure (FYM) rate in terms of the amount of nitrogen and organic carbon introduced into the soil, respectively. The amount of nitrogen introduced with mineral fertilizers and with (FYM) was identical. Organic fertilizers were applied together with complementary mineral fertilizers (PK), at half the rate introduced into the soil in the treatment with mineral fertilization (NPK). The experimental design and the amounts of nutrients introduced into the soil with fertilizers are presented in Table 1. Each treatment included six replications. The following crop rotations were used: potato, spring barley + undersown clover with grasses, clover with grasses, winter rape, winter wheat + rye as a catch crop, silage maize, spring barley, winter wheat. In the 30th year of the experiment potato cv. Oda was grown (Olsztyn Center for Potato Breeding and Seed Production OLZNAS-CN, Ltd.). Potatoes were harvested after 131 days.

Table 1
Experimental design and the amounts of nutrients introduced into the soil with fertilizers (annual means)

Treatments		N	P	K	Mg
		kg · ha^{-1}			
I	without fertilization	–	–	–	–
II	slurry I rate	131	46	76	17
III	slurry I rate + PK	131	68	137	17
IV	slurry II rate	389	140	232	51
V	slurry II rate + PK	389	161	293	51
VI	farmyard manure (FYM)	131	43	109	21
VII	FYM + PK	131	64	170	21
VIII	NPK	131	43	124	–

Ten kg of healthy, graded potato tubers (3.5 – 6 cm in diameter) were selected of each treatment for storage. Potatoes were put into clean plastic boxes and stored in a 12 m^3 storage chamber (PPUCh Tarczyn), at 6°C (±0.5°C) and relative air humidity of 90-95%. The tubers were stored for nine months (from September to May).

The amount of NO_3^- in potato tubers was determined immediately after harvest and during storage, by a standard analytical method (Orion 2001), using a potentiometer (Ionalyzer® Orion, Model 407) and an ion-selective electrode (Thermo Orion, Model 9307™). Reference solutions of NO_3^- (Orion) were used for analysis. The analysis was performed at two-month intervals.

The results were verified statistically by ANOVA at a significance level of $\alpha \leq 0.05$, using Statistica v. 6.0 software (StatSoft 2001).

Results and Discussion

The nitrate (V) content of potatoes, determined immediately after harvest, was widely differentiated. All fertilizers contributed to an increase in the concentration of this form of nitrogen in potato tubers, as compared with the control treatment. This increase ranged between 40.1 mg · kg^{-1} of fresh mass (FM) and 292.4 mg · kg^{-1} of FM. (Figure 1). Despite the fact that the amount of

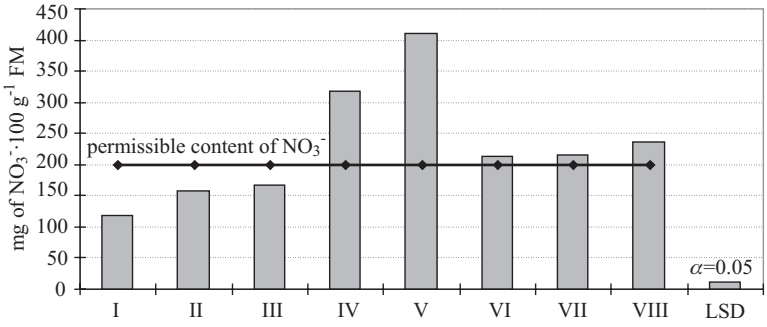


Fig. 1. Nitrate content of potato tubers after harvest: I – without fertilization, II – slurry I rate, III – slurry I rate + PK, IV – slurry II rate, V – slurry II rate + PK, VI – farmyard manure, VII – farmyrad manure + PK, VIII – NPK

nitrogen introduced into the soil with (FYM), slurry rate I and mineral fertilizers was identical, particular fertilizers had a different effect on nitrate (V) concentrations. The application of mineral fertilizers and (FYM) resulted in a slight exceedance of the maximum permissible nitrate concentrations in potato tubers. Slurry applied at rate I, equivalent to the (FYM) rate in terms of the amount of nitrogen introduced into the soil, did not cause an excessive increase in nitrate concentrations in potatoes. In potatoes fertilized with slurry applied at rate II, equivalent to the (FYM) rate in terms of the amount of organic carbon introduced into the soil, the NO_3^- content exceeded 1.6- to

2.1-fold the maximum permissible concentrations, determined in the Regulation of the Minister of Health of January 13, 2003 (Journal of Laws of 2003, No 37, item 326). The results obtained in the study indicate that overlapping application of organic fertilizers leads to nitrogen accumulation in the soil, followed by the accumulation of non-protein forms of this nutrient in plants.

During storage changes are observed in the concentrations of vitamin C, reducing and total sugars and alkaloids, but also in the composition of non-protein nitrogen. Authors vary in their opinions on the direction of changes in the nitrate (V) content of stored potato tubers. In our study nitrate concentrations decreased in all treatments during the first months of storage, except for the treatment fertilized with slurry rate I, where a slight increase in NO_3^- (by 6.0 $mg \cdot kg^{-1}$ of FM) was observed. Further storage contributed to an increase in nitrate (V) levels, as compared with the initial values (Figure 2). A statistically confirmed increase in nitrate concentrations was recorded in the treatments with organic and organic-mineral fertilization. This increase ranged between 55.3 $mg \cdot kg^{-1}$ of FM and 109.1 $mg \cdot kg^{-1}$ of FM. A non-significant increase in the nitrate content of potatoes was observed in the control treatment and in the treatment with NPK fertilization. CIEŚLIK and PAŁASIŃSKI (1997) as well as MAREČEK (2001) reported that nitrate concentrations in potato tubers decreased significantly over storage. On the other hand, FRYDECKA-MAZURCZYK and ZGÓRSKA (1996) found that an initial decrease in NO_3^- levels was followed by an increase towards the end of storage. This could be related to water transpiration in stored tubers and the natural process of cell sap concentration. In our experiment a relatively higher increase in this form of nitrogen was recorded in the treatments fertilized with slurry applied at rate I and II, in comparison with the treatments with (FYM) application and mineral fertilization. Complementary mineral fertilization (PK) combined with (FYM) and slurry rate II contributed to an increase in nitrate (V) concentrations in stored potato tubers, as compared with organic fertilizers applied alone, but this increase was statistically significant only when this fertilization variant was applied together with slurry. The opposite effect of mineral fertilizers (PK) was noted when they were applied together with slurry rate I – this combination caused a decrease in nitrate levels. The results of the study show that nitrate (V) concentrations in potato tubers may vary widely during storage, and that these changes are considerably affected by the type and rate of fertilizers. Therefore, particular attention should be paid to maintaining a low initial nitrate (V) content of potatoes designed for consumption, since it can ensure a safe level of NO_3^- in potato tubers after the storage period.

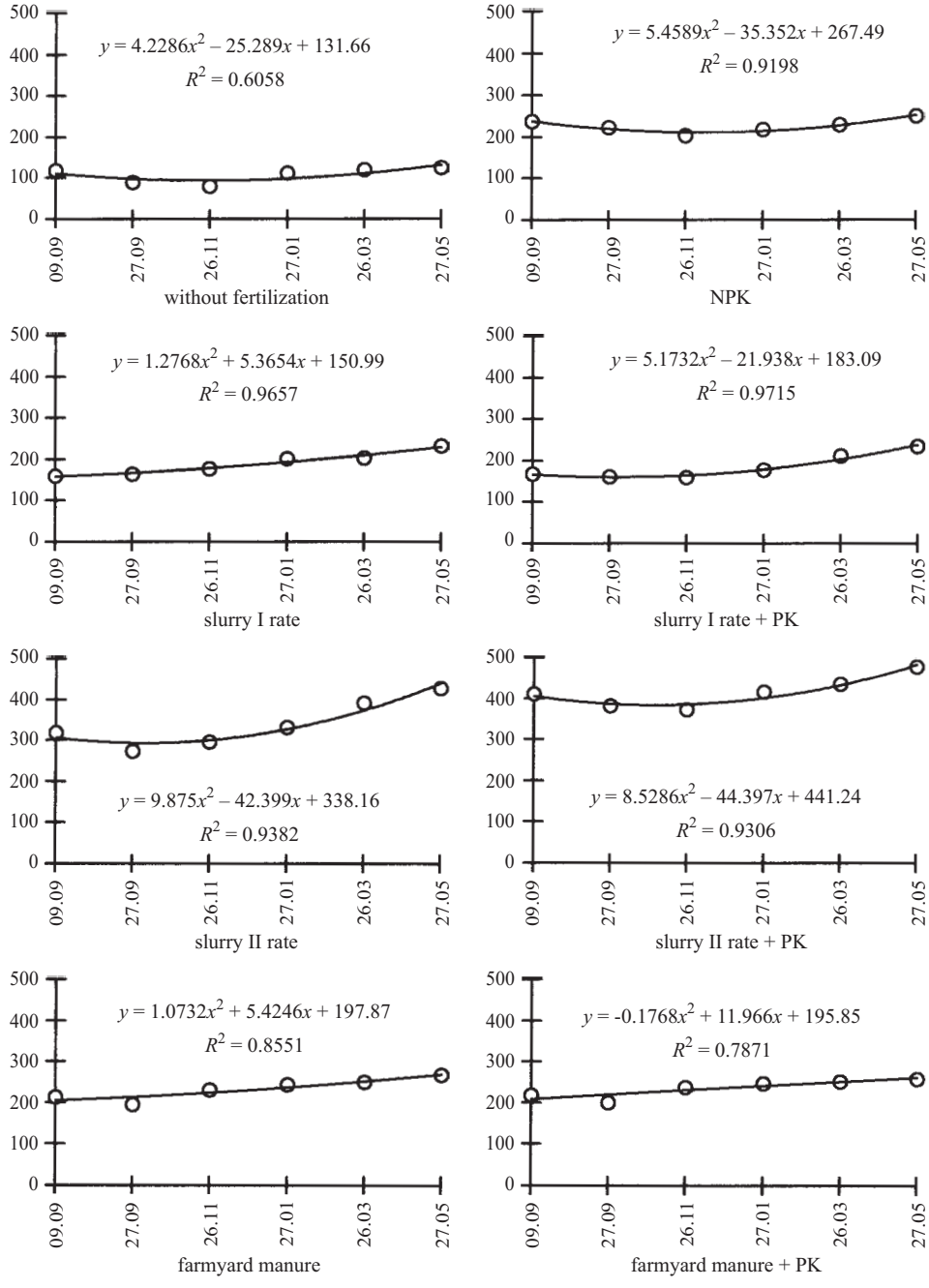


Fig. 2. Nitrate content of potato tubers during storage, mg $NO_3^- \cdot kg^{-1}$ of FM: $LSD_{\alpha=0.05}$ for factor I – storage time = 14.0, for factor II – storage time = 10.3, for interaction of factor I x factor II = 25.1

Conclusions

1. After harvest potato tubers had a high concentration of nitrates (V), which was related to the fertilization systems applied (only organic, only mineral and organic-mineral). Among the fertilizers used in the experiment only slurry applied at rate I, equivalent to the (FYM) rate in terms of the amount of nitrogen introduced into the soil, did not cause an excessive increase in nitrate concentrations in potatoes (i.e. above the permissible level of 200 mg $NO_3^- \cdot kg^{-1}$ in fresh mass).

2. All fertilization systems contributed to a significant increase in nitrate concentrations in potato tubers stored for nine months, as compared with the concentrations determined immediately after harvest (on average by about 57.9 mg $NO_3^- \cdot kg^{-1}$ of fresh mass). The increase in NO_3^- was higher in potatoes fertilized with slurry applied at rates equivalent to the manure rate in terms of the amount of both nitrogen and organic carbon introduced into the soil, as compared with potatoes fertilized with manure and mineral fertilizers.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 8.12.2006

References

CIEŚLIK E. 1995. *The effect of water conditions on the level of nitrates in tubers of some potato varieties.* Pol. J. Food Nutr. Sci., 4/45(3): 11-19.

CIEŚLIK E., PAŁASIŃSKI J. 1997. *Effect of storage temperature on nitrogen compounds transformation in potato tubers.* Zesz. Nauk. AR Kraków, 324(9): 23-30 (in Polish).

CIEŚLIK W., SIKORA E. 1998. *Correlation between the levels of nitrates and nitrites and the contents of potassium, calcium and magnesium in potato tubers*. Food Chem., 63(4): 525-528.

FRYDECKA-MAZURCZYK A., ZGÓRSKA K. 1996. *Factors which affect content of nitrates in potato tubers.* Biul. Inst. Ziemn., 47: 111-125. (in Polish).

GISLASON J., DAHLE H.K., BAERUG R., ROER L., RØNSEN K. 1984. *Nitrate in potatoes.* 1. *The effect of fertilization and storage on the nitrate content in 5 genotypes grown in widely separated lokalities.* Potato Res., 27: 331-337.

KARŁOWSKI K. 1990. *Nitrates in vegetables and potatoes – propasals for their limitation in Poland.* Rocz. PZH, 41(1-2): 1-9 (in Polish).

LIN J.K. 1990. *Nitrosamines as potential environmental carcinogens in man.* Ann. Clin. Biochem., 29: 39-44.

LIS B. 1996. *The influence of vegetation period time span and nitrogen fertilization on nitrates content in potato tubers.* Zesz. Probl. Post. Nauk Rol., 440: 217-222 (in Polish).

MAREČEK J. 2001. *Evaluation of selected qualitative parameters in several varieties of consumable potatoes*. Acta Fytotech. Zootech., 4: 224-225.

MIĘDZOBRODZKA A., CIEŚLIK E., SIKORA E., LESZCZYŃSKA T. 1992. *The effect of environment conditions on the level of nitrates and nitrites in various varieties of potato.* Pol. J. Food Nutr. Sci., 1/42(4): 45-56.

MÖHLER K. 1982. *Nitrat- und Nitritgehalte der Nährungsmittlel.* In: *Nitrat- und Nitrit- Nitrosoamine in Gemüsen*. Verlag Chemie.

NEUBAUER W., PIENZ G. 1993. *Der Nitratgehalt von Kartoffeln in Ergebnis von Feldexperimenten zu umweltschonender Anbautechnik.* Agrobiol. Res., 46: 120-125.

ORION 2001. *Methods Manual 93 series electrodes. thermo electron business formerly*. Orion Res., Inc., 22-24.

ROGOZIŃSKA I. 1995. *Wpływ nawożenia azotowego na bilans azotu oraz szkodliwych dla zdrowia substancji chemicznych w bulwach ziemniaków*. Post. Nauk Rol., 1: 59-65.

Rozporządzenie Ministra Zdrowia z dnia 13 stycznia 2003 r. w sprawie maksymalnych poziomów zanieczyszczeń chemicznych i biologicznych, które mogą znajdować się w żywności, składnikach żywności, dozwolonych substancjach dodatkowych, substancjach pomagających w przetwarzaniu albo na powierzchni żywności. Dz.U. z dn. 4 marca 2003 r., nr. 37, poz. 326.

StatSoft 2001. STATISTICA (data analysis software system) Statsoft Inc., version 6.0 www.statsoft.com

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 23-30, Y. 2007

DOI 10.2478/v100020-007-0003-x

APPLICATION OF ARTIFICIAL NEURAL NETWORKS TO PREDICT DRESSING PERCENTAGE IN CATTLE

Krzysztof Adamczyk, Zygmunt Gil, Małgorzata Golonka, Joanna Makulska, Piotr Zapletal

Chair of Cattle Breeding
Agricultural University in Kraków

K e y w o r d s: cattle, dressing percentage, artificial neural networks.

A b s t r a c t

The objective of this study was to evaluate the results of the application of two variants of the artificial neural networks (ANNs) to predict dressing percentage in slaughter cattle. In the Ist variant the input data included sex, genotype, muscularity, age and weight at slaughter as well as hot carcass weight. In the IInd variant hot carcass weight was omitted. Values of root-mean- square (RMS) indicated the proper course of network training in both variants. As it was expected, the prediction of dressing percentage by means of the Ist variant was much more accurate. On the contrary, high final values of RMS and low correlation between the network predictions and the actual values ($r = 0.27$*) in the IInd variant make questionable the usefulness of ANNs for predicting dressing percentage based on pre-slaughter data assumed in the present study. However, it should be emphasised that the increase of the observation number and the optimisation of the network structure may result in the improvement of its performance.

WYKORZYSTANIE SZTUCZNYCH SIECI NEURONOWYCH DO PRZEWIDYWANIA WYDAJNOŚCI RZEŹNEJ BYDŁA

Krzysztof Adamczyk, Zygmunt Gil, Małgorzata Golonka, Joanna Makulska, Piotr Zapletal

Katedra Hodowli Bydła
Akademia Rolnicza w Krakowie

S ł o w a k l u c z o w e: bydło, wydajność rzeźna, sztuczne sieci neuronowe.

Address: Krzysztof Adamczyk, Agricultural University in Kraków, al. Mickiewicza 24/28, 30-059 Kraków, Poland, e-mail: rzadamcz@cyf-kr.edu.pl

Abstrakt

W pracy oceniono wyniki zastosowania dwóch wariantów sztucznych sieci neuronowych (SSN) do przewidywania wydajności rzeźnej bydła. Dane wejściowe w I wariancie obejmowały: płeć, genotyp, wiek i masę ciała przy uboju, masę tuszy ciepłej oraz umięśnienie zwierząt. W wariancie II pominięto masę tuszy ciepłej.

Stwierdzono, że w obu wariantach proces uczenia się sieci przebiegał prawidłowo, o czym świadczy przebieg krzywej dla wartości błędu średniokwadratowego (RMS). Jak można było przypuszczać, predykcja wydajności rzeźnej z wykorzystaniem I wariantu sieci okazała się znacznie dokładniejsza. Wysokie wartości końcowe błędu RMS oraz niska korelacja między wskazaniami sieci w II wariancie a wartościami rzeczywistymi (r = 0,27*) stawiają pod znakiem zapytania przydatność SSN do przewidywania wydajności rzeźnej na podstawie przyjętych w pracy danych, dostępnych przed ubojem zwierzęcia. Należy jednak podkreślić, że zwiększenie liczby obserwacji i zastosowanie sieci o bardziej złożonej strukturze, mogłoby wpłynąć na poprawę skuteczności tej metody.

Introduction

The possibilities of accurate predicting cattle slaughter value based on live animal evaluation have been investigated since many years in all over the world. The studies were dealing with the application of various methods, from among the most important are: ultrasound and X-ray methods, digital image analysis, optical probes and magnetic resonance imaging (CROSS and BELK 1992, CROSS and WHITTAKER 1992, ROBINSON et al. 1993, SAKOWSKI et al. 1993). However, due to high costs, all these methods, besides the ultrasound one, are not recommended for breeding and production practice (GREINER et al. 2003, WALL et al. 2004).

Simultaneously, the investigations on the application of statistical methods to the prediction of slaughter value in cattle were carried out. It was proved that simple and multiple correlations were useful for predicting many parameters of slaughter value based on body weight and linear body measurements (GILBERT et al. 1993, ADAMCZYK 2002). Along with the development of computer techniques a particular attention has been paid to artificial neural networks (ANNs), the numerical method designed to mimic the human brain in order to perform complex functions such as reasoning and learning (TADEUSIEWICZ 1993, ADAMCZYK et al. 2002). The results of many investigations indicated the ability of ANNs to predict slaughter value from the data available before slaughter (BRETHOUR 1994, HATEM and TAN 1998, LI et al. 1999, HILL et al. 2000, ADAMCZYK et al. 2005).

The aim of the present study was to evaluate the effectiveness of two variants of the artificial neural networks used to predict dressing percentage in slaughter cattle.

Material and Methods

The investigations were carried out on 300 bulls and heifers purchased by the meat plant from the area of southern Poland. The average slaughter weight of the animals was 553 kg (Table 1). The data on sex, genotype, muscularity, age and weight at slaughter as well as hot carcass weight and hot dressing percentage were used in the analyses (Mc KIERNAN 2000) – Table 2.

Table 1

Number of heifers and bulls under study

Breed	Heifers	Bulls	Total
Black-and-White	25	39	64
Red-and-White	73	78	151
Simmental	20	25	45
Polish Red	13	27	40
Total	131	169	300

Table 2

Criteria of muscularity assessing in the animals under study (Mc KIERNAN 2000)

Muscularity class	Description (observing from behind)
Poor	narrower over topline, tapering through stifle, narrower stance, more prominent hip bones, stomach is more clearly visible
Average	not as wide or well-rounded over the topline, hip bones can be seen, has a narrow stance and the stomach is clearly visible
Good	wide, well-rounded topline, maximum width through stifle, has a wide stance and the stomach cannot be seen

According to the methodology of the artificial neural network the data set was randomly split into training (50% of data), validation (25%) and testing (25%) data sets. The training and validation sets were used in the process of ANN training whereas the testing set was used for verification of the trained network.

Hot dressing percentage was predicted by means of two variants of one-way, multiple-layered perceptron with the following parameters:

– continuous input data: age and weight at slaughter and hot carcass weight (I^{st} variant); age and weight at slaughter (II^{nd} variant);

– categorized input data (in both variants): sex, genotype, muscularity class;

– continuous output data (in both variants): hot dressing percentage;

– 6 (Ist variant) and 5 (IInd variant) of hidden neurons – calculated according the following formula (MASTERS 1996):

$$K = \sqrt{N \cdot M}$$

where:
K – number of hidden neurons,
N – number of neurons in the input layer,
M – number of output neurons;
– back-propagation training algorithm;
– other basic parameters of network training: logistic function of neuron activation; 2000 epochs*.

The performance of both variants of network in the course of training was measured by so-called "network training error" expressed by root-mean-square (RMS). The predictive abilities of the assumed network variants were compared by estimating coefficients of simple correlation and differences between the neural network predictions and the actual values in the testing set.

Thanks to considering hot carcass weight among the input data in the Ist variant a high accuracy of predictions was expected, however when the pre-slaughter estimation of hot dressing percentage is intended the IInd variant should be used.

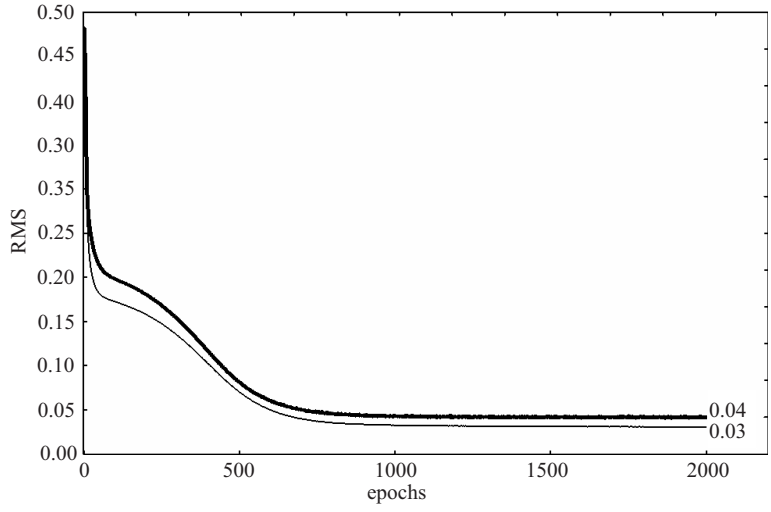
The calculations were performed using the computer program Statistica ver. 6.1.

Results and Discussion

The results of the analyses are presented in Figures 1-5.

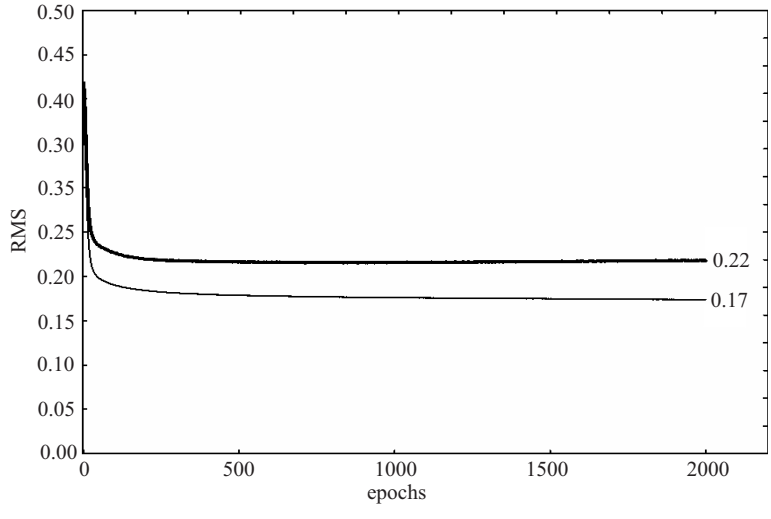
When evaluating the effectiveness of the assumed variants of the artificial neural network the so-called „network memorizing" was not observed. The values of RMS, describing the function of the network, were decreasing proportionally in the training and in the validation set until the horizontal trend indicating the minimum error has be achieved (Figures 1-2). However, the final results of training proved to be distinctly better in case of the Ist variant. The final values of RMS error both in the training and in the validation set of the Ist variant were as much as by 82% lower than in the IInd variant. The differences in the prediction of hot dressing percentage ranged

* epoch – network training cycle



Explanations: thin line – the training set, bold line – the validation set

Fig. 1. The course of neural network training in case of the Ist variant



Explanations – see Fig. 1.

Fig. 2. The course of neural network training in case of the IInd variant

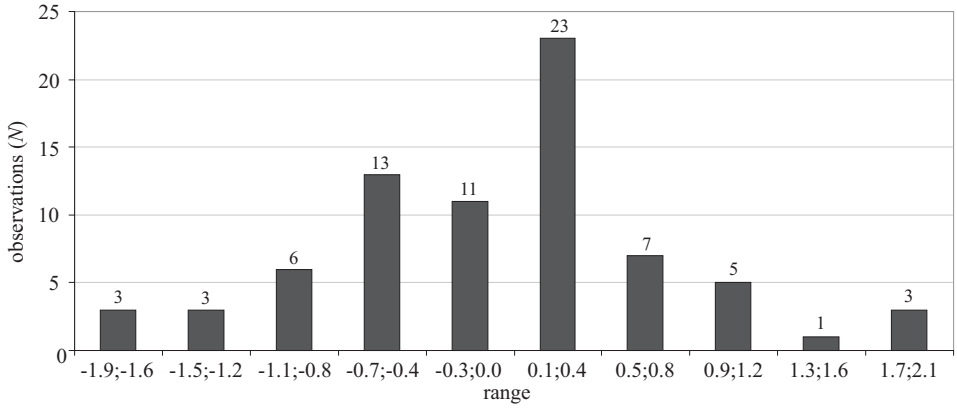


Fig. 3. Differences between the neural network predictions and the actual values of hot dressing percentage (%) for the Ist variant (based on 75 observations from the testing set)

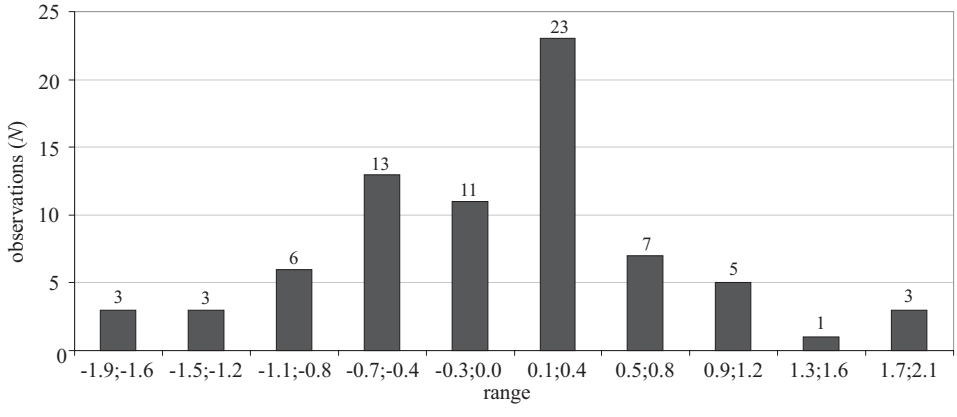
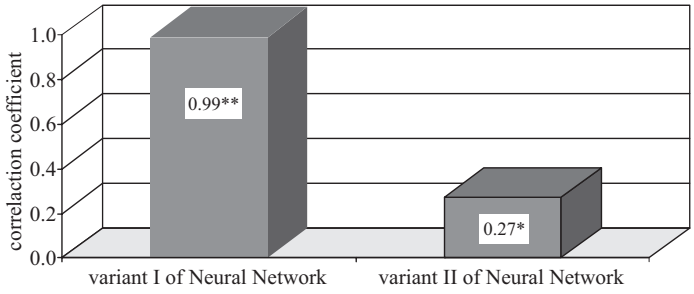


Fig. 4. Differences between the neural network predictions and the actual values of hot dressing percentage (%) for the IInd variant (based on 75 observations from the testing set)

between -1.9% and +2.1% for the Ist variant and between -13.8% and +11.6% for the IInd variant (Figures 3-4). It was found that the prediction differences for 87% of the observations in the Ist variant varied from -1.1% to 1.2% and as much as for 95% of the observations in the IInd variant from -8.7% to 6.4%. Also, the coefficient of simple correlation between the network predictions and the actual values in the testing set indicated that the Ist variant was definitely more accurate (r = 0,99**) – Figure 5. Relevant correlation in case of the IInd variant was only 0.27*. Generally, it can be said that the IInd variant performed much worse in dressing percentage prediction than the Ist variant.



* – $p \leq 0.05$; ** – $p \leq 0.01$

Fig. 5. Correlations between the artificial neural network predictions and the actual values of hot dressing percentage in the testing set

Some examples of the potential applications of neural networks to the support for other methods used in the evaluation of slaughter value of cattle were described in the following papers:

– analysis of the ultrasound images in the evaluation of beef marbling in live animals (BRETHOUR 1994);

– evaluation of cartilage ossification degree in beef cattle thoracic vertebrae aimed at slaughter value estimation (HATEM and TAN 1998);

– post-slaughter evaluation of beef tenderness (LI et al. 1999, HILL et al. 2000).

Also, ADAMCZYK et al. (2005) obtained very encouraging results in predicting hot carcass weight of bulls from growth data, expressed by a high value of the coefficient of correlation (0.97) between the ANNs outputs and the actual values.

Conclusions

The search for more and more precise methods of the estimation of slaughter value in live animals is practically and economically justified. Although the obtained results of the preliminary investigations did not prove the sufficient accuracy of artificial neural network predictions the improvement could probably be achieved by using larger data sets and optimization of network configuration. The collecting and analysing large sets of data seem to be possible thanks to the expected common application of computers in animal production. Hence, further research on the development of appropriate software for predicting slaughter value in cattle by means of well-trained artificial neural network should be carried out.

Translated by JOANNA MAKULSKA

Accepted for print 10.01.2007

References

ADAMCZYK K. 2002. *Określanie masy ciała, tempa wzrostu i wartości rzeźnej buhajków mieszańców*. Kat. Hodowli Bydła, AR Kraków, pp. 132 (rozprawa doktorska).

ADAMCZYK K., SZAREK J., MOLENDA K. 2002. *Wykorzystanie sztucznych sieci neuronowych (Neural Networks) w zakresie hodowli bydła*. Biul. Inf. IZ, 40(4): 65-75.

ADAMCZYK K., MOLENDA K., SZAREK J., SKRZYŃSKI G. 2005. *Prediction of Bulls; Slaughter value from growth data using artificial Neural Network*. J. Cent. Eur. Agric., 6(2): 133-142.

BRETHOUR J.R. 1994. *Estimating marbling score in live cattle from ultrasound images using pattern recognition and neural network procedures*. J. Anim. Sci., 72(6): 1425-1432.

CROSS H.R., BELK K. E. 1992. *Objective measurements of carcass and meat quality*. Proc. of 38th ICoMST Clermont – Ferrand, France, pp. 127-134.

CROSS H.R., WHITTAKER A.D. 1992. *The role of instrument grading in a beef value-based marketing system*. J. Anim. Sci., 70: 984-989.

GILBERT R.P., BAILEY D.R.C., SHANNON N. H. 1993. *Linear body measurements of cattle before and after 20 years of selection for postweaning gain when fed two different diets*. J. Anim. Sci., 71: 1712-1720.

GREINER S.P., ROUSE G.H., WILSON D.E., CUNDIFF L.V. WHEELER T.L. 2003. *Prediction of retail product weight and percentage using ultrasound and carcass measurements in beef cattle*. J. Anim. Sci., 81: 1736-1742.

HATEM I., TAN J.L. 1998. *Determination of animal skeletal maturity by image processing*. ASAE An. Int. Meeting, Orlando, Florida, 12-16.07.1998, ASAE Paper, 983019, pp. 13.

HILL B.D., JONES S.D.M., ROBERTSON W.M., MAJOR I.T. 2000. *Neural Network modeling of carcass measurements to predict beef tenderness*. Can. J. Anim. Sci., 80(2): 311-318.

LI J., TAN J., MARTZ F.A., HEYMANN H. 1999. *Image texture features as indicators of beef tenderness*. Meat Sci., 53(1): 17-22.

MASTERS T. 1996. *Sieci neuronowe w praktyce. Programowanie w języku C++*. WNT, Warszawa, pp. 455.

MC KIERNAN B. 2000. *Muscle scoring beef cattle*. NSW Department of Primary Industries, New South Wales, Australia (http://www.dpi.nsw.gov.au).

ROBINSON D.L., HAMMOND K., MCDONALD C.A. 1993. *Live animal measurement of carcass traits: estimation of genetic parameters for beef cattle*. J. Anim. Sci., 71: 1128-1135.

SAKOWSKI T., DE LAURANS A, CYTOWSKI J. 1993. *Świat stawia na obiektywną ocenę wartości rzeźnej zwierząt*. Prz. Hod., 12: 14-15.

TADEUSIEWICZ R. 1993. *Sieci neuronowe*. AOW, wyd. II, Warszawa, 12-19.

WALL P.B., ROUSE G.H., WILSON D.E., TAIT, JR. R.G., BUSBY W.D. 2004. *Use of ultrasound to predict body composition changes in steers at 100 and 65 days before slaughter*. J. Anim. Sci., 82: 1621-1629.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 31-42, Y. 2007

DOI 10.2478/v10020-007-0004-9

HATCHABILITY OF TURKEY EGGS AS DEPENDENT ON SHELL ULTRASTRUCTURE*

Emilia Mróz, Katarzyna Michalak, Aneta Orłowska

Chair of Poultry Science
University of Warmia and Mazury in Olsztyn

K e y w o r d s: egg, shell, hatchability, poult quality.

A b s t r a c t

The aim of the present study was to determine the hatchability of turkey eggs differing in shell structure and functional properties. Egg weight, the number of eggs in groups differing in shell ultrastructure and egg hatchability, as well as the body conformation and weight of heavy-type broad-breasted white poults were analyzed between 7 and 12 weeks of the laying season. The analysis was performed six times, at one-week intervals, on 2268 eggs (126 eggs with good-quality shells, 126 rough-shelled eggs and 126 eggs without shell surface pigmentation were examined each time). Sixty eggs of each group (10 eggs at a time) were used for shell ultrastructure analysis.

Rough-shelled eggs and eggs without shell surface pigmentation accounted on average for 4% of all tested eggs. Eggs without shell surface pigmentation were by 3.25 to 1.78 g lighter than eggs with good-quality shells and rough-shelled eggs. During 26 days of incubation weight losses in eggs without shell surface pigmentation and in rough-shelled eggs were by 1.76 and 0.50% higher, respectively, compared to eggs with normal-quality shells. The distinguishing features of normal shell microstructure, in comparison with rough shells and shells without surface pigmentation, were as follows: thicker crystal, palisade and mammillary layers, smaller mammillae and thinner inner shell membrane fibers. The layers of good-quality shells, except for the mammillary layer, were the thickest in the central part of the egg. Rough shells and their crystal layers were the thickest at the blunt end of the egg. Shells without surface pigmentation had the thickest crystal layer and the largest mammillae at the sharp end of the egg.

Eggs without surface pigmentation were characterized by the lowest hatching value. In this group the fertilization rate and the hatching rate were by 5.82 and 4.22%, and by 19.61 and 12.15% lower, respectively, compared to eggs with normal-quality shells and rough shells. The poults that hatched out in this group were by 1.8 g lighter and had more physical defects than those that hatched out in the other two groups.

Address: Emilia Mróz, University of Warmia and Mazury in Olsztyn, Michała Oczapowskiego 5, 10-719 Olsztyn, Poland, phone: 48 (089) 523 41 07, e-mail: emilia.mroz@uwm.edu.pl

* This work was conducted within the framework of research project no. 0104-0224.

ULTRASTRUKTURA SKORUPY A ZDOLNOŚĆ WYLĘGOWA JAJ INDYCZYCH

Emilia Mróz, Katarzyna Michalak, Aneta Orłowska

Katedra Drobiarstwa
Uniwersytet Warmińsko-Mazurski Olsztyn

S ł o w a k l u c z o w e: jajo, skorupa, wylęgowość, jakość indycząt.

A b s t r a k t

Celem pracy było określenie zdolności wylęgowej jaj indyczych, zróżnicowanych pod względem budowy i właściwości funkcjonalnych skorupy. W okresie od 7. do 12. tygodnia nieśności badano: masę i liczbę jaj o określonej budowie skorupy oraz wylęgowość, budowę i masę indycząt białych szerokopiersnych typu ciężkiego. Badania prowadzono 6-krotnie, w odstępach tygodniowych, na 2268 jajach (każdorazowo po 126 jaj z grup o skorupie prawidłowej, ziarnistej i bez pigmentu). Do badań ultrastruktury skorupy użyto po 60 jaj z każdej grupy (6-krotnie po 10 jaj).

Średni udział jaj o skorupie ziarnistej i bez pigmentu wynosił ok. 4%. Te ostatnie były lżejsze o 3,25-1,78 g od jaj prawidłowo ukształtowanych i o skorupie ziarnistej. Podczas 26 dni inkubacji ubytki masy jaj bez pigmentu i o skorupie ziarnistej były większe o 1,76 i 0,50% od strat masy jaj o skorupie prawidłowej. Jej mikrostruktura odznaczała się najgrubszą warstwą krystaliczną, palisadową i brodawkową, ale najmniejszymi brodawkami i najcieńszymi włóknami błony podskorupowej. Badane warstwy skorupy prawidłowej były najgrubsze w środkowej części jaja (z wyjątkiem warstwy brodawkowej i wielkości brodawek). Skorupa ziarnista i jej warstwa krystaliczna miały największą grubość w tej części jaja, a skorupa bez pigmentu w jego ostrej części, gdzie także wyróżniały ją największe brodawki.

Najniższą wartość wylęgową miały jaja o skorupie bez pigmentu. Procent zapłodnienia jaj o tym rodzaju skorupy był niższy niż w przypadku jaj o skorupie prawidłowej i ziarnistej odpowiednio o 5,82 i 4,22%, a wylęgowość gorsza o 19,61 i 12,15%. Indyczęta były lżejsze o 1,8 g i miały więcej fizycznych wad budowy w porównaniu z wyklutymi z dwóch pozostałych rodzajów jaj.

Introduction

The structure, chemical composition and functions of eggshells may vary widely (ROBERTS et al. 1995, SOLOMON 1996, MALEC et al. 1996, NYS et al. 1997, PANHELEUX et al. 1997, MALEC 1999). The outward appearance and features typical of a given bird species may undergo changes during shell formation (SOLOMON 1991, MRÓZ 1998, NIEDZIÓŁKA et al. 2001, MICHALAK and MRÓZ 2006). Structural faults of eggshells may be related to intensive farming, dietary problems and bird diseases, in some case also to genetic factors (ROBERTS et al. 1995, MALEC 1997, 1999, MRÓZ 1998, MALEC et al. 2002). Moreover, eggshell quality is considerably affected by environmental factors and breeding work, and usually deteriorates significantly in intensively farmed birds (BARAN et al. 1994, MRÓZ 1998, MRÓZ et al. 2002, 2002a).

Eggshell defects inhibit normal embryo growth, disturb the process of water loss, makes it difficult for the embryo to move and breathe, and negatively affect the hatching process (BELYAVIN 1994, KRYSTIANIAK 2002,

MRÓZ et al. 2002a). Structural faults are associated with increased embryo mortality. Studies on the correlation between eggshell structure and egg hatchability, carried out to date in Poland, have focused on chickens (MALEC 1999), emu (SZCZERBIŃSKA 2002) and geese (ROSIŃSKI et al. 1999). Research into the wide diversity of eggshell surface in turkeys (PUCHAJDA et al. 1997, MRÓZ 1998) has provided the basis for a detailed analysis of shell structure. Authors vary in their opinions on the suitability of non-typical eggs for incubation. Another problems that should be thoroughly examined is the relationship between the low hatching rates observed in turkeys and eggshell structure characteristics.

The aim of the present study was to determine the hatchability of turkey eggs differing in shell structure and functional properties.

Materials and Methods

The experimental materials comprised hatching eggs of heavy-type broad-breasted white turkeys, raised on deep litter. The eggs were laid between 7 and 12 weeks of the laying season and stored for 3 to 7 days. The eggs prepared for incubation were divided into three groups differing in shell surface characteristics (Table 1), according to the method developed by MRÓZ (1998). The number and weight of eggs, weight losses during incubation, shell microstructure, hatchability and poult quality were analyzed over the experimental period. The number of eggs per group is given as a percentage of the total number of tested eggs. The number of tested eggs depended on the number of eggs representing a given shell structure type. Due to the lower number of rough-shelled eggs and eggs without shell surface pigmentation it was necessary to analyze a greater number of hatching eggs. The selection was continued until 126 eggs with rough shells and 126 eggs without shell surface pigmentation were chosen. Egg weight was determined on an individual basis prior to incubation: 126 eggs of each group were weighed (a total of 2268 eggs). Egg weight losses were determined using eggs with living embryos. After 10 and 26 days of incubation 2054 and 1917 eggs were weighed, respectively. Shell microstructure was determined on 60 non-incubated eggs selected of each group (6 x 10 eggs), using a scanning electron microscope, as described by MICHALAK and MRÓZ (2006). The thickness of the shell, crystal, palisade and mammillary layers as well as the size of mammillae were estimated based on means of measurements taken at the thickest and thinnest region on the surface of a mammilla. The thickness of inner shell membrane fibers, measured in the central part of a single fiber, is given in μm. The number of mammillae is given per mm-2. The results of measurements were analyzed by digital image analysis (SCAO).

Table 1

Outward appearance of eggshells

Eggshell characteristics		Egg groups		
		group I normal-quality shell	group II rough shell	group III shell without pigmentation
Surface	color	cream	light-cream, white	white
	structure	smooth, shiny	rough; thickened areas of shell mass of various size and shape that can be abraded	smooth, shiny
Pigmentation	color	brown	brown, gray	white
	pattern, distribution	round, oval or elongated spots	round spots around thickened areas of shell mass, oval or elongated spots, no pigmentation visible at some places	invisible over the entire surface area

Six incubation series were carried out in Petersime incubators in accordance with the relevant technological standards. The eggs were candled on day 10 and 26 of incubation, to determine the percentage of fertilized eggs and eggs containing dead embryos. The percentage of eggs containing dead embryos in the hatching unit, the percentage of eggs containing living unhatched embryos as well as the hatching rate of set and fertilized eggs were determined at the completion of incubation. The quality of newly-hatched poults was evaluated after incubation based on the percentages of poults with and without physical defects, applying the method described in Polish Standards (*Drób. Pisklęta jednodniowe* 1998) and adjusted for the purpose of the present analysis by MRÓZ et al. (2005). The obtained results were verified statistically based on means and coefficients of variation, using Duncan's test.

Results and Discussion

Table 2 shows that the quality of hatching eggs improved between 7 and 12 weeks of the laying season. The number of eggs with rough shells and without shell surface pigmentation decreased. The average proportion of rough-shelled eggs and eggs without shell surface pigmentation slightly exceeded 4%, which means that it was quite low, compared to previous results (MRÓZ 1996, 1998). Such a low number of eggs with shell surface defects could be related to the

Table 2

Number of tested eggs

Week of the laying season	Number of tested eggs	Number of eggs (%)		
		normal-quality shell	rough shell	shell without pigmentation
7	2646	92.21	3.02	4.77
8	5292	95.52	2.38	2.09
9	5418	95.51	2.32	2.15
10	6678	96.52	1.58	1.89
11	6174	96.22	1.73	2.04
12	7182	97.38	1.76	0.86
7-12	33 390	95.98	2.01	2.00

Table 3

Weight of hatching eggs and weight losses during incubation

Specification	Statistical measures	Egg groups		
		group I normal-quality shell	group II rough shell	group III shell without pigmentation
Egg weight before incubation (g)	x	91.76^{A}	90.30^{B}	88.51^{C}
	$v\%$	6.37	6.83	8.13
Egg weight losses (g)				
– to day 10	x	3.74^{C}	4.20^{B}	4.38^{A}
	$v\%$	21.11	26.25	29.56
– to day 26	x	10.71^{B}	11.09^{B}	11.89^{A}
	$v\%$	15.25	19.96	22.36
Egg weight losses (%)				
– to day 10	x	4.19^{C}	4.77^{B}	5.35^{A}
	$v\%$	24.92	35.39	50.63
– to day 26	x	11.71^{B}	12.21^{B}	13.47^{A}
	$v\%$	32.57	37.84	43.14

Explanation:
Mean values in lines followed by different letters differ significantly:
ABC – at a level of $p \leq 0.01$
abc – at a level of $p \leq 0.05$

origin of birds and to the fact that they were kept on litter. Many turkey flocks are characterized by a high proportion (> 30%) of eggs with shell surface faults (MRÓZ 1996, 1998, MRÓZ et al. 1997, 2002). Varied percentages of eggs with defective shells (7.5-27.6%) are observed in inbred turkey flocks (BARAN et al. 1994). A higher inbreeding rate is associated with an increase in the number of eggs considered unsuitable for incubation. Studies on chickens (ZIĘBA et al. 1996) and heavy-type turkeys (MRÓZ et al. 1997, MRÓZ 1998) suggest that more

eggs with defective shells are produced in the cage system. However, this tendency has not been observed in light-type turkeys (JANKOWSKI 1983).

Table 3 presents the average weights of turkey hatching eggs. Eggs with good-quality shells were heavier ($p \leq 0.01$) than eggs without shell surface pigmentation and rough-shelled eggs, and the coefficient of variation was the lowest in this group. During the first 10 days of incubation weight losses were the lowest in eggs with normal-quality shells. Over 26 days of incubation weight losses in eggs with good-quality shells and in rough-shelled eggs were comparable and lower ($p \leq 0.01$) than in the group of eggs without shell surface pigmentation. The lowest variation in weight losses was recorded in the group of eggs with normal-quality shells (15.2-21.1%), while the highest – in the group of eggs without shell surface pigmentation (22.3-29.5%).

The average weights of eggs with good-quality shells and rough-shelled eggs were high, but did not exceed the optimum values given in the Polish Standard (*Jajo wylęgowe indycze...* 1998). Eggs without shell surface pigmentation were characterized by medium weight. Eggs whose shells differed from the relevant standard were found to be lighter, which agrees with the results obtained by other authors (KRYSTIANIAK 2002, MRÓZ et al. 2002a). Our results as well as those reported by LERNER et al. (1993), SOLIMAN et al. (1994), BORZEMSKA and KOSOWSKA (1996) show that weigh losses increased over incubation in eggs with defective and pigmented shells. This was caused by lower shell permeability and a slower growth rate of embryos in these eggs. Eggs with shell surface faults are characterized by excessive evaporation. Weight losses observed in eggs with good-quality shells (Table 3) were comparable to those observed in pervious studies (CHRISTENSEN et al. 1996, MRÓZ and PUDYSZAK 1997).

Table 4 presents shell microstructure characteristics of turkey hatching eggs. Eggs with normal shell structure were by 57.1 to 71.0 μm and by 15.56 to 34.0 μm thicker ($p \leq 0.01$), in comparison with shells without surface pigmentation and rough shells. The crystal, palisade and mammillary layers were the thickest in good-quality shells and the thinnest in shells without surface pigmentation ($p \leq 0.01$). Eggs with normal-quality shells had the smallest mammillae and the thinnest inner shell membrane fibers. The size of mammillae had no significant influence on their number per mm^{-2}, which was correlated with shell surface area (not determined in the study).

The layers of good-quality shells, except for the mammillary layer, were the thickest in the central part of the egg. The mammillary layer was the thickest and the mammillae were the largest at the blunt end of the egg (Table 4). In rough shells and in shells without surface pigmentation the crystal layer was the thickest and the number of mammillae was the highest at the blunt or sharp end of the egg.

Table 4

Eggshell microstructure

Eggshell characteristics	Egg part	Statistical measures	Egg groups		
			normal-quality shell	rough shell	shell without pigmentation
Eggshell thickness (μm)	blunt		340.8^{A}	325.2^{B}	274.90^{C}
			9.22	7.92	15.42
	central	x	352.4^{A}	318.4^{B}	295.3^{C}
		$v\%$	6.78	6.42	14.97
	sharp		328.2^{A}	309.1^{B}	257.2^{C}
			8.28	12.81	19.82
Thickness of the crystal layer (μm)	blunt		29.6^{A}	28.6^{A}	19.90^{B}
			25.04	49.96	30.55
	central	x	34.3	25.0	18.10
		$v\%$	24.47	31.30	37.49
	sharp		29.5^{A}	21.8^{B}	22.80^{B}
			37.08	35.82	38.95
Thickness of the palisade layer (μm)	blunt		162.0^{A}	155.2^{A}	135.10^{B}
			11.34	17.0	24.54
	central	x	188.0^{A}	166.2^{B}	149.00^{C}
		$v\%$	16.50	13.49	15.27
	sharp		168.0^{A}	161.5^{B}	113.50^{B}
			15.42	27.79	23.23
Thickness of the mammillary layer (μm)	blunt		149.2^{A}	141.2^{A}	119.90^{B}
			19.49	15.44	28.66
	central	x	130.1	127.2	128.20
		$v\%$	18.90	18.54	24.22
	sharp		130.7	125.8	120.90
			23.54	13.56	24.60
Size of mammillae (μm)	blunt		55.00	61.61	62.00
			30.51	25.72	30.62
	central	x	50.5^{Bb}	57.00^{Ba}	66.80^{A}
		$v\%$	28.59	26.75	28.22
	sharp		50.2^{C}	59.00^{B}	69.80^{A}
			26.86	30.60	26.51
Number of mammillae per mm^{-2}	blunt		276.9	239.5	250.50
			31.17	17.59	36.88
	central	x	294.5	241.7	210.90
		$v\%$	6.68	20.58	47.22
	sharp		279.1	250.7	219.70
			20.94	18.18	23.72
Thickness of inner shell membrane fibers (μm)	blunt		2.37^{a}	1.90^{b}	2.30^{a}
			52.13	33.93	43.83
	central	x	2.40^{b}	2.40^{b}	3.00^{a}
		$v\%$	41.60	40.26	43.82
	sharp		2.20	1.99	2.20
			38.51	34.37	38.73

Explanation:
Mean values in lines followed by different letters differ significantly:
ABC – at a level of $p \leq 0.01$; *abc* – at a level of $p \leq 0.05$

A relationship between the external appearance and thickness of eggshells has been observed not only in turkeys (CHENG-TAUNG et al. 1995, MALEC 1999, RICHARDS and DEEMING 2001, MRÓZ et al. 2002a). Larger mammillae have been found in eggs with thin and deformed shells (ROBERTS et al. 1995, RICHARDS, DEEMING 2001, NIEDZIÓŁA et al. 2001), while thicker inner shell membrane fibers have been observed in eggs without shell surface pigmentation (KRYSTIANIAK 2002), which is consistent with our results.

Particular parts of the egg differ in the thickness of the shell and its layers. This has been confirmed in our study as well as in the experiments conducted by CHENG-TAUNG et al. (1997) and SZCZERBIŃSKA (2002). The central part of the eggshell is the thickest since it is most intensively used by the embryo. The thickest mammillary layer and the largest mammillae can be found at the blunt end of the egg, which is related to high gas permeability.

The hatchability of turkey eggs in particular groups varied widely (Table 5). Eggs without surface pigmentation were characterized by a significantly lower fertilization rate (by 5.82 and 4.22%) and higher embryo mortality rates in all analyzed periods, compared to the other groups. The hatching rate determined at the completion of the experiment was by 19.61 and 7.46% lower in eggs without surface pigmentation and in rough-shelled eggs, respectively, compared to eggs with normal-quality shells. Irregular shell surface pigmentation decreases egg hatchability to a higher degree than rough shell surface.

54.73% of poults that hatched out in the group of eggs with normal-quality shell surface and only 28.33% of poults that hatched out in the group of eggs without shell surface pigmentation had no physical defects. Poults with anatomical abnormalities of the umbilicus and plumage dominated among birds with physical defects (86% and 27%, respectively). Differences in the quality of poults were also reflected by their different body weights (Table 5). The lightest poults (60.8 g) hatched out in the group of eggs without shell surface pigmentation. The variation of this trait in particular groups was low, and ranged from 7.1% to 8.5%.

Turkeys are characterized by high fertilization rates, exceeding 96% after the peak of the laying season (MRÓZ et al. 2002a, GODWIN et al. 2005, ORŁOWSKA and MRÓZ 2006). A lower fertilization rate was recorded in turkey eggs with irregular shell pigmentation, which is in agreement with results of previous studies (MRÓZ 1998, MRÓZ et al. 2002, 2002a). Our results confirm the opinion that the fertilization rate is influenced also by the female.

In chicken eggs with irregularly pigmented shells embryo vitality is reduced by 2% to 20% (MALEC 1999, HODGETTS 2000). Increased early embryo mortality can be also observed in turkey eggs with rough shells (9.35%) and with shells showing irregular pigmentation (13.27%), in comparison to those with normal-quality shells (6.25%). At further stages of

Table 5

Hatchability of turkey eggs

Specification	Statistical measures	Egg groups		
		group I normal-quality shell	group II rough shell	group III shell without pigmentation
Set eggs (number)		756	756	756
Fertilized eggs (%)	*x*	97.35^{a}	95.75^{a}	91.53^{b}
	v%	1.34	2.03	5.94
Dead embryos (%)				
– to day 10	*x*	2.31^{a}	4.25^{a}	7.74^{b}
	v%	47.11	54.18	65.72
– between day 11	*x*	2.70^{A}	5.60^{A}	11.26^{B}
and 26	*v*%	71.65	36.94	34.14
Dead embryos in the hatching unit and unhatched poults (%)	*x*	2.45	5.07	8.07
	v%	65.34	71.58	53.37
Hatching rate				
– of set eggs (%)	*x*	90.07^{A}	82.00^{A}	66.79^{B}
	v%	4.29	6.45	13.99
– of fertilized	*x*	92.54^{A}	85.08^{A}	72.93^{B}
eggs (%)	*v*%	4.69	6.47	11.46
Poults				
– with normal body	*x*	54.73^{Aa}	40.67^{b}	28.33^{B}
conformation (%)	*v*%	18.14	27.35	41.46
– with physical	*x*	45.25^{Bb}	59.31^{a}	71.65^{A}
defects (%)	*v*%	21.95	18.75	16.40
Weight of poults (g)	*x*	62.6^{A}	62.6^{A}	60.8^{B}
	v%	7.16	7.52	8.51

Explanation:
Mean values in lines followed by different letters differ significantly:
ABC – at a level of $p \leq 0.01$
abc – at a level of $p \leq 0.05$

incubation the mortality rate of turkey embryos has been found to increase by 2.94% in eggs with irregularly pigmented shells and by 3.5% in rough-shelled eggs (MRÓZ 1998). Both the present and previous results show that early embryo mortality rates are higher and the hatching process is more difficult in eggs with shell surface faults.

The occurrence of high numbers of hatchlings with physical anomalies has been described in both Polish and foreign literature on the subject. Only 48 to 62% of chicks (TONA et al. 2003) and 44 to 57% of poults can be classified as best-quality ones (ORŁOWSKA and MRÓZ 2006). The quality of hatchlings depends on a variety of egg traits (TAZAWA, WHITTOW 2000, MRÓZ, PUDYSZAK 1997, ORŁOWSKA, MRÓZ 2006). The present study suggests that poult quality is affected by eggshell quality.

Conclusions

1. Eggs with good-quality and rough shells are characterized by higher weight and lower weight losses during incubation than eggs without shell surface pigmentation.

2. The distinguishing features of normal shell microstructure, in comparison with rough shells and shells without surface pigmentation, are as follows:

– thicker crystal, palisade and mammillary layers, smaller surface area of mammillae and thinner inner shell membrane fibers,

– the thickest crystal and palisade layers, the highest number of mammillae and the thickest inner shell membrane fibers in the central part of the egg,

– the thickest mammillary layer and the largest surface area of mammillae at the blunt end of the egg.

3. Eggs without surface pigmentation have a low hatching value, which is related to a low fertilization rate, a high embryo mortality rate over the incubation period and a very low hatching rate. In addition, poults that hatched out in this group were the lightest.

4. The number of poults with physical defects is higher in the groups of rough-shelled eggs and eggs without shell surface pigmentation, compared to eggs with good-quality shells.

Translated by Aleksandra Poprawska

Accepted for print 17.01.2007

References

Baran S., Jankowski J., Faruga A. 1994. *Wpływ inbredu na wartość cech reprodukcyjnych indyczek*. Zesz. Nauk. Prz. Hod., 8: 66-73.

Belyavin Ch. 1994. *Good egg handling*. Poult. Int., 4: 14-18.

Borzemska W., Kosowska G. 1996. *Ważniejsze problemy w patologii lęgów u drobiu*. Zesz. Nauk. Prz. Hod., 10: 25-29.

Cheng-Taung W., Tien-Chun W., Ching-Moo P., Yeong-Hsiang Ch. 1995. *Comparison of physical-chemical properties between greenish and whitish shell egg of brown tsaiya duck*. Proc. the VI Europ. Symp. *Egg and egg products quality*. Zaragoza – Spain, September, pp. 123-30.

Christensen V.L., Donaldson W.E., McMutry J.P. 1996. *Physiological differences in late embryos from turkey breeders at different ages*. Poult. Sci., 75: 172-178.

Drób. Pisklęta jednodniowe. 1998. PN-R-78566.

Godwin J.L., Grimes J.L., Christensen V.L., Wineland M.J. 2005. *Effect of dietary phosphorus and phytase levels on the reproductive performance of Large White Turkey breeder hens*. Poult. Sci., 84: 485-493.

Hodgetts B. 2000. *Hatching egg quality*. Int. Hatchery Practice, 5(14): 7-21.

Jajo wylęgowe indycze, kacze i gęsie. 1998. PN-R-78564.

Jankowski J. 1983. *Wpływ sposobu chowu i okresu w cyklu nieśności na jakość jaj indyczych*. Zesz. Nauk. ART Olszt. Zoot., 25: 95-104.

Krystianiak S. 2002. *Nieśność bażanta łownego oraz jakość jaj i ich wylęgowość*. AR Poznań (paca doktorska).

LENER.P., FRENCH., MCINTYRE D., BAXTER-JONES C. 1993. *Age – related changes in egg production, fertility, embryonic mortality and hatchability in commercial turkey flocks*. Poult. Sci., 72: 1025--1039.

MALEC H. 1997. *Wykrywanie mikroanalizą rentgenowską uszkodzeń skorupy jaj przy syndromie dużej głowy u kur*. Med. Wet., 53(2): 95-98.

MALEC H. 1999. *Biologiczna wartość kurzych jaj wylęgowych o różnej zawartości porfiryny*. Zesz. Nauk. 251. Rozpr. AR Kraków.

MALEC H., BORZEMSKA W., NIEDZIÓŁKA J. 1996. *Przypadek hipertermii u zarodków indyczych w ostatnim etapie lęgu*. Med. Wet., 52(10): 645-646.

MALEC H., BORZEMSKA W., NIEDZIÓŁKA J., PIJALSKA J. 2002. *Wartość wylęgowa jaj o tzw. skorupach z naddatkami*. Med. Wet., 1 (58): 49-51.

MICHALAK K., MRÓZ E. 2006. *Ultrastructure of the turkey hatching egg shell*. Pol. J. Natur. Sc., 21(2): 671-689.

MRÓZ E. 1996. *Niektóre cechy morfologiczne powierzchni skorupy jaj indyczych i ich związek z wylęgowością*. Zesz. Nauk. Prz. Hod., 24: 77-84.

MRÓZ E. 1998. *Studia nad zmiennością cech powierzchni skorupy jaj indyczych i ich związkiem z wylęgowością*. ART Olsztyn, Rozpr. i Monogr. 4.

MRÓZ E., PUDYSZAK K. 1997. *Analiza wyników wylęgowości i jakości jednodniowych indycząt w zależności od wieku indyczek*. Zesz. Nauk. Prz. Hod., 32: 97-102.

MRÓZ E., PUDYSZAK K., PUCHAJDA H. 1997. *Wpływ różnych czynników na jakość skorupy i wylęgowość jaj indyków*. Zesz. Nauk. Prz. Hod., 31: 59-66.

MRÓZ E., PUCHAJDA H., MICHALAK K., PUDYSZAK K. 2002. *Analiza biologiczna wylęgowości jaj indyczych*. Rocz. Nauk. Zoot., Supl., 16: 61-66.

MRÓZ E., PUCHAJDA H., PUDYSZAK K. 2002a. *Structure and pigmentation of eggshell and biological value of turkey hatching eggs*. Pol. J. Natur. Sc., 10(1): 141-152.

MRÓZ E., MICHALAK K., ORŁOWSKA A. 2005. *Indyczęta jednodniowe – ocena budowy*. Indyk Pol. (11) 2: 4-12.

NIEDZIÓŁKA J., MALEC H., BORZEMSKA W., MALEC L., PIJALSKA J. 2001. *Effect of ovulation disorders in hens on eggshell ultrastructure and course of hatching*. Ann. Anim. Sci., 1 (1): 87-96.

NYS Y., HINCKE M.T., ARIAS J.L., GARCIA- RUIZ J.M., SOLOMON S.E. 1997. *Biochemical characterisation and in vitro functonal properties of gg shell matrix extracts and of uterine fluid in hens*. Proc. of the VII Europ. Symp. *Egg and egg products quality*. Poznań – Poland, September, pp. 107-126.

ORŁOWSKA A., MRÓZ E. 2006. *Effects of age turkey-hens and egg storage period on embryo mortality andpoults quality*. XVIII Int. Poultry Symp. PB WPSA *Science for poultry practice – poultry practice for science.* Rogów – Poland, September, pp 49-54.

PANHELEUX M., MAUREEN B., FERNANDEZ M.S., GAUTRON J., ARIAS J., SOLOMON S., HINCKE M., NYS Y. 1997. *Comparative analysis of organic matrix andof eggshell ultrastructure in various domestic birds*. Proc. of the VII Europ. Symp. *Egg and egg products quality*. Poznań – Poland, September, pp. 162-172.

PUCHAJDA H., CYWA-BENKO K., FARUGA A., PUDYSZAK K., MRÓZ E. 1997. *Porównanie budowy makroskopowej i ultrastruktury skorup jaj indyków różnego pochodzenia*. Rocz. Nauk. Zoot., 24 (1): 33-43.

ROBERTS J.R., BRACKPOOL C.E., SOLOMON S.E. 1995. *The ultrastructure of good and poor quality eggshells from Australian layer strains*. VI Europ. Symp. *Egg and egg products quality*. Zaragoza – – Spain, September, pp. 107-116.

ROSIŃSKI A., ELIMINOWSKA-WENDA G., CYWA-BENKO K., KŁOSOWSKA D. 1999. *Eggshell ultrastructure and its relation to hatchability in the goose*. I Word Waterfowl Conf. Taiwan – Taichung, December, pp. 476-481.

SOLOMON S.E. 1991. *Egg and eggshell quality*. Wolfe publishing LTD, London, England.

SOLOMON S.E. 1996. *The eggshell-a barier*? Turkeys, 44(4): 8-10.

SOLIMAN F.N.K., RIZK R.E., BRAKE J. 1994. *Relationship between shell porosity, shell thickness, egg weight loss, and embryonic development in Japanese quail eggs*. Poult. Sci., 73: 1607-1611.

SZCZERBIŃSKA D. 2002. *Charakterystyka użytkowości reprodukcyjnej emu (Dromaius Novaehollandiae) ze szczególnym uwzględnieniem ultrastruktury skorupy i jej związku ze wskaźnikami wylęgowości jaj*. AR Szczecin, Rozpr. 210.

TAZAWA H., WHITTOW G.C. 2000. *Incubation physiology*. Ch. 24. In: *Sturkie's avian physiology*. 5th ed. Acad. Press, San Diego, pp. 617-634.

TONA K., BAMELIS F., DE KATELAERE B., BRUGGEMAN V., MORAES V.M.B., BUYES J., ONAGBESAN O., DECUYPERE 2003. *Effects of storage time on spread of hatch quality and juvenile growth*. Poult. Sci., 82: 736-741.

ZIĘBA G., WITKOWSKI A., HORBAŃCZUK J. 1996. *Występowanie wybranych cech jakości skorupy jaj u dwóch ras kur nieśnych*. Zesz. Nauk. Prz. Hod., 24: 121-126.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 43-49, Y. 2007

DOI 10.2478/v10020-007-0005-8

COAT COLOUR VERSUS PERFORMANCE IN THE HORSE (*EQUUS CABALLUS*)

***Anna Stachurska*[1], *Anne P. Ussing*[2]**

[1] Chair of Horse Breeding and Use
Agricultural University, Lublin, Poland
[2] The Royal Library, Copenhagen University Library North Copenhagen N., Denmark

K e y w o r d s: horse, coat colour, performance, genes.

A b s t r a c t

The coat colour in the horse being mainly a protective trait is also a factor which contributes to the maintenance of the thermal equilibrium in the body. A relationship between the colour and performance in the horse is possible since the genes producing the colour may be linked with genes which affect the horse's performance or can be pleiotropic simultaneously influencing both kinds of traits. Current studies performed on racehorses have not shown a considerable relationship between the loci controlling the grey colour as well as eumelanogenesis in bay and black horses and the racing records. Instead, a few loci are known which control certain colours and involve pathologic signs in the organism or are lethal in homozygotes.

MAŚĆ A DZIELNOŚĆ KONI (*EQUUS CABALLUS*)

Anna Stachurska, Anne P. Ussing

[1] Katedra Hodowli i Użytkowania Koni
Akademia Rolnicza w Lublinie
[2] The Royal Library, Copenhagen University Library North Copenhagen N., Dania

S ł o w a k l u c z o w e: koń, maść, dzielność, geny.

A b s t r a k t

Barwa włosów u koni odgrywająca głównie rolę ochronną jest też czynnikiem wspomagającym utrzymanie równowagi cieplnej organizmu. Związek rodzaju maści z dzielnością konia jest możliwy, gdyż geny powodujące powstanie maści mogą być sprzężone z genami wpływającymi na dzielność

Address: Anna Stachurska, Agricultural University, Akademicka 13, 20-950 Lublin, Poland, anna.stachurska@ar.lublin.pl

konia, a także oddziaływać równocześnie na oba rodzaje cech. Najnowsze badania przeprowadzone na koniach wyścigowych nie wykazały istotnego związku genów maści siwej oraz wytwarzania eumelaniny u koni gniadych i karych z wynikami wyścigów. Znanych jest natomiast kilka loci kontrolujących pojawienie się określonych maści, które równocześnie powodują patologiczne zmiany ustrojowe, a u homozygot są letalne.

As far as it is known, different wild horses were of different colours, e.g. the Tarpan (*Equus caballus gmelini*) according to old chronicles was blue dun (PRUSKI 1959). Paleolithic cave paintings in Middle Asia and Europe (Altamira, Spain and Lascaux, France; 15.000-10.000 BC) show, among horses of basic (bay, chestnut and black) and dun (wild type pattern) colours also leopard pattern (white areas with spots, most pronounced on the hips). The Przewalski Horse *(Equus caballus przewalskii)*, the last living wild representative of the species, is of a yellow dun colour with wild type pattern. White markings on the head and legs probably appeared in the domestic horse. Domestication in horses and later breeding caused a great coat colour diversity among breeds. Some of them are of a particular coat colour, whereas others show a wide variety of colour phenotypes (KLUNGLAND, VÍ GE 2000).

The coat colour is a protective trait which helps the wild horses hide and escape predators (STACHURSKA et al. 2004). Currently, in California-Nevada a significant difference is observed in the proportion of variously coloured foals killed by pumas *(Felis concolor)* relative to the distribution of colours born into the feral horse population. The pumas select for sorrel (light chestnut) foals, probably because this colour more closely approaches the colour of the mule deer *(Odocoileus hemionus)* of this region than any other foal colour does (TURNER, MORRISON 2001). The coat colour also plays a role in maintaining the body temperature: dark colours quickly absorb sunrays and easily eliminate the heat, while light colours retain the heat longer and are advantageous when it is cold (CENA et al. 1957).

Horse breeders and users have been always interested in knowing whether the performance of variously coloured horses differ. Usually, the preferences for certain coat colours are the consequence of a belief that they can be associated with better or worse performances. Such relationship is possible, since the genes producing the colour may be linked with genes affecting the horse's systemic traits. Certain genes can also be pleiotropic, i.e. they can influence both the colour and the systemic features (FALCONER 1981). The colour is a quality trait which is controlled by several loci (SPONENBERG 2003, STACHURSKA 2002, USSING 2000). Most of the loci have already been assigned to particular chromosomes (Table 1). They are closely linked to some genes producing e.g. various blood protein variants, which may be important for the systemic functions (ANDERSSON, SANDBERG 1982). The performance traits

depend on the activity of additive polygenes and possible Quantity Trait Loci (QTLs) of a great phenotypic effect (EDWARDS et al. 1987). Up to date, no QTLs affecting the horse's performance have been found. The characters which make it up are complex and difficult to measure. Moreover, they are considerably influenced by the rider, exercise, feeding and many other factors (SAASTAMOINEN, BARREY 2000). The polygenetic nature of the horse's performance hinders the search for its relationship with the coat colour.

Table 1

Loci controlling the horse coat colours assigned to chromosomes

Locus	Chromosome	Colour/Colours	Locus bibliography
Lp	ECA1	leopard	TERRY et al. 2004
MC1R (E)	ECA3	chestnut, bay, black	ANDERSSON, SANDBERG 1982, MARKLUND et al. 1996, RIEDER et al. 2001
To	ECA3	tobiano	ANDERSSON, SANDBERG 1982, BROOKS et al. 2002
Rn	ECA3	roan	ANDERSSON, SANDBERG 1982, MARKLUND et al. 1999
Sb	ECA3	sabino	BROOKS, BAILEY 2005, MAU et al. 2004
EDNRB	ECA17	associated with overo and other white patterns	SANTSCHI et al. 1998, 2001
MATP (C)	ECA21	palomino, buckskin, cremello	LOCKE et al. 2001, MARIAT et al. 2003
ASIP (A)	ECA22	bay, black	RIEDER et al. 2001
G	ECA25	grey	HENNER et al. 2002, LOCKE et al. 2002, SWINBURNE et al. 2002
W	ECA3	dominant white	MAU et al. 2004

Opinions on such connections are reported to be common worldwide (KEELER 1947, SPONENBERG 2003, STACHURSKA et al. 2001). The issue has been investigated since a long time. Many studies have been performed on racehorses since the racing records are more numerous and scored under more similar circumstances than show jumping, three day eventing or other equestrian sports (RICARD et al. 2000). However, the racehorses are not much differentiated with regard to the colour (usually bay, black, chestnut and grey) and thus only few loci controlling the colour could have been investigated. The results are mostly negative, and solely SKORKOWSKI (1976) found a considerable difference in the endurance and speed between bay and chestnut Thoroughbreds. The bay mares were more enduring and won more at the age of three years, particularly over distances above 2000 m, compared to the chestnut mares, which were faster at the age of two years, over shorter

distances. These findings do not agree with results of other studies. An investigation performed on winners of five English classical races in 1777-1938 showed no difference associated with the colour (GALIZZI VECCHIOTTI 1986). According to DUŠEK (1980), the performance in chestnuts, bays, blacks and greys, as well as in chestnuts with different amounts of white markings, is similar. Recently, STACHURSKA et al. (2006) and STACHURSKA, PIĘTA (2006) examined nearly 15 thousand starts of a great number of Thoroughbreds and Purebred Arabians, with regard to current knowledge on the loci controlling the colour. The results indicate that G locus assigned to ECA25 is not associated with the performance, i.e. grey and non-grey horses gain similar scores. Regarding locus MC1R located on ECA3, eumelanic (bay and black) horses show an insignificant tendency towards better results than phaeomelanic (chestnut) horses. However, it cannot be excluded that the effect might be expressed in homozygous grey (GG genotype) and eumelanic (EE genotype) horses since dominance of G and E genes may be not complete. Most of Thoroughbreds are heterozygous (Gg and Ee genotypes, respectively). Physiological actions ruled by the combined action of dominant and recessive genes can lead to phenotypic traits a little bit similar to those found in recessive genotypes (gg and ee). Horses of overall darker colours had inconsiderably better scores than lighter ones. Such tendencies may be due to the physiological properties related to the brightness of the hair. The ASIP locus on ECA22 was not studied since black horses were few. The relationship between the coat colour and racing performance in horses at particular age, suggested by SKORKOWSKI (1976) has not been found.

The difference in the temperament in horses of various colours was not proved (ESTES WORTH 1948). JAKUBEC at al. (1997) found the conformation of grey and black Old Kladrub horses varied. Nevertheless, this could result from the isolated selection of those subpopulations and not from the relations among the traits in question and the colour. BLESA et al. (1999) and DRING et al. (1981) did not find any correlation between the mare's colours and gestation length in Thoroughbreds.

Although an important relationship of the coat colour with the performance and physiological traits in the horse was not found, the fact that certain loci controlling the coat color involve pathological signs in the organism was documented. For instance, 67%-80% of grey horses older than 15 years develop melanomas (neoplasms) which turn malignant in 66% of the cases (SÖLKNER et al. 2004). Probably that is the reason the life span of the grey horses is on average by two years shorter than usual (GALIZZI VECCHIOTTI 1986). Some observations have not been scientifically investigated, yet, e.g. homozygous leopard horses are reported by Danish Knabstrub breeders to have impaired night vision (USSING 2000) and some splashed white horses in Australia seem

to be deaf (GOWER 1999). In horses, like in many other mammalian species, certain colours are produced by lethal or semi lethal alleles. It was proved that among dominant white horses, homozygotes WW are lethal at early stages of embryo development (PULOS, HUTT 1969). Similarly, dominant homozygotes in Roan (Rn) locus may be lethal in intrauterine life (HINTZ, VLECK VAN 1979). Overo lethal white syndrome (OLWS) is an inherited fatal disease of foals born to overo parents. Affected foals are white and die within a few days from intestinal aganglionosis (VONDERFECHT et al. 1983). The syndrome is caused by a mutation in the endothelin receptor B (EDNRB) gene in homozygotes (SANTSCHI et al. 1998, 2001). Considering the ratio of foals different than expected from matings of variously coloured parents, KNYAZEV et al. (1999) suggest that embryos of grey heterozygotes may be less vital than others.

To conclude: currently, only relationships between the horse coat colour and some physiological functions of the organism are known. No important relationship between the coat colour and the horse's performance has been found. The research to date on the racing performance of horses of different colours has considered solely two loci controlling the colour. In future, provided the genetic basis of the performance is known better, a molecular approach may give an unequivocal answer whether such relationship exists and if so, which particular traits are associated with which colours.

Translated by ANNA STACHURSKA

Accepted for print 21.12.2006

References

ANDERSSON L., SANDBERG K. 1982. *A linkage group composed of three colour genes and three serum protein loci in horses.* J. Hered., 73(2): 91-94.

BLESA F., VALERA M., VINUESA M., MOLINA A. 1999. *The gestation length of the Andalusian and Arabian Horse*. Book of Abstracts 5, 50th An. Meeting of Europ. Ass. for Animal Prod., Zurich (Switzerland). Copyright Wageningen Pers, Wageningen, pp. 348.

BROOKS S.A., BAILEY E. 2005. *Exon skipping in the KIT gene causes a Sabino spotting pattern in horses*. Mamm. Gen., 16(11): 893-902.

BROOKS S.A., TERRY R.B., BAILEY E. 2002. *Association of PCR-RFLP for* KIT *with Tobiano coat color in horses*. Anim. Genet., 33: 301-303.

CENA M., CZYSZEK W., SŁOMKA J. 1957. *Badania nad wpływem umaszczenia koni na pochłanianie promieniowania słonecznego*. Med. Wet., 6: 261-264.

DRING L.A., HINTZ H.F., VAN VLECK L.D. 1981. *Coat color and gestation length in Thoroughbred mares.* J. Hered., 72: 65-66.

DUŠEK J. 1980. *Přispěvek k řešeni vztahu výkonnosti a barev u anglických plnokrevniku.* Výzkumná Stanice pro Chov Koní, Slatiňany: Bull., 34: 77-87.

EDWARDS M.D., STUBER C.W., WENDEL J.F. 1987. *Molecular-marker-facilitated investigations of quantitative-trait loci in maize.* I. *Numbers, genomic distribution and types of gene action.* Genetics, 116: 113-125.

ESTES WORTH B. 1948. *Lack of correlation between coat colour and temperament.* J. Hered., 39: 84.

FALCONER D.S. 1981. *Introduction to quantitative genetics.* Longman Group Ltd, New York, USA, pp. 41, 281.

GALIZZI VECCHIOTTI G. 1986. *Mendelian inheritance in horses*. Manuscript. Abstract in Proc. 37th An. Meeting of Europ. Ass. for Animal Prod., Budapest (Hungary).

GOWER J. 1999. *Horse colour explained: a breeder's perspective*. Marlborough: Crowood, p. 144.

HENNER J., PONCET P.A., GUERIN G., HAGGER CH., STRANZINGER G., RIEDER S. 2002. *Genetic mapping of the (G)-locus, responsible for the coat colour phenotype "progressive greying with age" in horses (Equus caballus).* Mamm. Gen., 13(9): 535-537.

HINTZ H.F., VLECK L.D. VAN 1979. *Lethal dominant roan in horses*. J. Hered., 70: 145-146.

JAKUBEC V., SCHLOTE W., JELINEK J., SCHOLZ A., ZÁLIŠ N. 1997. *Linear type trait analysis in the genetic resource of the Old Kladrub Horse.* Book of Abstracts 3, 48th An. Meeting of Europ. Ass. for Animal Prod., Vienna (Austria). Copyright Wageningen Pers, Wageningen, pp. 384.

KEELER C.E. 1947. *Coat color, physique, and temperament*. J. Hered., 38: 271-279.

KLUNGLAND H., VÍ GE D. I. 2000. *Molecular genetics of pigmentation in domestic animals*. Curr. Genomics, 1(3): 223-242.

KNYAZEV S.P., GUTOROVA N.V., NIKITIN S.V., STAROVEROVA O.I., DUBROVSKAYA R.M. 1999. *The relationships between horse gray coat color and prenatal vitality of Orlov trotters*. Book of Abstracts 5, 50th An. Meeting of Europ. Ass. for Animal Prod., Zurich (Switzerland). Copyright Wageningen Pers, Wageningen, pp. 341.

LOCKE M.M., RUTH L.S., MILLON L.V., PENEDO M.C.T., MURRAY J.D., BOWLING A.T. 2001. *The cream dilution gene, responsible for the palomino and buckskin coat colours, maps to horse chromosome 21.* Anim. Genet., 32(6): 340-343.

LOCKE M.M., PENEDO M.C.T., BRICKER S.J., MILLON L.V., MURRAY J.D. 2002. *Linkage of the grey colour locus to microsatellites on horse chromosome 25.* Anim. Genet., 33(5): 329-337.

MARIAT D., TAOURIT S., GUERIN G. 2003. *A mutation in the MATP gene causes the cream coat colour in the horse.* Gen. Sel. Evol., 35(1): 119-133.

MARKLUND L. S., MOLLER M. J., SANDBERG K., ANDERSSON L. 1996. *A missense mutation in the gene for melanocyte-stimulating hormone receptor (MC1R) is associated with chestnut coat color in horses*. Mamm. Gen., 7(12): 895-899.

MARKLUND L. S., MOLLER M. J., SANDBERG K., ANDERSSON L. 1999. *Close association between sequence polymorphism in the KIT gene and the roan coat colour in horses.* Mamm. Gen., 10(3): 283-288.

MAU C., PONCET P.A., BUCHER B., STRANZINGER G., RIEDER S. 2004. *Genetic mapping of dominant white (W), a homozygous lethal condition in the horse (Equus caballus).* J. Anim. Breed. Genet., 121(6): 374-383.

PRUSKI W. 1959. *Dzikie konie wschodniej Europy*. Rocz. Nauk Rol., D-85: 1-131.

PULOS W.L., HUTT F.B. 1969. *Lethal dominant white in horses*. J. Hered., 60: 59-63.

RICARD A., BRUNS E., CUNNINGHAM E.P. 2000. *Genetics of performance traits.* In: *The genetics of the horse*. Eds. BOWLING A.T., RUVINSKY A. CABI Publ., Wallingford, pp. 411-438.

RIEDER S., TAOURIT S., MARIAT D., LANGLOIS B., GUÉRIN G. 2001. *Mutations in the agouti (ASIP), the extension (MC1R), and the brown (TYRP1) loci and their association to coat color phenotypes in horses (Equus caballus).* Mamm. Gen., 12(6): 450-455.

SAASTAMOINEN M.T., BARREY E. 2000. *Genetics of conformation, locomotion and physiological traits*. In: *The genetics of the horse*. Eds. BOWLING A.T., RUVINSKY A. CABI Publ., Wallingford, pp. 447, 452.

SANTSCHI E.M., PURDY A.K., VALBERG S.J., VROTSOS P.D., KAESE H., MICKELSON J.R. 1998. *Endothelin receptor B polymorphism associated with lethal white foal syndrome in horses.* Mamm. Gen., 9(4): 306-309.

SANTSCHI E.M., VROTSOS P.D., PURDY A.K., MICKELSON J.R. 2001. *Incidence of the endothelin receptor B mutation that causes lethal white foal syndrome in white-patterned horses.* Am. J. Vet. Res., 62(1): 97- 103.

SKORKOWSKI E. 1976. *Colour, types and shapes and the principles of horse breeding*. World Rev. Anim. Prod., 12(1): 45-50.

SÖLKNER J., SELTENHAMMER M., CURIK I., NIEBAUER G. 2004. *Genetic relationships between speed of greying, melanoma and vitiligo prevalence in grey horses.* Book of Abstracts 10, 55th An. Meeting of Europ. Ass. for Animal Prod., Bled (Slovenia), G5.3, Copyright Wageningen Pers, Wageningen.

SPONENBERG D.P. 2003. *Equine Color Genetics*. Iowa State Univ. Press, Ames, USA.

STACHURSKA A. 2002. *Identyfikacja koni*. Wyd. AR Lublin.

STACHURSKA A., PIĘTA M. 2006. *Is there a relationship between coat colour, age and racing performance in horses?* Ann. Anim. Sci., 6(2): 249-255.

STACHURSKA A., PIĘTA M., JAWORSKI Z., USSING A. P., BRUŚNIAK A., FLOREK M. 2004. *Colour Variation in Blue Dun Polish Konik and Biłgoraj Horses.* Livest. Prod. Sci., 90: 201-209.

STACHURSKA A., PIĘTA M., ŁOJEK J., SZULOWSKA J. 2007. *Performance in racehorses of various colours.* Livest. Sci., 106(2-3): 282-286.

STACHURSKA A., USSING A. P., NESTAAS T. 2001. *Maści rozjaśnione na przykładzie koni fiordzkich*. Med. Wet., 57(12): 876-879.

SWINBURNE J.E., HOPKINS A., BINNS M.M. 2002. *Assignment of the horse grey coat colour gene to ECA25 using whole genome scanning*. Anim. Genet., 33(5): 338-342.

TERRY R.B., ARCHER S., BROOKS S., BERNOCO D., BAILEY E. 2004. *Assignment of the appaloosa coat colour gene (LP) to equine chromosome 1*. Anim. Genet., 35(2): 134-139.

TURNER J.W., MORRISON M.L. 2001. *Influence of predation by mountain lions on numbers and survivorship of a feral horse population*. Southwest. Nat., 46(2): 183-190.

USSING A.P. 2000. *Hestenes farver*. Nucleus Forlag ApS, Arhus, Denmark.

VONDERFECHT S.L., TROMMERSHAUSEN BOWLING A., COHEN M. 1983. *Congenital Intestinal Aganglionosis in White Foals*. Vet. Pathol., 20: 65-70.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 50-60, Y. 2007

DOI 10.2478/v10020-007-0006-7

MILK PRODUCTION EFFICIENCY AS DEPENDENT ON THE SCALE OF PRODUCTION AND COW MANAGEMENT SYSTEMS ON DAIRY FARMS

Marek Wroński, Marek Cichocki, Katarzyna Borkowska, Jan Redmer

Chair of Cattle Breeding and Milk Quality Evaluation
University of Warmia and Mazury in Olsztyn

K e y w o r d s: dairy cows, production efficiency, costs, milk price.

A b s t r a c t

The study was performed on five farms located in the Province of Warmia and Mazury, Administrative District of Olecko, and in the Pomeranian Province, Administrative District of Nowy Dwór Gdański. The aim of the study was to estimate milk production efficiency in herds differing in size, milk yield and housing system. The annual maintenance costs per cow decreased along with an increase in the number of cows in a herd. In the free-stall system and PMR feeding, the costs of feed (mostly purchased) were higher, while the labor costs were almost twofold lower, compared with the tie-stall system and traditional feeding. An increase in the scale of production and average milk yield enabled to improve milk production profitability as well as to increase the agricultural income per cow.

WPŁYW SKALI PRODUKCJI ORAZ RÓŻNYCH TECHNOLOGII UTRZYMANIA KRÓW NA EFEKTYWNOŚĆ PRODUKCJI MLEKA W GOSPODARSTWACH FARMERSKICH

Marek Wroński, Marek Cichocki, Katarzyna Borkowska, Jan Redmer

Katedra Hodowli Bydła i Oceny Mleka
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: krowy mleczne, efektywność produkcji, koszty, cena mleka.

Address: Marek Wroński, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 5, 10-957 Olsztyn, Poland, phone: 48 (089) 523 37 59, e-mail: khb@uwm.edu.pl

A b s t r a k t

Badaniami objęto pięć gospodarstw zlokalizowanych w woj. warmińsko-mazurskim – powiat Olecko i w woj. pomorskim – powiat Nowy Dwór Gdański. Celem badań było oszacowanie efektywności produkcji mleka w stadach różniących się liczebnością, mlecznością oraz technologią utrzymania.

Koszty utrzymania w przeliczeniu na jedną krowę rocznie malały wraz ze wzrostem liczby krów w stadzie. Przy wolnostanowiskowym utrzymaniu zwierząt i żywieniu systemem PMR udział kosztów pasz, w tym głównie z zakupu, był wyższy, a udział kosztów pracy ponad 2-krotnie niższy niż przy uwięziowym utrzymaniu i żywieniu tradycyjnym. Wraz ze wzrostem skali produkcji i przeciętnej wydajności krów w stadach wzrastała opłacalność produkcji i dochód rolniczy w przeliczeniu na jedną krowę.

Introduction

High-quality milk production is one of the most capital-intensive branches of agricultural production. Milk production efficiency is affected primarily by cow performance, direct and indirect costs as well as by milk price at collection points. Although producers have no direct influence on milk price, they can make efforts to provide best-quality milk, to improve the milk productivity of cows and to reduce production costs, including labor costs and especially the costs of feed (JASIOROWSKI 2000, OKULARCZYK 2001).

The scale of production usually plays a major role with respect to profitability of low-income lines of business, such as animal production (especially cow raising). The minimal livestock density generating an agricultural income comparable to the average pay in the non-agricultural sector depends mostly on milk yield (OKULARCZYK 2000, OKULARCZYK, SZUMIEC 2000, PARZONKO 2003).

The costs of feed and labor dominate in the structure of milk production costs (BRZUSKI et al. 1994, OKULARCZYK et al. 1997). Permanent grasslands are the main source of feed for dairy cattle. Feed production on permanent grassland is by about 46 to 180% cheaper, compared with arable land. Therefore, an increase in the proportion of grasslands may contribute to both reducing the costs of cow farming and increasing milk production profitability (OKULARCZYK et al. 1997, OKULARCZYK 2000, OKULARCZYK, SZUMIEC 2000). Milk production efficiency is also higher when cows are fed farm fodder (OKULARCZYK 2001, OKULARCZYK, SOWULA-SKRZYŃSKA 2002). Milk production intensification through milk yield maximization brings the best results if based on farm-produced feed. A high proportion of purchased feed in the ration makes it hardly possible to increase milk production profitability in this way (OKULARCZYK 2000).

The tie-stall system is much more labor-consuming than the free-stall system. In the former direct-to-can or direct-line milking takes place in the

cowshed, whereas in the latter milking is carried out in the milking parlor. The work of a milker in the milking parlor is not only easier and more efficient, but also has a beneficial effect on animals and on milk quality, in comparison with milking in the tying stall (STADNIK et al. 2002).

Studies on milk production profitability in Poland show that during the years 1997-1999 milk production costs were higher than its selling price. A substantial increase in milk production profitability was observed in the year 2000 and 2002, mostly due to the increase in the selling price of milk, from 0.70 PLN/l in 1999 to 0.87 PLN/l in 2000 and to 0.90 PLN/l in 2001 (OKULARCZYK, SZUMIEC 2000, OKULARCZYK 2001).

Before our accession to the European Union, Poland was one of the cheapest milk producers in Europe and worldwide. The prices offered for milk to Polish farmers over the 1995-2003 period (except for the year 2001) were by approximately 50% lower, compared with UE member states. In 2004 the price of prime-quality milk exceeded 105 PLN/100 l, i.e. was comparable to that offered to milk producers in France, Ireland, Great Britain and Germany. This increase in milk price allowed dairy farms to earn a higher income despite the rise in price of means of production (SEREMAK-BULGE 2005).

Milk production efficiency is estimated based on an economic account, which involves the determination of production outlays and comparing them with incomes. The most adequate method for assessing milk production profitability is to calculate a direct surplus per cow, defined as the difference between the value of production obtained annually per cow (including sale of milk and sale of culled calves and cows) and direct/real costs (OKULARCZYK 2000).

The aim of the study was to estimate milk production efficiency in herds differing in stock density, productivity and housing system.

Materials and Methods

The study was conducted on five herds (A, B, C, D, E) of Polish Holstein-Friesian Black-and-White dairy cows. Herds A and B were kept on farms located in the Province of Warmia and Mazury, Administrative District of Olecko, while herds C, D, E were kept on farms situated in the Pomeranian Province, Administrative District of Nowy Dwór Gdański. The characteristics of farms and herds are given in Table 1.

The data used in the study come from RW-1 tabulograms (milk yield records), breeding records, milk receipts and delivery notes, milk quality evaluation records, veterinary certificates, purchase invoices and accounts.

Table 1

Characteristics of farms

Specification	Farm				
	A	B	C	D	E
Arable land area (ha) including:	134	79	50	160	750
– grassland area (ha)	50	34	20	36	50
Number of cows (head)	49	40	14	55	185
Housing system	free-stall	tie-stall	tie-stall	free-stall	tie-stall
Manure removal	twice a year	manual	manual	twice a year	with a front casting bulldozer
Feeding	PMR	traditional	traditional	PMR	TMR, annually
Watering	automated	manual	automated	automated	automated
Milking method	herringbone milking parlor	direct-to-line	direct-to-can	tandem milking parlor	direct-to-line
Annual yield:					
– milk (kg)	5714	5306	6333	6612	7448
– fat (kg)	259.4	205.8	266.0	257.2	348.6
– fat (%)	4.54	3.88	4.20	3.89	4.68
– protein (kg)	182.3	167.6	200.8	208.3	244.3
– protein (%)	3.19	3.16	3.17	3.15	3.28
Average somatic cell count (SCC)	198	149	242	295	316
Average price of sold milk (PLN/kg)	1.17	1.16	0.88	1.12	1.12

The economic analysis was performed employing the *Methods for Calculating Direct Surplus in Agriculture* (*Metodyki*. 1999), developed by the Institute of Agricultural Economics and Food Economy. The costs of feed were calculated based on outlays for feed production on the farm and for the purchase of concentrate and mineral feed. Non-feed direct costs (herd depreciation, veterinary care service and preventive treatment, insemination, milk yield testing, veterinary hygiene products) were determined based on documents provided by farmers. Labor costs related to milking and herd maintenance were calculated as the product of the actual working time and pay per man-hour (PLN 7). The other indirect costs (depreciation, repair and maintenance of buildings, machinery and equipment, charges for electricity and water supply, insurance fees) were determined on the basis of records kept on farms.

χ^2 test was applied to determine the effects of the scale of production, cow performance, milk price and various components of the housing system on the values of the following economic indices of milk production:
– number of cows: below 20 head, 21 to 50 head, above 100 head;
– milk yield: below 6000 kg, 6000 to 7000 kg, above 7000 kg;
– milk price: 0.88, 1.12, 1.16 to 1.17 PLN/l;
– housing system: free-stall, tie-stall;
– feeding system: traditional, PMR, TMR;
– watering system: automated, manual;
– manure removal system: twice a year with a front casting bulldozer.

Results and Discussion

The highest mean annual milk yield was recorded on farm E (7448 kg) (Table 1). Milk from this farm had also the highest concentrations of fat and protein. Farm E had the largest arable land area, the most numerous herd of cows and the most modern machinery and equipment. Milk productivity was at the lowest level on farm B, where cows were housed in a traditional system and work was only partly mechanized. Milk delivered by the farms under analysis was of prime quality (*Mleko surowe...* PN-99/A-86002), but there were considerable differences in the average somatic cell count (SCC). According to BRZOZOWSKI et al. (2001) and SAWA & PIWCZYŃSKI (2002), the somatic cell count in milk is affected, among others, by the proportion of HF genes, milk yield, stage of lactation, as well as human-related factors, such as house climate, husbandry conditions, veterinary care, performance of milking equipment and the application of proper milking procedures. In all herds higher mean milk yields were accompanied by higher average somatic cell counts.

Milk price depends on its quality (concentrations of major components, somatic cell count) as well as on production scale and proper milk handling procedures after milking. Dairy plants fix the purchase price of milk on an individual basis. Each of the farmers delivered milk to a different milk-processing plant, so the prices they got differed significantly irrespective of milk chemical composition (cf. the results achieved by farms D and E). Farm C, characterized by the lowest total milk production, was offered the lowest price – 0.88 PLN/kg.

Table 2 presents the effects of the factors analyzed in the study on the economic indices of milk production. Most of them were highly significantly correlated with the costs and value of production. The housing system had no considerable impact on the total costs and value of production, while direct costs were not affected by the milking method. There was a highly significant

Table 2

Effects of the factors analyzed in the study on the economic indices of milk production

	Number of cows (head)	Milk yield (kg)	Milk price (PLN)	Housing system	Feeding system	Watering system	Milking method	Manure removal system
Direct costs including:	***	***	***	***	***	-	***	***
– farm-made feed	***	***	***	*	***	***	***	***
– purchased feed	***	***	***	***	***	***	***	***
Indirect costs including:	***	***	***	***	***	***	***	***
– maintenance and milking costs	***	***	***	***	***	***	***	***
Total costs	***	***	***	–	***	***	***	***
Production value (PLN/head)	***	***	***	–	***	***	***	***
Direct surplus (PLN/head)	***	***	***	***	***	***	***	***
Direct surplus (PLN/kg milk)	***	***	***	–	–	–	–	–
Production costs per kg of milk (PLN)	***	***	***	–	–	–	–	–
Production profitability ratio (%)	***	***	***	–	*	*	***	*
Agricultural income (PLN/head)	***	***	***	***	***	***	***	***

* – significance at a level of 0.05; ** – significance at a level of 0.01; *** – significance at a level of 0.005

correlation between the obtained results, direct surplus and agricultural income per cow.

The production profitability ratio was highly significantly correlated with the number of cows, milk yield and price as well as with the milking method. A significant effect was observed for the systems of feeding, watering and manure removal. The production costs and direct surplus per kg of milk were affected only by the number of cows, their performance and the selling price of milk.

The annual direct maintenance costs (over 3 000 PLN/head) were found to be the highest on farms A and D (PMR feeding, milking in the milking parlor) – Table 3. Indirect costs were much lower on these farms, and the costs of herd maintenance and milking were two- to threefold lower than the other costs. Comparable results were obtained in Germany, where annual maintenance of a single cow took 70 to 120 man-hours in the tie-stall system, and 50 man-hours in the free-stall system (VEANTHIE, LEŚNIAKOWSKI 2002). The total annual maintenance costs per cow were dependent on herd size and decreased along with an increase in the number of cows in a herd. They ranged between 4512 PLN/head on farm C (14 cows) to 3954 PLN/head on farm E (185 cows). The structure of maintenance costs was affected by the housing system (Figure 1). On farms A and D (free-stall system, PMR feeding, milking in the milking parlor) the costs of purchased feed dominated in the cost structure (30.15% and 28.38%, respectively). Total feed costs on these farms amounted to 55%, compared with 38% on farm C and about 45% on farms B and E. On farms A, B and C, where grassland area corresponded to herd size and where cows were pasture-grazed over summer, the costs of farm fodder did not exceed 25% in the total cost structure. A similar proportion of expenses for feed production and purchase in the total cost structure was reported by SZALUNAS (2005), whereas a higher contribution of costs of feed to overall milk production costs (approx. 60%) was observed by OKULARCZYK (2001).

Table 3

Annual cow management costs (PLN/head)

Specification	Farm				
	A	B	C	D	E
Direct costs including:	3178	2715	2642	3233	2596
– farm-made feed	1027	1025	992	1186	1286
– purchased feed	1277	960	721	1195	417
– total	2304	1985	1713	2381	1703
Indirect costs including:	1058	1651	1870	977	1358
– maintenance and milking costs	469	1150	1363	498	894
Total costs	4236	4366	4512	4210	3954

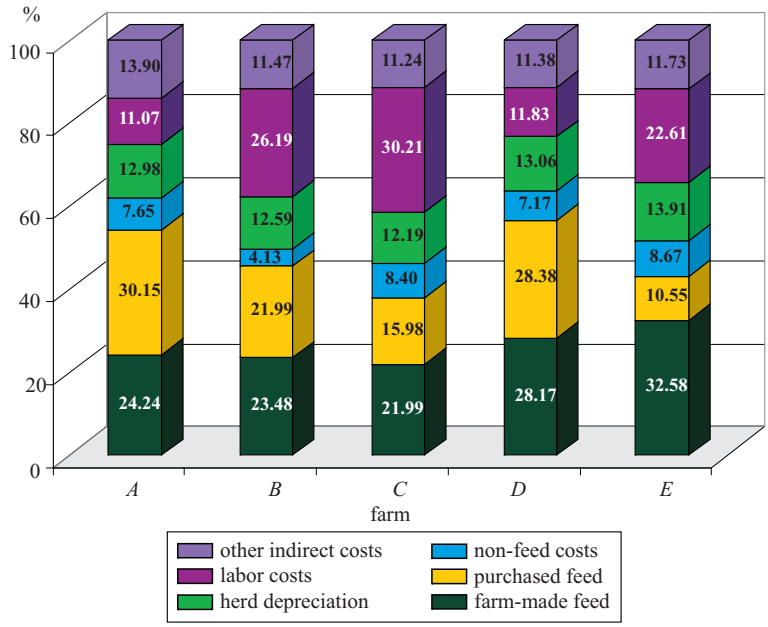


Fig. 1. Structure of cow management costs

The cow management system had a significant effect on the proportion of labor costs in the cost structure. On farm C (low mechanization/automation, direct-to-can milking) labor costs accounted for over 30% of total maintenance osts. On farm B (direct-to-line milking) they were reduced to 26.19%. On farm E, TMR feeding enabled to decrease labor costs to 22.61%. On farms A and D (high mechanization, automated milking) labor costs made up only about 11% of total maintenance costs.

The value of milk production per cow was affected by the value of milk sold, which varied from 93% to 95%, depending on the farm (Table 4). The lowest production value (below 6000 PLN/head) was achieved on farm C. This was a consequence of the lowest selling price of milk on this farm. Small-scale producers most often deliver milk to small dairies whose market position is relatively weak and which are not able to compete with the prices offered by large milk-processing plants. On the other farms the production value was related to the annual sales of milk obtained from a single cow. A comparison of milk production effectiveness ratios on the tested farms shows that on farm C the low value of production resulted in the lowest direct surplus per cow and per kg of sold milk, as well as in the lowest milk production profitability and agricultural income. An increase in the number of cows on a farm was accompanied by an increase in the direct surplus per head.

Table 4

Milk production efficiency

Specification	Farm				
	A	B	C	D	E
Production value including: (PLN/head)	7093	6577	5953	7756	7942
– milk sale	6705	6177	5510	7405	7522
– sale of culled calves and cows	388	400	443	351	420
Direct surplus (PLN/head)	3915	3862	3311	4523	5346
Direct surplus (PLN/ kg of milk)	0,68	0,72	0,52	0,68	0,72
Production costs per kg of milk (PLN)	0,74	0,83	0,71	0,63	0,53
Production profitability ratio (%)	167	151	132	184	201
Agricultural income (PLN/head)	2857	2211	1441	3546	3988

On the other farms the value of direct surplus per kg of sold milk was dependent on direct costs. On farms A and D, where direct maintenance costs per cow exceeded 3000 PLN, the direct surplus per kg of sold milk was 0.68 PLN. On farms B and E, where direct costs were lower, this surplus was equal to 0.72 PLN/kg of milk. The production costs per kg of milk were affected by herd size and housing system to a slight degree only. They depended primarily on the quantity of milk obtained from one cow and sold, i.e. production costs decreased along with an increase in milk yield.

The highest milk production profitability and agricultural income per cow were attained on farm E. Cow performance on this farm was the highest, high-quality fodder produced there enabled to minimize the costs of purchased feed, and due to a high level of mechanization and automation the costs of maintenance and milking did not exceed 900 PLN per cow. On farm D, where the housing system and maintenance costs per cow were comparable to those on farm A, milk production profitability was by 17 percentage points higher, agricultural income per cow was by 700 PLN higher, while the production costs per kg of milk were by 0.11 PLN lower. The differences in milk production effectiveness ratios between herds were caused by a much higher (by approx. 900 kg) milk yield on farm D. On farm B, where the price per kg of milk was comparable to that on farm A and the direct surplus was higher, milk production profitability was by 16 percentage points lower and agricultural income per cow was by over 600 PLN lower. Such milk production effectiveness ratios on this farm resulted from lower, by about 400 kg of milk, mean

annual productivity per cow as well as from higher costs of maintenance and milking (at a lower level of mechanization). In consequence, indirect costs per cow were by about 600 PLN higher. A comparison of herds from farms A, B and D indicates that milk production efficiency increases with an increase in the number of cows in a herd and their average annual productivity.

It may be concluded that the total annual maintenance costs per cow decreased along with an increase in the number of cows in a herd. The structure of maintenance costs was affected by the housing system. In the free-stall system and PMR feeding, the costs of feed (mostly purchased) were higher, while the labor costs were almost twofold lower, compared with the tie-stall system and traditional feeding. The selling price of milk depended to a greater extent on the milk processing plant to which it was supplied than on its chemical composition. An increase in herd size and average milk yield enabled to improve milk production profitability as well as to increase the agricultural income per cow.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 17.01.2007

References

BRZOZOWSKI P., DZIARSKI K., LUDWICZUK K. 2001. *Wpływ wybranych czynników na zawartość komórek somatycznych i składu chemicznego mleka*. Zesz. Nauk PTZ Prz. Hod., 55: 123-131.

BRZUSKI P., GŁUŚ GŁOŚ, SZAREK I. 1994. *Wstępna analiza wybranych wskaźników ekonomicznych hodowli bydła ncb w gospodarstwie należącym do skarbu państwa*. Zesz. Nauk. AR Wrocław, 245: 251-257.

JASIOROWSKI H. 2000. *Niektóre kluczowe problemy rozwoju hodowli bydła w Polsce*. Zesz. Nauk PTZ Prz. Hod., 51: 17-26.

OKULARCZYK S., TYBURCY A., KAPŁON B., SOWULA-SKRZYŃSKA E., KWAŚNIEWSKA M. 1997. *Koszty składników pasz w różnych regionach kraju*. Rocz. Nauk. Zoot., 24: 3, 253-262.

OKULARCZYK S. 2000. *Determinanty opłacalności produkcji zwierzęcej w warunkach polskich*. Mat. Konf. Nauk. nt.: Zarządzanie przedsiębiorstwem w warunkach konkurencji. UWM, Olsztyn, 15-16 czerwca, ss. 109-119.

OKULARCZYK S., SZUMIEC A. 2000. *Wpływ zmian cen zbóż i ich dostępność w kraju na intensywność produkcji mleka*. Zesz. Nauk PTZ Prz. Hod., 54: 125-137.

OKULARCZYK S. 2001. *Jakość mleka i czynniki rynkowe w opłacalności jego produkcji*. Zesz. Nauk PTZ Prz. Hod., 55: 113-121.

OKULARCZYK S., SOWULA-SKRZYŃSKA E. 2002. *Wykorzystanie cech regionów, skali produkcji i jakości mleka w jego konkurencyjności na rynku krajowym i europejskim*. Rocz. Nauk. Zoot., supl., 15: 233-236.

Metodyka liczenia nadwyżki bezpośredniej dla działalności produkcji rolniczej. 1999. Pr. zbior. IERiGŻ, Warszawa.

PARZONKO A. 2003. *Wyniki ekonomiczne gospodarstw wyspecjalizowanych w chowie bydła mlecznego w zależności od skali produkcji mleka*. Rocz. Nauk. Zoot. Ser. G, 90(1): 133-138.

Mleko surowe do skupu. Wymagania i badania. PN-99/A-86002.

SAWA A., PIWCZYŃSKI D. 2002. *Komórki somatyczne a wydajność i skład mleka krów mieszańców cb x hf*. Med. Wet., 58: 636-640.

SEREMAK-BULGE J. 2005. *Stan i perspektywy rozwoju gospodarstw mlecznych w Polsce*. Prz. Mlecz., 5: 4-10.

STADNIK L., LOUDA F., NOVA V., TOUSOVA R. 2002. *Ocena ekonomiki produkcji mleka z uwzględnieniem systemu utrzymania*. Biul. Inf. IŻR, 2: 91-97.

SZALUNAS T. 2005. *Wartość użytkowa krów i efektywność produkcji mleka w gospodarstwie specjalistycznym*. Kat. Hodowli Bydła, UWM Olsztyn (rozprawa doktorska).

VEANTHIE G., LEŚNIAKOWSKI R. 2002. *Jak wydajnie pracować w oborze*. Top Agrar Polska, 5: 135-137.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 61-72, Y. 2007

DOI 10.2478/v10020-007-0007-6

ORGANICS REMOVAL AND NITRIFICATION IN MUNICIPAL LANDFILL LEACHATE TREATED IN SBRs WITH CLINOPTILOLITE CARRIER

Dorota Kulikowska, Joanna Racka

Chair of Environmental Biotechnology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: municipal landfill leachate, organics removal, nitrification, clinoptilolite, SBR, hydraulic retention time (HRT).

A b s t r a c t

The effect of clinoptilolite addition to activated sludge system on organics removal and nitrification in real municipal landfill leachate was studied. Experiments were carried out at hydraulic retention time (HRT) 4 d and 2 d in three SBRs operated in parallel. The reactors were operated in a 24-h cycle mode (filling 0.25 h, anoxic 3 h, aeration 18 h, settling 2.5 h, and discharge 0.25 h). Clinoptilolite concentration in SBRs was maintained at 2 $g \cdot dm^{-3}$ (SBR 2) and 5 $g \cdot dm^{-3}$ (SBR 3). SBR 1 worked as a control (without clinoptilolite). It was shown that at HRT 4 d clinoptilolite carrier did not have an effect on efficiency of BOD_5 removal and only moderate effect on efficiency of COD removal. However, clinoptilolite addition caused increase of ammonium removal rates from 12.8 mg $N_{NH4} \cdot dm^{-3} \cdot h^{-1}$ in SBR 1 to 19.2 mg $N_{NH4} \cdot dm^{-3} \cdot h^{-1}$ in SBR 3. In the effluent from all reactors nitrate were predominant product of nitrification. HRT shortening to 2 d caused decrease both BOD_5 and COD removal efficiency in all reactors. Moreover, in SBRs with clinoptilolite carrier nitrite were the main form of nitrification, while in SBR without clinoptilolite high concentration of ammonium (over 300 mg $N_{NH4} \cdot dm^{-3}$) was found.

USUWANIE ZWIĄZKÓW ORGANICZNYCH I NITRYFIKACJA PODCZAS OCZYSZCZANIA ODCIEKÓW WYSYPISKOWYCH W REAKTORACH SBR Z WYPEŁNIENIEM Z KLINOPTYLOLITU

Dorota Kulikowska, Joanna Racka

Katedra Biotechnologii w Ochronie Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: odcieki z wysypisk odpadów komunalnych, substancje organiczne, nitryfikacja, klinoptylolit, reaktory SBR, czas zatrzymania.

Address: Dorota Kulikowska, University of Warmia and Mazury in Olsztyn, Słoneczna St. 45G, 10-907 Olsztyn, Poland, phone: 48 (089) 523 41 45, e-mail: dorotak@uwm.edu.pl

Abstrakt

Badano wpływ klinoptylolitu na efektywność usuwania związków organicznych i nitryfikację podczas oczyszczania odcieków z wysypisk odpadów komunalnych. Badania prowadzono w trzech równolegle pracujących reaktorach SBR. Czas zatrzymania (HRT) wynosił 4 i 2 d, cykl pracy reaktorów – 24 h (napełnianie 0,25 h, mieszanie 3 h, napowietrzanie 18 h, sedymentacja 2,5 h, dekantacja 0,25 h). Stężenie wprowadzonego klinoptylolitu wynosiło 2 $g \cdot dm^{-3}$ (SBR 2) i 5 $g \cdot dm^{-3}$ (SBR 3). SBR 1 był reaktorem kontrolnym (bez klinoptylolitu). Wykazano, że w przypadku czasu zatrzymania 4 d, wprowadzenie klinoptylolitu nie wpłynęło na efektywność usuwania związków organicznych wyrażonych BZT_5, i tylko w niewielkim stopniu poprawiło efektywność usuwania ChZT. Klinoptylolit spowodował natomiast wzrost szybkości usuwania azotu amonowego z 12,8 mg $N_{NH4} \cdot dm^{-3} \cdot h^{-1}$ w SBR 1 do 19,2 mg $N_{NH4} \cdot dm^{-3} \cdot h^{-1}$ w SBR 3. W odpływie ze wszystkich reaktorów dominowały azotany, będące głównymi produktami nitryfikacji. Skrócenie HRT do 2 d spowodowało spadek efektywności usuwania związków organicznych (zarówno BZT_5, jak i ChZT). Ponadto, w reaktorach z wypełnieniem z klinoptylolitu głównym produktem nitryfikacji były azotyny, w odpływie z reaktora kontrolnego – azot amonowy w stężeniu powyżej 300 mg $N_{NH4} \cdot dm^{-3}$.

Introduction

Leachate from municipal solid waste landfills are defined as hazardous wastewater. The choice of their appropriate treatment method is difficult, mainly because of leachate composition, which varies depending on landfill age and biological activity within the landfill. The mature leachate contain relatively low concentrations of degradable organic materials and high concentrations of ammonium (KNOX 1985).

The leachate with high ammonium content are difficult to effective treatment by conventional biological method due to its toxic inhibition to nitrifying microorganisms. Moreover, in activated sludge the nitrification process may be inhibited by metals and hazardous compounds.

Recent studies examined the leachate treatment with high ammonium loading using chemical precipitation of ammonium as a pre-treatment process (LI, ZHAO 2001). However, the high salinity formed in treated leachate because of using chemicals affected negatively on microbial activity in the following steps of biological treatment.

In activated sludge, the ammonium oxidation proceeds slowly because of slow nitrifying bacteria growth. So, it is necessary to provide high residence time of nitrifying cells (sludge age). Thereby, in order to obtain nitrification there are employed combined methods, such as activated sludge processes with biomass carriers. So far, nitrification was studied in reactors with activated sludge contained plastic carrier material as small cubes of macroporous cellulose or tubes made of polyethylene with an addition of ammonium chloride (WELANDER et al. 1997). Commonly used appliances to nitrification are moving-bed biofilm process, based on the use of small, free-floating polymeric

(polyurethane) elements, while biomass is being grown and attached as biofilm on the surface of these porous carriers (LOUKIDOU, ZOUBOULIS 2001).

Recently, many authors employed zeolite to improve nitrification in activated sludge systems. From literature view it is concluded that two different mechanisms could cause enhancement of nitrification in activated sludge with that carriers. Added minerals adsorb some compounds working as nitrification inhibitors and nitrification may be improved as a consequence of decreased inhibition (NG et al., 1987), and enable nitrifiers to colonize the attached biofilm, improving nitrification (LEe et al. 2002). However, it is worth remarking that most of these researches have been made on synthetic wastewater (CAMPOS et al. 2002, LEE et al. 2002, JUNG et al. 2004).

The aim of this study was to determine the effects of clinoptilolite addition to activated sludge system on organics removal and nitrification in sequencing batch reactors (SBRs) treating municipal landfill leachate.

Materials and Methods

Description of SBRs and operating conditions

Experiments were carried out at HRT 4 d and 2 d in three SBRs operated in parallel. The reactors were operated in a 24-h cycle mode (filling 0.25 h, anoxic 3 h, aeration 18 h, settling 2.5 h, and discharge 0.25 h). Clinoptilolite concentration in SBRs was maintained at 2 $g \cdot dm^{-3}$ (SBR 2) and 5 $g \cdot dm^{-3}$ (SBR 3). SBR 1 worked as a control reactor (without clinoptilolite).

The reactors with the working volume of 6 l were made of plexiglass and were equipped with a stirrer at the regulated rotation speed (36 r min^{-1}). Dissolved oxygen was supplied using porous diffusers, placed at the bottom of reactors. In all reactors, the amount of the oxygen supplied to the reactor in the aeration phase was regulated in order to maintain oxygen concentration between 2.5-4.0 $mgO_2 \cdot dm^{-3}$ at the end of the phase. All reactors were operated at a room temperature (19±3°C) for about 2 months both at HRT 4 d and 2 d.

Activated sludge

SBRs were supplied with the sludge from municipal wastewater treatment plant "Łyna" (Olsztyn, Poland). The activated sludge was adapted to the leachate by cultivation of microorganisms in diluted leachate in SBRs during 3 weeks.

Characteristic of clinoptilolite

Clinoptilolite used in the experiment originated from mine located in Nižny Hrabovec. Clinoptilolite showed 0.8 mval · g^{-1} of ammonium capacity. The medium size of the clinoptilolite was 0.3-2 mm and the composition of powder consisted dominantly of SiO_2 (60.68-71.78%) and Al_2O_3 (11.47-12.83%). The characteristic of the zeolite was given in Table 1.

Table 1

Characteristic of clinoptilolite

Parameters	Value
Physical properties	
ammonium exchange capacity (mval · g^{-1})	0.8
density (kg · m^{-3})	2000-2450
specific surface area (m^2 · g^{-1})	20-30
medium size (mm)	0.3-2
Chemical composition (wt. %)	
SiO_2	60.68-71.76
Al_2O_3	11.47-12.83
TiO_3	0.3-0.33
Fe_2O_3	1.5-1.7
CaO	3.5-7.1
MgO	0.5-1.26
Na_2O	0.55-0.7
K_2O	2.26-3.02
P_2O5	0.02-0.05

Landfill leachate characteristic

Leachate used in this study were collected from a municipal landfill located in Wysieka near Bartoszyce in the Warmia and Mazury Province, Poland. The landfill has been in operation since January of 1996.

The leachate were delivered to the laboratory twice a month and stored at 4°C.

Table 2

Characteristic of municipal landfill leachate

Parameter	Unit	Value
pH	–	8.1 (± 0.3)
Chemical oxygen demand (COD)	mg COD · dm^{-3}	997 (± 53)
Biochemical oxygen demand (BOD_5)	mg BOD_5 · dm^{-3}	169 (± 14)
BOD_5/COD	–	0.17
Total nitrogen	mg N · dm^{-3}	874 (± 21)
Ammonium nitrogen	mg N_{NH4} · dm^{-3}	816 (± 18)
Total phosphorus	mg P · dm^{-3}	14.2 (± 2.3)
Total solids	mg · dm^{-3}	6904 (± 439)

The characteristic of investigated landfill leachate is shown in Table 2. As leachate originated from old landfill, they contained mainly refractory organics (low BOD_5/COD ratio) and high concentration of ammonium.

Required amounts of KH_2PO_4 were added to leachate deficient in the phosphate to adjust COD/P ratio to 100:1.

Analytical methods

The raw leachate and effluent from SBRs were analysed for: pH, organic compounds (COD) (dichromate reflux method – according to HERMANOWICZ et al. (1999) and BOD_5 – with the use of the OxiTop® according to DIN EN 1899-1/EN 1899-2 official EPA method using OxiTop® made by WTW company), nitrogen compounds (total Kjeldahl nitrogen, Kjeldahl Method), ammonium nitrogen (distillation/Nesslerization method), nitrite nitrogen (colorimetric method with sulfanilic acid and 1-naphthylamine) and nitrate nitrogen (colorimetric method with phenolsulfonic acid), phosphorus (ascorbic acid method) and total solids – according to HERMANOWICZ et al. (1999).

The mixed reactor content was measured for volatile suspended solids (MLVSS), according to HERMANOWICZ et al. (1999) and oxygen concentration using an oxygen controller (model: HI 9142 made by Hanna Instruments).

Results and Discussion

Organics removal

It was shown that at HRT 4 d clinoptilolite additive did not have an effect on BOD_5 removal efficiency – in all SBRs it was over 90%, and only moderate effect on COD removal efficiency (on average it increased from 14% in SBR 1 to 19% in SBR 3) – Table 3. After HRT shortening to 2 d BOD_5, removal efficiency decreased to 86% (on average) and COD removal efficiency was below 10% (Table 3).

Table 3

Organics removal efficiency in SBRs 1-3 at HRT 4 and 2 d

SBR No.	HRT 4 d		HRT 2 d	
	COD (%)	BOD_5 (%)	COD (%)	BOD_5 (%)
SBR 1	14.3	92.5	8.9	85.3
SBR 2	14.2	91.8	9.3	86.0
SBR 3	19.1	92.8	9.4	87.2

Similarly, WELANDER et al. (1998) and BARBUSIŃSKI et al. (1997) showed low COD removal (about 20% and 7.5%, respectively) during landfill leachate treatment from old site. The low COD removal achieved in this work is in agreement with the low BOD_5/COD ratio of the raw leachate. The low biodegradability of the organics is typical for leachate from landfill in the methanogenic phase, for which most of biodegradable matter is converted to biogas already in the landfill and, in consequence, the produced leachate contain mainly refractory compounds.

Nitrification

The investigations revealed that at HRT 4 d nitrification was achieved in all reactors, but at different time. In SBR 1 (without clinoptilolite), a complete ammonium removal was achieved after 41 days of operation. In the effluent from SBR 2 (2 $g \cdot dm^{-3}$ clinoptilolite) and SBR 3 (5 $g \cdot dm^{-3}$ clinoptilolite) ammonium concentration below 1 mg $N_{NH4} \cdot dm^{-3}$ was observed after 34 and 18 days, respectively (Figure 1).

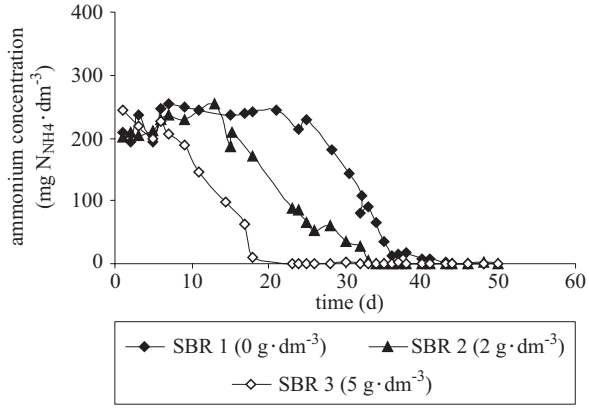


Fig. 1. Ammonium concentration in the effluent from SBRs 1-3 (HRT 4 d)

The presence of clinoptilolite influenced greatly as well oxidation products of ammonium. In SBR 1 during the first 36 days, nitrite were the main product of nitrification. But as the ammonium concentration reached a low level, nitrite concentration dropped dramatically and a complete nitrification to nitrate was observed (Figure 2a). In SBR 2, nitrite production during start-up was nearly at the same level as in SBR 1. After about 40 days, however, nitrite concentration was always below 1 mg $N_{NO2} \cdot dm^{-3}$ and complete nitrification

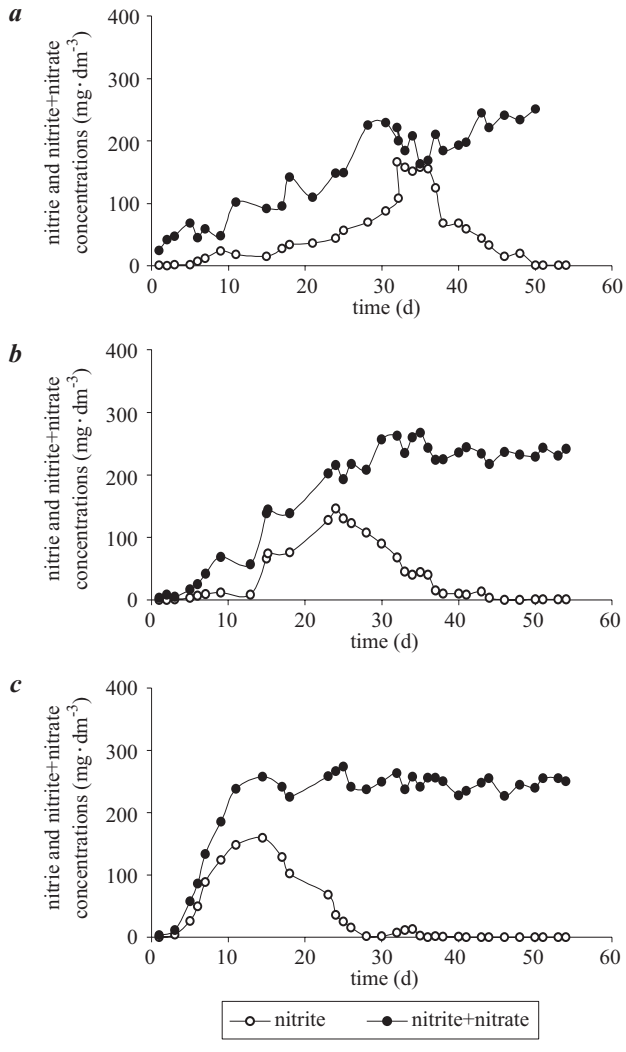


Fig. 2. Nitrite and nitrite plus nitrate concentrations in the effluent from SBRs 1-3 (HRT 4 d): *a* – SBR 1 (without clinoptilolite), *b* – SBR 2 (2 g · dm^{-3} clinoptilolite), *c* – SBR 3 (5 g · dm^{-3} clinoptilolite)

was obtained (Figure 2b). In SBR 3, nitrification ended up when nitrite were only presented during the first 18 days. Full nitrification with nitrate as a main product was observed after 3 weeks of operation (Figure 2c).

After reaching steady-state, nitrate were formed in all reactors in amounts approximately stoichiometric to the consumed ammonium.

In the present research, HRT in all SBRs was kept at 4d during 56 days of experiment, after which it was shortened to 2 d. It resulted in a considerable increase in the ammonium concentration to over 300 mg $N_{NH4} \cdot dm^{-3}$ in the effluent from SBR 1 (Figure 3a). In SBRs 2 and 3 ammonium was nitrified to nitrite. In SBR 3, nitrite were stable final product of nitrification (Figure 3c), while in SBR 2 apart from nitrite in the effluent, ammonium nitrogen appeared in concentration in the level 50-60 mg $N_{NH4} \cdot dm^{-3}$ (Figure 3b).

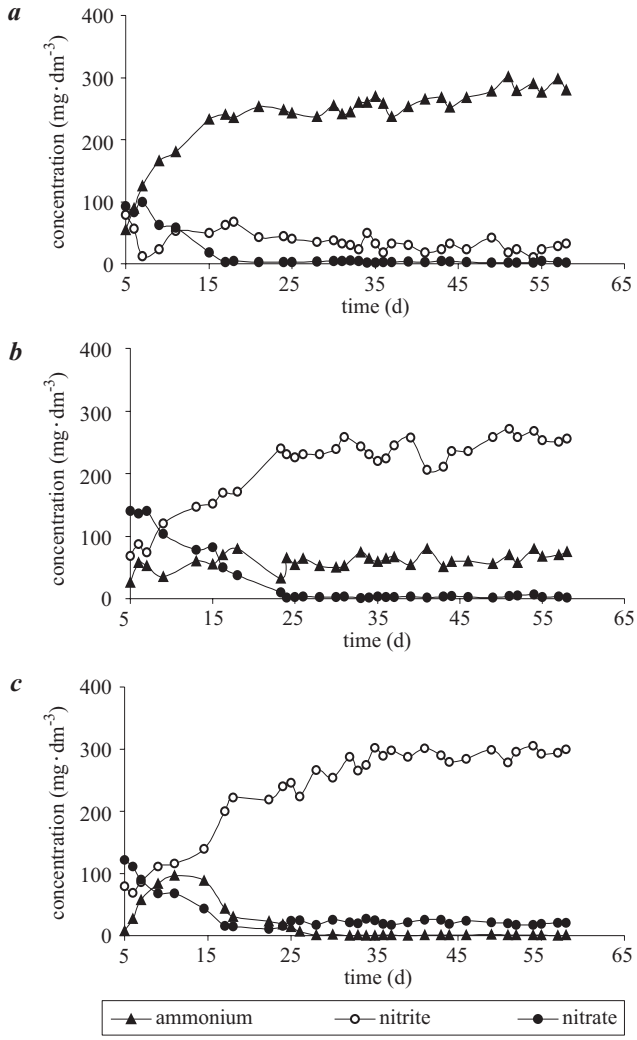


Fig. 3. Ammonium, nitrite and nitrate concentrations in the effluent from SBRs 1-3 (HRT 2 d): *a* – SBR 1 (without clinoptilolite), *b* – SBR 2 (2 $g \cdot dm^{-3}$ clinoptilolite), *c* – SBR 3 (5 $g \cdot dm^{-3}$ clinoptilolite)

The reason of improved nitrification in SBRs 2 and 3 (partially nitrification) towards to SBR 1 (lack of nitrification) may be linked with application of clinoptilolite, but the mechanism of clinoptilolite action is not clear. Therefore, explanation of the role of minerals added to activated sludge there has been an on-going research.

From literature review it is concluded that clinoptilolite is a kind of natural mineral, which is well known for its ability to remove ammonium ion from water, due to preferential selectivity for this ion (ROIĆ et al. 2000). In this research, ammonium exchange capacity was about 0.8 mval $\cdot$ g^{-1}. Considering that leachate contained high concentration of ammonium nitrogen due to ammonium exchange capacity and the fact that consumed ammonium and formed nitrate occurred stoichiometrically, it may be supposed that ammonium adsorption onto clinoptilolite was negligible.

CAMPOS et al. (2002) were studied the effects of the addition of kaolin powdered particles to nitrifying activated sludge. The authors showed that the specific nitrifying activities of ammonium and nitrite oxidizing processes were enhanced up to 75% and 50%, respectively, when kaolin was added. However, additional ammonium, nitrite and nitrate adsorption tests showed that these compounds were not adsorbed on kaolin. Different results were obtained by KARGI and PAMUKOGLU (2004). They compared two different adsorbents, PAC (powdered activated carbon) and zeolite, alone and with that adsorbent added to biological treatment of pre-treated landfill leachate. The percent of ammonium removal only by adsorption was 28% and 16% for clinoptilolite and PAC, respectively. Adsorbents added to biological system caused increase in ammonium removal to 40% (clinoptilolite) and 30% (PAC).

In present work, higher MLVSS in SBRs with clinoptilolite in contrary SBR without clinoptilolite was obtained (in SBRs 2 and 3 the average biomass concentrations were 2460 and 2820 mg MLVSS $\cdot$ dm^{-3}, whereas in SBR 1 – merely 1840 mg MLVSS $\cdot$ dm^{-3}). Therefore, it is supposed that clinoptilolite was significant mainly as bio-carrier for bacteria. Other authors made similar notices. LEE et al. (2002) proved that minerals addition into activated sludge reactors caused significant increase in the concentration of microorganisms. KIM et al. (2003) showed that in reactor with clinoptilolite (4 g $\cdot$ dm^{-3}) biomass concentrations expressed as MLSS and MLVSS were 9 580 mg $\cdot$ dm^{-3} and 4 760 mg $\cdot$ dm^{-3}, respectively. The microbial concentration of the control (conventional activated sludge) was 2 850 and 2 030 mg $\cdot$ dm^{-3}, respectively. In all discussed cases, increase of the amount of microorganisms may indicate that the biofilm is formed on the surface of the powdered mineral (CHUDOBA, PANNIER 1994, SON et al. 2000). In our study lack of nitrification in SBR 1 and partial nitrification in SBRs 2 and 3 may be linked with the fact that both *Nitrosomonas* and *Nitrobacter* are slow growing bacteria and could be washed

out from reactor without long HRT unless the microorganisms were kept there by adsorption onto added minerals. ROSTRON et al. (2001) in the reactor with immobilised biomass, complete nitrification were observed at HRT 2.2-8d. After HRT shortening to 1.5d build-up of nitrite took place. According to authors, the lower cell yield of *Nitrobacter* in comparison to *Nitrosomonas* (0.042 mg cells · mg^{-1} N and 0.142 mg cells · mg^{-1} N, respectively) indicated that a lack of *Nitrobacter* was probably the reason for the build-up of the nitrite. When HRT was further decreased to 1d, the concentration of ammonia increased, indicating washout of *Nitrosomonas*.

Considering that our investigations were carried out on real leachate, there is necessary to notice the ability of clinoptilolite to remove refractory organic compounds and heavy metals (OUKI, KAVANNAGH 1997, INGLEZAKIS, GRIGOROPOULOU 2004, BEKTAŞ, KARA 2004, MIER et al. 2001). HRT shortening might has caused the increase of toxic substances concentration e.g. heavy metals at the cycle beginning, and in consequence inhibit nitrification in SBR 1. In SBRs 2 and 3, zeolite addition to activated sludge could effectively increase nitification by adsorbing inhibitors to *Nitrosomonas* and *Nitrobacter*. According to the literature data it results that *Nitrobacter* is much more sensitive to inhibitors than *Nitrosomonas*. Therefore, the nitrite accumulation is observed in many cases where 2nd phase of nitrification is inhibited (RHEE et al. 1997, HWANG et al. 2000, AKTAS, ÇEÇEN 2001).

After 52 days of experiment (at HRT 4 d) measurements of changes in ammonium concentration during SBRs cycle were conducted. In all reactors, ammonium removal rate was described by zero-order kinetics and defined by the following differential equation:

$$r_N = -\frac{dC_N}{dt} = -k_N \tag{1}$$

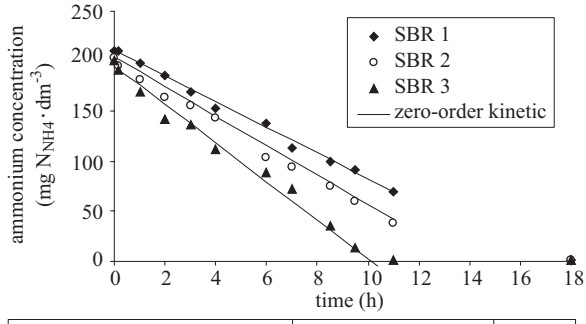
The solution for this could be fitted to the experimental data according to (2):

$$C_N = C_{0,N} - k_N \cdot t \tag{2}$$

where:

r_N – ammonium removal rate (mg $N_{NH_4} \cdot dm^{-3} \cdot h^{-1}$),
k_N – constant of ammonium removal rate (mg $N_{NH_4} \cdot dm^{-3} \cdot h^{-1}$),
C_N – ammonium nitrogen concentration after time t (mg $N_{NH_4} \cdot dm^{-3}$),
t – time (h),
$C_{0,N}$ – ammonium nitrogen concentration at the beginning of the aeration phase (mg $N_{NH_4} \cdot dm^{-3}$).

The values of ammonium removal rate were estimated from equation (2). It was shown that clinoptilolite carrier improved ammonium removal rates – in SBR 1 it was 12.8 mg $N_{NH4} \cdot dm^{-3} \cdot h^{-1}$, whereas in SBR 3 it was about 1.5-fold higher (Figure 4). Similarly, ROSTRON et al. (2001) in reactors containing suspended biomass and reactors with biomass adsorption onto particles (Kaldnes, Linpor) and PVA (polyvinyl alcohol) encapsulated nitrifiers, showed that volumetric nitrification rates were 0.36, 0.53, 0.57 and 0.70 kg $N \cdot m^{-3} \cdot d^{-1}$ for suspended biomass reactor, Kaldnes, Linpor and PVA-encapsulated, respectively.



SBR No.	r (mg $N_{NH4} \cdot dm^{-3} \cdot h^{-1}$)	φ^2
SBR 1 (without clinoptilolite)	12.8	0.005
SBR 2 (2 g · dm^{-3} clinoptilolite)	14.7	0.003
SBR 3 (5 g · dm^{-3} clinoptilolite)	19.2	0.009

Fig. 4. Concentrations profiles of ammonium in leachate during aerobic conditions and reaction rates described by zero-order kinetics at HRT 4 d

Conclusions

SBRs employing clinoptilolite as bio-carrier were compared to the control, and the following results were obtained:

– at both monitored HRTs clinoptilolite did not enhance BOD_5 removal efficiency and had only moderate effect on increase of COD removal efficiency;

– at HRT 4 d clinoptilolite addition improved ammonium nitrogen removal rate – in SBR 3 (5 g · dm^{-3} of clinoptilolite) it was 1.5 fold higher in comparison to SBR 1 (control, without clinoptilolite);

– at HRT 2 d in SBR 1, there was observed lack of nitrification. In SBRs 2 and 3 the nitrification had been improved by the addition of clinoptilolite.

Translated by ZYGMUNT GUSIATIN

Accepted for print 19.01.2007

References

AKTAS Ö., ÇEÇEN F. 2001. *Nitrification inhibition in landfill leachate treatment and impact of activated carbon addition.* Biotechnol. Lett., 23: 1607-1611.

BARBUSIŃSKI K., KOŚCIELNIAK H., MAJER M. 1997. *Oczyszczanie wód podziemnych zalegających pod składowiskiem odpadów przemysłowych.* V Ogólnopol. Symp. Nauk.-Tech. „Biotechnologia Środowiskowa".

BEKTAŞ N., KARA S. 2004. *Removal of lead from aqueus solutions by natural clinoptilolite: equilibrium and kinetic studies.* Sep. Purif. Technol., 39: 189-200.

CAMPOS J. L., GARRIDO R., MÉNDEZ R., LEMA J. M. 2002. *The effect of kaolin particles on the behavior of nitrifying activated sludge units*. Biores. Technol., 81: 225-231.

CHUDOBA P, PANNIER M. 1994. *Use of powdered clay to upgrade activated sludge process.* Environ. Technol., 15: 863-870.

HERMANOWICZ W., DOŻAŃSKA W., DOJLIDO J., KOZIOROWSKI B., 1999. *Fizyczno-chemiczne badanie wody i ścieków*. Arkady, Warszawa.

HWANG B. H., HWANG K. Y., CHOI D. K., JUNG J. Y. 2000. *Enhanced nitrite build-up in proportion to increasing alkalinity/NH_4^+ ratio of influent in biofilm reactor.* Biotechnol. Lett., 22: 1287-1290.

INGLEZAKIS V. J., GRIGOROPOULOU H. 2004. *Effects of operating conditions on the removal of heavy metals by zeolite in fixed bed reactors.* J. Hazard. Mater., B112: 37-43.

JUNG J-Y., CHUNG Y-CH, SHIN H-K, SON D-H. 2004. *Enhanced ammonia nitrogen removal using consistent biological regeneration and ammonium exchange of zeolite in modified SBR process*. Wat. Res., 38: 347-354.

KARGI F., PAMUKOGLU M. Y. 2004. *Adsorbent supplemented biological treatment of pre-treated landfill leachate by fed-batch operation.* Biores. Technol., 94: 285-291.

KIM CH. G., LEE H. S., YOON T. I. 2003. *Resource recovery of sludge as a micro-media in an activated sludge process.* Adv. Environ. Res., 7: 629-633.

KNOX K. 1985. *Leachate treatment with nitrification of ammonia.* Wat. Res., 19: 895-904.

LEE Y-W., ONG S-K., SATO CH. 1997. *Effects of heavy metals on nitrifying bacteria*. Wat. Sci. Technol., 36: 69-74.

LEE H. S.,. PARK S. J, YOON T. I. 2002. *Wastewater treatment in a hybrid biological reactor using powdered minerals: effects of organic loading rates on COD removal and nitrification.* Proc. Biochem., 38: 81-88.

LI X. Z., ZHAO Q. L. 2001. *Efficiency of biological treatment affected by high strength of ammonium-nitrogen in leachate and chemical precipitation of ammonium-nitrogen as pretreatment.* Chemosphere, 44: 37-43.

LOUKIDOU M. X., ZOUBOULIS A. I. 2001. *Comparison of two biological treatment processes using attached-growth biomass for sanitary landfill leachate treatment.* Environ. Pollut., 111: 273-281

MIER M. V., CALLEJAS R. L., GEHR R., CISNEROS B. E. J., ALVAREZ P. J. J. 2001. *Heavy metal removal with Mexican clinoptilolite: multi-component ionic exchange.* Wat. Res., 35: 373-378.

NG A., STENSTROM M. K., MARRS D R. 1987. *Nitrification enhancement in the powdered activated carbon-activated sludge process for the treatment of petroleum refinery wastewater.* J. WPCF, 59: 199-211.

OUKI S. K., KAVANNAGH M., 1999. *Treatment of metals-contaminated wastewaters by use of natural zeolites.* Water Sci. Technol., 39: 115-122.

RHEE S. K., LEE J. J., LEE S. T. 1997. *Nitrite accumulation in sequencing batch reactor during aerobic phase of bilogical nitrogen removal.* Biotechnol. Lett., 19: 195-198.

ROIĆ M., CERJAN-STEFANOWIĆ S., KURAJICA S., VANČINA V., HODŽIĆ E. 2000. *Ammoniacal nitrogen removal from water by treatment with clay and zeolites.* Wat. Res. 34, 3675-3681.

ROSTRON W. M., STUCKEY D. C., YOUNG A. A. 2001. *Nitrification on high strength ammonia wastewaters: comparative study of immobilization media.* Wat. Res., 35: 1169-1178.

SON D. H., KIM D. W., CHUNG Y. Ch. 2000. *Biological nitrogen removal Rusing a modified oxic./anoxic reactor with zeolite circulation.* Biotechnol. Lett., 22: 35-38.

WELANDER U., HENRYSSON T., WELANDER T. 1997. *Nitrification of landfill leachate using suspended-carrier biofilm technology.* Wat. Res., 31: 2351-2355.

WELANDER U., HENRYSSON T., WELANDER T. 1998. *Biological nitrogen removal from municipal landfill leachate in a pilot scale suspended carrier biofilm process.* Wat. Res., 32: 1564-1570.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 73-82, Y. 2007

DOI 10.2478/v10020-007-0008-5

COMPUTER SIMULATION OF LATENT COAGULATION

Lech Smoczyński, Regina Wardzyńska, Beata Załęska-Chróst, Zbigniew Bukowski

Chair of Chemistry
University of Warmia and Mazury in Olsztyn

K e y w o r d s: computer simulation, coagulation, flocculation, aggregation.

A b s t r a c t

The paper presents the results of a study carried out using the computer program ZB1 simulating the destabilization of suspended solids. The description of the program is followed by an analysis of the effects of such parameters as sol concentration, size and initial velocity of colloidal particles, and size of sedimenting flocs on the duration of latent coagulation.

KOMPUTEROWA SYMULACJA PROCESU KOAGULACJI SKRYTEJ

Lech Smoczyński, Regina Wardzyńska, Beata Załęska-Chróst, Zbigniew Bukowski

Katedra Chemii
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: symulacja komputerowa, koagulacja, flokulacja, agregacja.

A b s t r a k t

W pracy opisano wyniki badań uzyskane za pomocą komputerowego programu ZB1, który symuluje proces destabilizacji zawiesin ściekowych. Przedstawiono charakterystykę opracowanego programu, a następnie badano wpływ takich parametrów, jak: stężenie zolu, wielkość i prędkość początkowa cząstki koloidalnej, wielkość kłaczka sedymentującego podczas trwania koagulacji skrytej.

Address: Lech Smoczyński, University of Warmia and Mazury, pl. Łódzki 4, 10-727 Olsztyn, Poland, phone: 48 (089) 523 36 68, e-mail: lechs@uwm.edu.pl

Introduction

The degrees of dispersion in a colloidal system as well as its destabilization are affected by a variety of physicochemical factors. Successive stages of this process comprise aggregation, flocculation, and sedimentation of gel/sludge. Coagulation-flocculation is universally employed for water and wastewater treatment as well as in many other technological processes that require solid phase separation from the colloidal system (DUTKIEWICZ 1998). Coagulation starts with particle aggregation, i.e. with a decrease in the degree of system dispersion. No manifestations of aggregation-flocculation, such as changes in color or turbidity, can be observed at this stage, sometimes referred to as latent coagulation. Easily noticeable changes, like the formation of sludge/gel flocks, take place at the next stage, known as flocculation or visible coagulation. The duration of latent coagulation of lyophobic colloids is so short that for many years researchers have focused exclusively on visible coagulation, although latent coagulation often decides about the overall efficiency of coagulation, flocculation or even sedimentation (STUMM, MORGAN 1981).

Industrial or semi-technological scale coagulation experiments are always carried out on natural wastewater, whereas laboratory tests are performed on natural or model wastewater (SMOCZYŃSKI, WARDZYŃSKA 1996, 2003). Model wastewater guarantees unlimited reproducibility of experimental conditions and a high number of repetitions, which enables adequate fitting of a mathematical model (SMOCZYŃSKI 2000) to the database obtained in the study.

Computer simulation may be a viable alternative to laboratory tests. It has become a reliable tool in many braches of science, thus filling the gap between theory and practice. Computer-based simulation of coagulation-flocculation offers practically unlimited experimental options, because it permits, among others, direct observation and recording of e.g. slow latent coagulation. Computer-based simulation is also applied in studies on the kinetics of rapid perikinetic and orthokinetic coagulation, as well as for experimentation including the modeling and modification of aggregation-flocculation and sedimentation (ELIMELECH et al. 1995)

The model of computer simulation used most frequently is the so called Monte Carlo' simulation (KOŁODZIEJSKI 2002). This method, based on thè theory of probability and statistical mechanics, has a wide range of applications. It is employed, among others, to determine the fractal size (LATTUADA et al. 2003, LEE et al. 2002) and structure (PUSHKAR, ROSNER 1999) of post-coagulation sludge aggregates, as well as to analyze the mechanism of coagulation, aggregation and flocculation (NAZZARRO et al. 2002, XIAO-YAN LI et al. 2003).

The paper presents the results of a study carried out using the computer program ZB1 simulating destabilization of suspended solids. The effects

of such parameters as sol concentration, size and initial velocity of colloidal particles, and size of sedimenting flocks on latent coagulation were determined. Latent coagulation is difficult to record experimentally due to the lack of noticeable manifestations. The system performance was positively verified against the classical theory of the kinetics and diffusion of dispersoids (SMOCZYŃSKI et al. 2006). It was found that the program ZB1 operates in accordance with the logic and general principles of colloidal system destabilization.

Material and Methods

The simulation model applied in our study, ZB1, is a stochastic-dynamic one (PERKOWSKI 1980), based on random variables. The state of the system changes due to the passage of simulation time. The kernel of the operating system is a module solving equations of motion for a given number of units of matter (particles) in a closed vessel. The program simulates the process of destabilization of spherical particles of a homodisperse sol. The initial values of particle positions are generated randomly in uniform distribution within the vessel. The particles move according to Brownian motion. The program computes the trajectories of individual particles, and the direction of motion is selected randomly (RANDOM function). The angle at which the particle bounces off the vessel wall is equal to the incident angle. A collision of two particles which have different velocities results in the formation of a cluster (e.g. a dimer) whose direction and velocity are resultants calculated as vectors. In this way aggregates composed of three, four and more particles are formed. During the process of aggregation the number of single particles decreases gradually, and the original homodisperse sol turns into a polydisperse system. Having reached the adequate size, the aggregates undergo sedimentation and are eliminated from the system.

The program was written in Pascal, using an easily available tool, i.e. "Turbo Pascal" version 6.0 (Borland Inc.). Widely variable input parameters of the simulation program were:

N_0 – number of particles,
V_0 – initial velocity equal to the simulated initial velocity of each particle,
D – size of sedimenting flocks determined by the number of particles forming an agglomerate,
H – particle diameter,
Q – rate of sedimentation, i.e. a systemic parameter specifying the range of "sedimentation values" assumed by clusters.

Variable parameters were: a) number of particles N_0 (1000 to 2000), b) particle diameter (1 to 10), c) initial velocity V_0 (10 to 100), d) size D of

sedimenting agglomerates (5 to 50). The constant parameter was the rate of sedimentation $Q = 0.02$.

The ZB1 program is equipped with a particle counter, a simulated clock and a STOP function, stopping the counter and the clock. All measurements were performed in five replications. The graphs were plotted for mean values, showing standard deviations.

Results and Discussion

The rate of coagulation is dependent on the Brownian motion of particles and interactions between them. For an effective collision, the distance between two particles must be shorter than the radius of the attraction range. However, this is possible only when: 1) the particles lost their electric charge and acquired electrokinetic potential equal to zero, or 2) the particles discharged below the so called critical potential. In the present study the particles had no electrolytic potential and each collision was considered to be effective.

Figure 1 presents the relationships between the duration of latent coagulation and particle size. The duration of latent coagulation is the period of time that passes from the beginning of the process to the sedimentation of the first aggregate. The measurements were carried out at initial concentrations N_0 equal to 1000, 1200 1400, 1600, 1800, 2000, at a constant initial velocity $V_0 = 50$ and at a constant size of a sedimenting flock $D = 20$. The optimal mathematical fitting of R^2 (coefficient of determination) was obtained for a power function of the $y = ax^b$ type. R^2 values and corresponding equations are presented in Table 1.

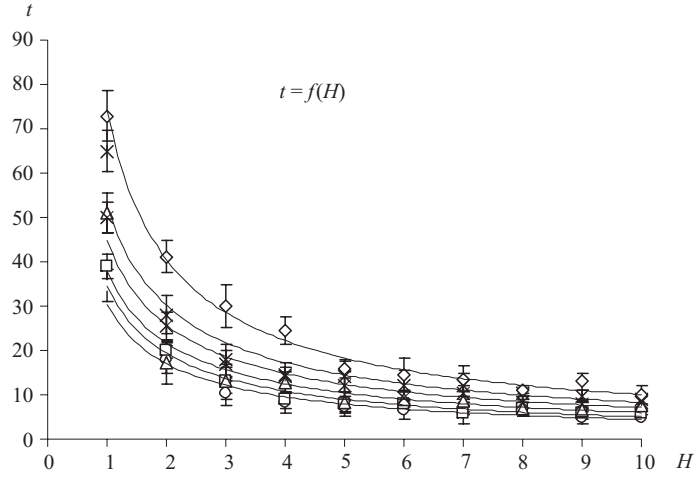


Fig. 1. Effect of the particle size H on the duration of latent coagulation

Table 1

Relationships between the duration of latent coagulation and particle size

Series	Sol concentration	Equation	R^2
O	1000	$73.983x^{-0,8683}$	0.98
✳	1200	$52.518x^{-0,8015}$	0.96
×	1400	$44.807x^{-0,8015}$	0.98
□	1600	$37.827x^{-0,8004}$	0.92
△	1800	$34.521x^{-0,8371}$	0.96
◇	2000	$30.417x^{-0,8367}$	0.97

The equations given in Table 1 show that the values of the constant "b" in the "$y = ax^b$" type equations remained within a relatively narrow range, i.e. -0.8003 to -0.8683, which may indicate that this constant is independent of the initial concentration N_0 of the sol and of the particle size H, i.e. of the degree of system dispersion. The constant "a" is responsible for different courses of curves in Figure 1, since its value was over twofold higher ($a \approx 74$) for the sol of the initial concentration $N_0 = 1000$, compared with the sol of the concentration $N_0 = 2000$ ($a \approx 30$). This means that the lower the initial concentration of the sol (1000 to 1400) the more significant the effect of particle size on the duration of latent coagulation. An increase in particle size reduces the time of latent coagulation. A tenfold increase in particle diameter allows to shorten the duration of the process over sevenfold. Generally, for a sol of a constant initial concentration the greatest influence of particle size (H) on the duration of latent coagulation was observed for the smallest particles ($H = 1$ to 5). In the case of bigger particles ($H \geq 5$ to 10), the degree of system dispersion had a slight impact on the time of latent coagulation for all initial concentrations of the sol.

According to SMOLUCHOWSKI (1917), in homodisperse systems the probability W that one particle meets another is directly proportional to a displacement in a unit of time, resulting from Brownian motion, as well as to the range of attraction A between particles with identical radii:

$$W = 4\pi A D_f \tag{1}$$

where:
D_f is the coefficient of diffusion of the system, being a measure of the above displacement. If two particles are to collide, the distance between them must be shorter than the radius of the attraction range A (Figure 2).

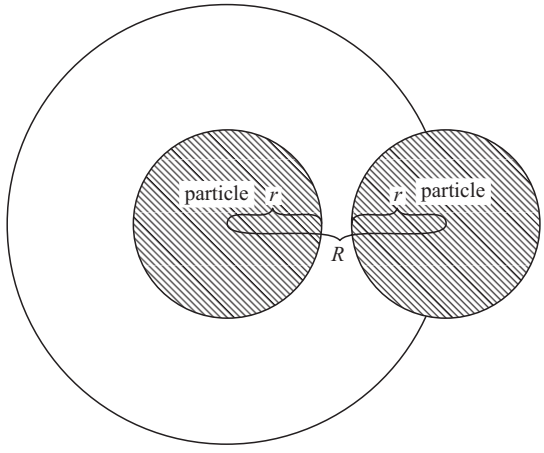


Fig. 2. Range of coagulation effectiveness (WEISER 1950)

It was proved experimentally that the radius of the attraction range is only a little bigger than the particle diameter H, which means that an increase in H should be followed by a higher probability of collisions between particles, i.e. a shorter time of latent coagulation. This relationship is easily noticeable for small particles (H = 1 to 5). However, it must be kept in mind that the probability of collisions between particles (W) is also affected by the displacement resulting from Brownian motion. The measure of displacement (per unit time) in Brownian motion is the diffusion coefficient D_f. According to the concept formulated by Graham (FORSTERLING, KUHN 1976) this coefficient is inversely proportional to the radius r of a particle:

$$D_f \text{ (cm}^2 \text{ s)} = f(1\ r^{-1}) \tag{2}$$

Aggregated particles (dimers) move according to Brownian motion at a velocity reduced proportionally to their increased size. Therefore, it seems that in the case of bigger particles (H = 5 to 10) the increased probability of collisions resulting from the bigger radius of the attraction range is balanced by smaller displacement in Brownian motion.

Figure 3 illustrates the effect of a simulated initial velocity of a particle, V_0 = 10 to V_0 = 100, on the duration of latent coagulation for sols of various initial concentrations N_0 (1000, 1200, 1400, 1600, 1800, 2000). The size of a colloidal particle and the size of a sedimenting floc were constant, H = 5 and D = 10 respectively. The optimal fitting of results ($R^2 \geq 0.96$) was obtained for a power dependence of the $y = ax^b$ type. R^2 values and corresponding equations are presented in Table 2.

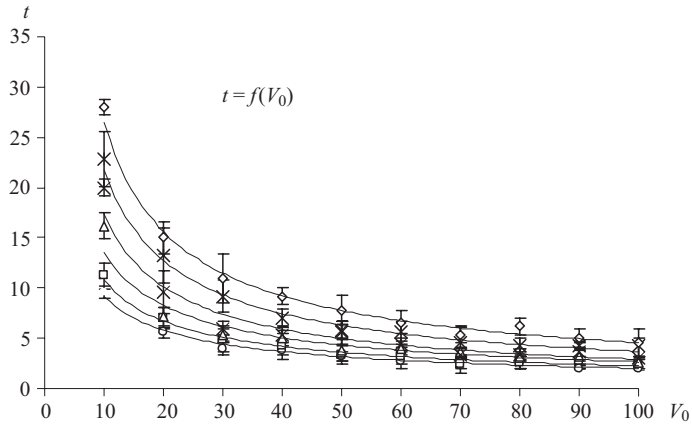


Fig. 3. Effect of the initial velocity V_0 of a particle on the duration of latent coagulation

Table 2

Relationships between the duration of latent coagulation and the initial velocity V_0 of a particle

Series	Sol concentration	Equation	R^2
◇	1000	$153.36x^{-0,7627}$	0.99
✳	1200	$128.34x^{-0,771}$	0.98
×	1400	$103.29x^{-0,7756}$	0.96
△	1600	$68.578x^{-0,7065}$	0.96
□	1800	$51.56x^{-0,6803}$	0.97
O	2000	$41.26x^{-10,6558}$	0.98

An increase in the simulated velocity of a particle results in the shortening of the time of latent coagulation for all sols, irrespective of their initial concentrations, although the stronger the concentration, the shorter the coagulation. A tenfold increase in the initial velocity of a particle allows to reduce the duration of the process about fivefold. The optimal value of the initial velocity V_0 of a particle can be observed for each sol. A further increase in V_0 permits only an insignificant reduction in the duration of latent coagulation. This value depends on the initial concentration of a sol. For instance, for N_0 = 1000 V_0 = 70, whereas for N_0 = 2000 the optimal value V_0 is as low as 30.

According to the theory proposed by SMOLUCHOWSKI (1917), the probability that a given particle meets another increases along with an increase in the above-mentioned displacement (per unit time) in Brownian motion. In practice, the displacement defined in this way must be directly related to the initial

velocity of a particle in the ZB1 simulation program. Applying the Fick law (FORSTELING, KUNH 1976) for concentrated solutions, where D_f is dependent on the concentration of the system, we obtain an equation showing the correlation between the so called diffusion current $\delta c/\delta t$ and concentration gradient $\delta c/\delta x$:

$$\delta c/\delta t = \delta/\delta x[D_f(\delta c/\delta x)] \tag{3}$$

where:
x may denote a generalized coordinate.

Equation (3) shows that an increase in the value of diffusion current $\delta c/\delta t$ is directly proportional to D_f. Thus, an increase in concentration should be theoretically accompanied by an increase in the diffusion coefficient D_f as well as the probability of collisions between particles. This was confirmed by simulated experimental data.

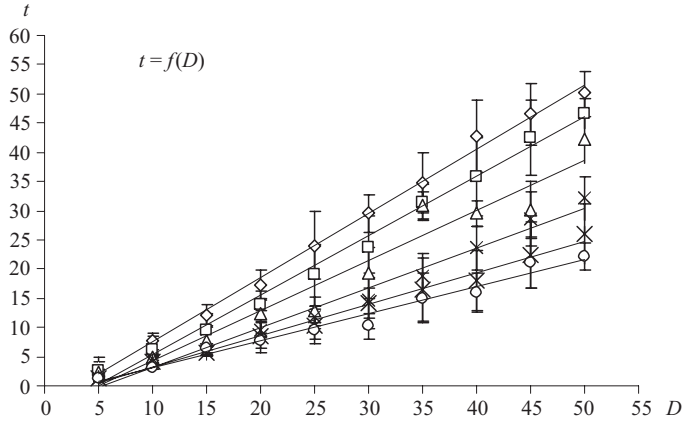


Fig. 4. Effect of the size D of a floc-aggregate on the duration of latent coagulation

Figure 4 presents the relationships between the simulated time of latent coagulation and the size D of a floc-aggregate for sols of various initial concentrations N_0 (1000, 1200, 1400, 1600, 1800, 2000). The initial velocity V_0 and diameter R of a particle were constant, 50 and 5 respectively. As expected, the relationships $t = f(D)$ are of the straight-line type, while the values of $R^2 > 0.95$ indicate a high probability of fitting of this mathematical model (a straight-line equation) to the experimental database – Table 3.

Table 3
Relationships between the duration of latent coagulation and the size D of a floc-aggregate

Series	Sol concentration	Equation	R^2
◇	1000	$0.9024x + 3.3332$	0.99
□	1200	$0.9768x + 4.8958$	0.99
△	1400	$1.1021x + 6.2947$	0.95
×	1600	$1.4357x + 5.807$	0.98
✳	1800	$1.8454x + 4.1374$	0.99
O	2000	$2.1076x + 3.7687$	0.98

An increase in the size D of a flock-aggregate extends the duration of latent coagulation. A tenfold increase in size D prolongs the time of latent coagulation around 16-fold. A bigger size of agglomerate requires the agglomeration of a higher number of particles, which takes more time at a specified probability of active collisions. The probability of collisions increases with an increase in the initial concentration N_0 of a sol, and the duration of coagulation becomes shorter. The increasing values of slopes of straight-lines, obtained in the study, are given in Table 3.

Conclusion

The ZB1 computer simulations performed in the study provided data on latent coagulation. This process would be difficult to monitor under laboratory conditions. The obtained relationships are consistent with universally known theories concerning the processes of coagulation, aggregation and flocculation, leading to the destabilization of a colloidal system. Similarly as results of laboratory tests, the database created in simulation tests is burdened with a certain experimental error. Thus, the computer program ZB1 is not an automatic mathematical machine, but simulates natural behavior patterns of a sol, characterized by variation and diversity. ZB1 can be successfully applied in practice, e.g. to optimize the chemical coagulation of wastewater.

The statistical significance of measurements was verified by the Q-Dixon test (PAWLACZYK, ZAJĄC 2001) at a significance level $\alpha_{0.05}$ ($P = 95\%$). All results were found to be reliable.

1. In contrast to simple jar-tests, the computer program simulating the destabilization of wastewater suspension permits precise and thorough monitoring of latent coagulation which often decides about the overall efficiency of coagulation, aggregation, flocculation and sedimentation.

2. The simulation program ZB1 allows to determine the effects of some parameters on latent coagulation:

– the concentration of the dispersed phase of a colloidal system, the size of a colloidal particle and the size of a sedimenting floc have a significant impact on the duration of the process,

– the initial velocity of a colloidal particle has the slightest influence on the duration of the process.

3. Results of measurements obtained using the ZB1 program are consistent with the classical theories of destabilization of colloidal systems.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 15.12.2006

References

DUTKIEWICZ E. T. 1998. *Fizykochemia powierzchni*. WNT, Warszawa.

ELIMELECH M., GREGORY J., JIA X., WILLIAMS R., A. 1995. *Particle deposition and aggregation – measurement, modeling and simulation.* Elsev., Amsterdam, 441 pp.

FORSTERLING H.D., KUHN H. 1976. *Eksperymentalna chemia fizyczna*. WNT, Warszawa.

KOŁODZIŃSKI E. 2002. *Symulacyjne metody badania systemów*. PWN, Warszawa.

LATTUADA M., HUA WU, MORBIDELLI M. 2003. *A simple model for structure of fractal aggregates.* J. Col. Interface Sci., 268(1): 106-20.

LEE D.G., BONNER J.S., GARTON L.S., ERNEST A.N.S., AUTENRIETH R.L. 2002. *Modeling coagulation kinetics incorporating fractal theories: comparison with observed data.* Wat. Res., 36: 1056-1066.

NAZZARRO M., NIETO F., RAMIREZ-PASTOR A.J. 2002. *Influence of surface heterogeneities on the formation of diffusion-limited aggregates.* Surface Sci., 497(1-3): 275-284.

PAWLACZYK J., ZAJĄC M. 2001. *Walidacja metod analizy chemicznej*. AM, Poznań.

PERKOWSKI P. 1980. *Technika symulacji cyfrowej*. WNT, Warszawa.

PUSHKAR T., ROSNER D.E. 1999. *Monte Carlo Simulation of Particle Aggregation and Simultaneous Restructuring*. J. Coll. Interface Sci., 213: 273-286.

SMOCZYŃSKI L. 2000. *Aggregation of the silica suspension by Al-coagulants.* Pol. J. Chem., 74: 1617-1624.

SMOCZYŃSKI L., WARDZYŃSKA R. 2003. *Fractal characteristics of post-coagulation sludge from sewage.* Environ. Protect. Eng., 29: 65-72.

SMOCZYŃSKI L., WARDZYŃSKA R. 1996. *Study on macroscopic aggregation of silica suspensions and sewage.* J. Coll. Inter. Sci., 183: 309-314.

SMOLUCHOWSKI M. 1917. *Versuch einer mathematischen Theorie der Koagulations-kinetic kolloider Lsungen.* Z. Phys., 92, 129.

STUMM W., MORGAN J. J. 1981. *Aquatic chemistry: an introduction emphasizing chemical equlibria in natural waters.* New York: Wiley.

WAISER H. B. 1950. *A Textbook of Colloid Chemistry*. JohnWiley & Sons, NewYork.

WARDZYŃSKA R., BUKOWSKI Z., ZAŁĘSKA-CHRÓST B, SMOCZYŃSKI L. 2006. *Computer Simulation of Wastewater Flocculation.* Environ. Protect. Eng. (in print).

XIAO-YAN LI, JIAN-JUN ZHANG 2003. *Numerical simulation and experimental verification of particle coagulation dynamics for a pulsed input*. Coll. Interface Sci., 262: 149-161.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 83-91, Y. 2007

DOI 10.2478/v10020-007-0009-4

EFFECT OF SELECTED ENVIRONMENTAL PARAMETERS ON SEWAGE SLUDGE VERMICOMPOSTING

Andrzej Walkowiak

Chair of Environmental Protection Engineering
University of Warmia and Mazury in Olsztyn

K e y w o r d s: utilization of sewage sludges, vermicomposting process, Eisenia fetida.

A b s t r a c t

Vermicomposting of sewage sludges is one of the methods of their utilization. Not adhering to appropriate conditions of that process decreases the nutritive value of the vermicompost obtained to a significant extent, as only compost that does not pose any sanitary hazard can be utilized for natural purposes.

An inappropriate content of oxygen in culture medium, too high bulk density of the medium (a degree of compression), too low or too high temperature of the vermicomposting process, improper pH, improper humidity, and a variety of other factors may negatively affect the quality of the vermicompost produced, and consequently the degree of sewage sludge neutralization.

Some constraints of the vermicomposting process are strictly linked with the quantity and quality of sewage sludge. An excessive content of heavy metals in the sludge may completely exclude the possibility of sewage sludge utilization with that method.

Yet, adherence to specified rules and performance of different treatments in the course of sewage sludge vermicomposting reduces, to a considerable extent, the negative impact of the above-mentioned factors on vermiculture as well as on the quality of processes proceeding over the period of vermicomposting.

Relatively precise determination of threshold values of factors determining the vital activity of earthworms and establishing optimal conditions of the process in which vermicompost with a high nutritive value is produced are feasible due to the possibility of running a pot culture under laboratory conditions. Results obtained this way will provide a basis for determining conditions of the vermicomposting process modified by the introduction of a physical factor, namely an electromagnetic field.

Address: University of Warmia and Mazury, Andrzej Walkowiak, Prawocheńskiego 1, 10-957 Olsztyn, Poland, e-mail: a.walkowiak@uwm.edu.pl

WPŁYW WYBRANYCH PARAMETRÓW ŚRODOWISKOWYCH NA WERMIKOMPOSTOWANIE OSADÓW ŚCIEKOWYCH

Andrzej Walkowiak

Katedra Inżynierii Ochrony Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: utylizacja osadów ściekowych, wermikompostowanie, Eisenia fetida.

A b s t r a k t

Wermikompostowanie osadów ściekowych jest jednym ze sposobów ich utylizacji. Nieprzestrzeganie odpowiednich warunków tego procesu wysoce obniża wartość nawozową uzyskanego wermikompostu. Wykorzystany przyrodniczo może być tylko kompost nie stwarzający zagrożenia sanitarnego.

Nieodpowiednia ilość tlenu w podłożu hodowlanym, zbyt duża gęstość podłoża (stopień ubicia), zbyt niska lub wysoka temperatura procesu wermikompostowania, niewłaściwe pH, nieodpowiednia wilgotność oraz wiele innych czynników mogą negatywnie wpłynąć na jakość wyprodukowanego wermikompostu, a tym samym na stopień neutralizacji osadów ściekowych.

Niektóre ograniczenia procesu wermikompostowania są ściśle związane z ilością oraz jakością osadów ściekowych. Zbyt duża zawartość metali ciężkich w osadach może całkowicie wykluczyć możliwość utylizacji osadów ściekowych tą metodą.

Przestrzeganie określonych zasad i wykonywanie różnych zabiegów w trakcie wermikompostowania osadów ściekowych w znaczny sposób obniża negatywny wpływ ww. czynników na wermikulturę oraz jakość procesów zachodzących w trakcie wermikompostowania.

W miarę precyzyjne ustalenie progowych wartości czynników determinujących aktywność życiową dżdżownic oraz wyznaczenie optymalnych warunków procesu, w którym powstaje wermikompost o wysokiej wartości nawozowej, jest możliwe dzięki prowadzeniu hodowli wazonowej w warunkach laboratoryjnych. Uzyskane w ten sposób wyniki mogą stanowić podstawę do wyznaczenia warunków procesu wermikompostowania, zmodyfikowanego wprowadzeniem czynnika fizycznego, jakim jest pole elektromagnetyczne.

Introduction

The number of sewage treatment plants has been observed to increase each year. An increasing bulk of sewage sludge prompts the necessity of appropriate sludge management. Nevertheless, more and more often sanitary and ecological properties of sludges do not meet expectations despite the application of highly advanced processes of their treatment. Such sludges are still detrimental to the natural environment, hence they cannot be utilized for natural purposes. Still, as a result of applying proper processes of sludge treatment, factors that impair their natural utilization can be eliminated.

A vermiculture is defined as a high-density culture of earthworms on different organic wastes (KOSTECKA 1995). An earthworm species of the highest recognition at our latitude is *Eisenia fetida* Sav. One of the methods of sewage sludge utilization is the process of vermicomposting. The rate of sewage sludge treatment with that method depends on the number and

biomass of earthworms. As claimed by KOSTECKA (after FLECKENSTEIN and GRAF): ,,one tone of earthworm biomass is capable to process one tone of sludge into vermicompost within five days''. Production of good vermicompost is linked with strictly following the process of its production. Only good vermicompost, consisting practically of sole earthworm excreta, is characterized by a high nutritive value.

Material and Methods

Investigations were carried out on Californian earthworm Eisenia Fetida. The culture was run on three types of culture media:

– variant I- culture pots without drainage (No 1*a* and 1*b*), on the surface of which homogenized sewage sludge (150 mL) with hydration ranging from 93 to 95 % were poured once a weak. Inspections of the developing vermiculture were carried out every six weeks;

– variant II- culture pots with drainage (No 2*a* nad 2*b*), on the surface of which 150 mL of water-homogenized sewage sludges with hydration ranging from 96 to 99% were poured 2-3 times a week. Inspections of the developing vermiculture were carried out every six weeks;

– variant III- culture pots without drainage (No. 3a and 3b), on the surface of which 150 mL of sewage sludges with hydration ranging from 93 to 95% were poured once a week. Those pots were not subjected to inspections.

Bottom of the pots with drainage was perforated. Between the perforated bottom and culture medium there were three layers of gravel with different diameters. So prepared drainage enabled feeding the earthworms with liquid sludges in a considerable excess (2-3 times a week, 150 cm^3 each time). That treatment was aimed at monitoring the effect of the humidity of culture medium on the vermiculture. Having been filtrated through a layer of the culture medium, the homogenized sewage sludges escaped off the culture pot in the form of an efflux.

In all culture pots the process of vermicomposting proceeded at a temperature ranging from 22 to 25°C.

In the case of the culture pots without drainage, homogenized sewage sludges were poured onto their surfaces also at a dose of 150 cm^3, but only once a week. In addition, depending on the measurement of dry matter content of the culture medium, they were sprinkled with water. This way, medium humidity in those pots was kept at a level optimal for the earthworms, i.e. 75-80%.

Over the six-month experimental period, a treatment of medium ventilation ("inspection") was performed in pots No 1*a* and 1*b* as well as No 2*a* and 2*b*

every six weeks. The treatment consisted in pouring the culture media containing the earthworms out of the pots. It was aimed at supplying oxygen into the culture medium, enabled measurements of the number and individual biomass of the earthworms as well as loosened the medium.

The culture medium and vermiculture present in the pots without drainage (No 3*a* and 3*b*) were not subjected to periodical inspections. That procedure was aimed at determining whether a lack of inspections and, consequently, an increase in medium density, affect the intensity of channel boring by the earthworms.

In order to provide conditions similar to open-air vermicultures run on the industrial scale, the access of light to the culture pots was eliminated.

The earthworms were fed with sewage sludges collected from sewage plots of the Municipal Wastewater Treatment Plant in Olsztyn.

In research proceedings answers were sought to the three basic questions:

1. How does the vermiculture develop depending on different humidity of the culture medium?

2. What is the effect of an increase in total and individual biomass and in the number of earthworms on the degree of medium loosening and, consequently, on oxygen content of the medium?

3. How does the respiratory activity of different types of culture media changes?

During colonizing with earthworms, sawdust were added to all culture pots so as to improve the C/N ratio. Cellulose present in the sawdust is indispensable for building egg cocoons laid by the earthworms. PH was kept at a level optimal for the earthworms, i.e. from 6.8 to 7.2.

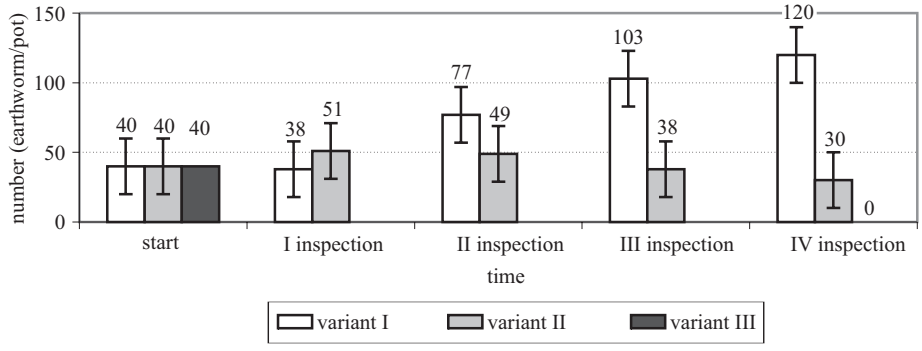
An assumption was made that the intensity of earthworms development is an indicator of the degree of sewage sludge mineralization. It was assumed that the parameter indicating mineralization processes occurring in the composted sewage sludges is the respiratory activity of the culture medium expressed as a decrease in oxygen pressure. The activity was determined after the six-month experimental period with the use of a measuring system OxiTop Control according to the following procedure: 50 mg of the composted medium and 50 cm^3 of sodium hydroxide (carbon dioxide adsorbent) were transferred to tightly closed measuring vessels (with a volume of 1 dm^3). The vessels were fixed in a thermostatic room at a temperature of 20°C. For 5 consecutive days, a measuring head of the apparatus registered pressure changes resulting from disintegration of biologically-degradable organic compounds. The produced CO_2 was bound by the alkaline absorbent, thus not affecting pressure changes in the measuring vessel. Therefore, the pressure changes recorded resulted from the consumption of oxygen occurring as a free gas in the measuring vessel.

Values of the number as well as total and individual biomass observed for particular variants are an arithmetic mean recorded for respective objects.

Results and Discussion

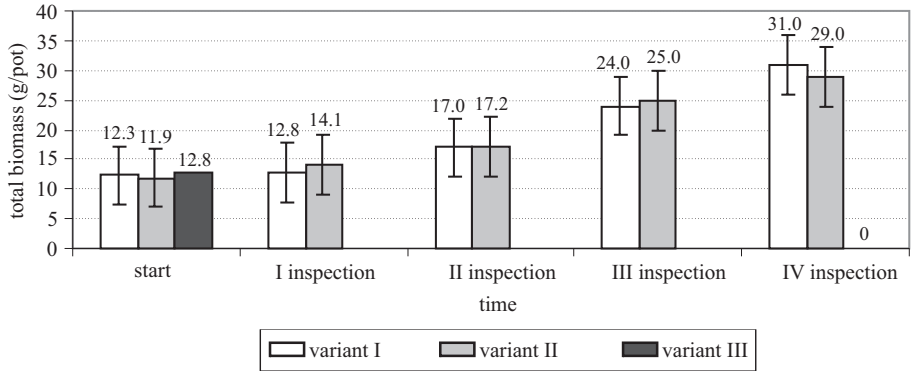
In the context of the objective adopted and research questions raised, determinations were carried out for the number as well as total and individual biomass of earthworms.

Changes in the number, total and individual biomass were analyzed in six-week intervals (Figures 1, 2, 3).



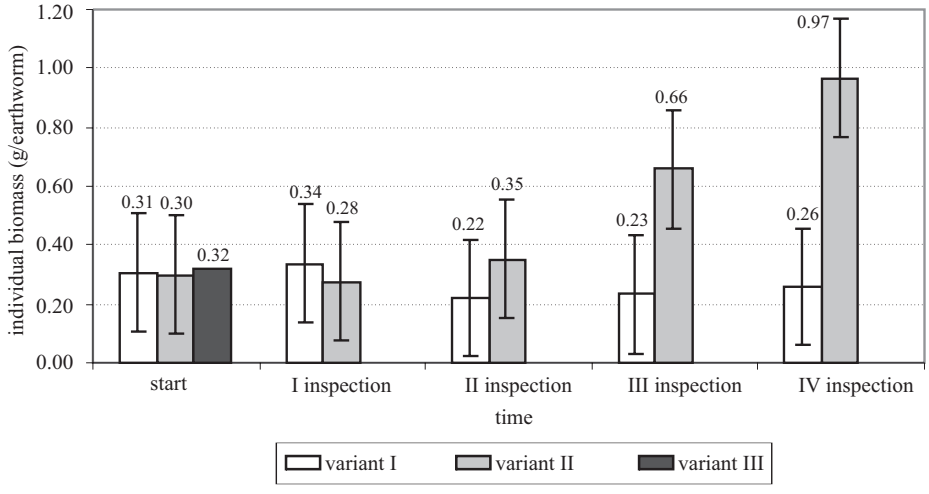
Source: own study

Fig. 1. Changes in the number of earthworms



Source: own study

Fig. 2. Changes in the total biomass of earthworms



Source: own study

Fig. 3. Changes in the individual biomass of earthworms

The greatest increase in the number of earthworms was observed in pots without drainage (variant I). Over the 6-month experimental period, the number of earthworms increased in those pots three times. In the pots with drainage (variant II), such an increase was reported in the first three months. Yet, in the consecutive months of the experiment the number of earthworms was noted to decrease below the initial number. The initial increase followed by the decrease in the number of earthworms could have been caused by an increase in the density of medium that was systematically compressed in the course of distributing homogenized sewage sludges onto the surface of the pots. The increased medium density was accompanied by the decreased intensity of pore boring by the earthworms. This, in turns, reduces their vital activity (PIEKARZ et al. 1996). As claimed by those authors, one of the factors reducing the boring of galleries in excessively thickened soil is the fact that during boring the earthworms only ingest the soil but are unable to move the soil aside, as it occurs in the case of less compressed soil.

In pots without drainage (variant III) no inspections were performed in the six-month experimental period, and after that time no earthworms were found in the pots. This could have resulted from a variety of reasons.

As a result of not performing "inspections" oxygen present in the culture medium depleted very fast. It was used (most of all) in the biodegradation process of easily-degradable substances occurring in the medium. The concentration of ammonia nitrogen higher than 0.5 mg $\cdot$ g^{-1} of substrate also contributes to a decrease in the physiological activity of earthworms (KALISZ et al.

2000). Precise determination of the date of earthworm population decay in those objects appeared to be difficult.

As reported by Staliński (after PEIJNENBURG and WELTIJE), earthworms tolerate an increased level of heavy metals, yet the presence of those metals is likely to inhibit their reproductive processes (STALIŃSKI et al. 2000).

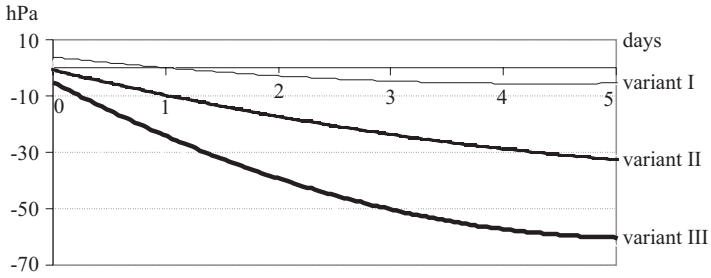
The highest total biomass was observed in the pots without drainage (variant I). Over the six-month experimental period it was increasing almost threefold. In the pots with drainage (variant II) the increase in the total biomass of earthworms was slightly smaller. In pots without drainage (variant III), no earthworms were found after six months of the culture. According to KALISZ et al. (2000), in the vermicomposting process, channel-boring earthworms cause the mixing of substrate processed, aerate and loosen it. The fact that in the pots without drainage and with no inspections performed (variant III) the population of earthworms decayed suggests that in the case of pot culture carried out in 2 dm^3 beakers at the surface of which 150 cm^3 of sewage sludges with hydration of 93-95% were poured out once a week channels bored by the earthworms are not sufficient enough to supply air in the quantity meeting the vital needs of the earthworms.

The individual biomass of earthworms was definitely the highest in the earthworms from the pots with drainage (variant II). It was over three times higher as compared to the individual biomass at the beginning of the experiment. Such a remarkable increase in the individual biomass of those earthworms was caused by the fact that while developing in a humid medium those oligochaeta absorb water, thus growing bigger (KALISZ et al. 2000). Nevertheless, when comparing the individual biomass reported for that variant with the respiratory activity we can observe that the fact that the earthworms were bigger had no effect on the degree of culture medium mineralization.

In the pots without drainage (variant I) the individual biomass of earthworms was considerably lower.

The assumption that the degree of medium mineralization is proportional to the number and biomass of earthworms was verified based on measurements of the respiratory activity of the culture media. Figure 4 presents representative plots of the respiratory activity of culture media in variants I, II and III.

The highest respiratory activity is typical of the culture media collected from the pots without drainage (variant III). That medium caused the greatest pressure drop as a result of decomposition of biologically-degradable organic compounds. Thus, that medium appeared to be mineralized to the least extent. The content of organic matter in those pots was also increased by dead earthworms.



Source: own study

Fig. 4. Respiratory activity of culture media

The culture medium originating from the pots without drainage (variant I) was found to be best mineralized. Those pots were also characterized by the highest numbers of earthworms. According to the accepted assumption, the medium contained in those pots was mineralized to the greatest extent. That dependency was confirmed by the measurement of respiratory activity.

Only slightly lower total biomass observed at the end of the experiment for variant II could suggest that in those pots the degree of culture medium mineralization was similar to that recorded in the case of variant I. Still the plot of respiratory activity of the culture medium originating from the pots with drainage (variant II) indicates that the mineralization was not sufficient. This, in turn, confirms the fact that due to water absorption earthworms developing in the medium with a high humidity are bigger but simultaneously weaker and less active. Their ability for reproduction decreases as well, which is reflected in their low population numbers (KALISZ et al. 2000).

Conclusions

1. The greatest increase in the number and biomass of the earthworms was observed for variant I.

2. The respiratory activity of the culture media, expressing the degree of their mineralization, correlates with the assays of earthworm number.

3. The degree of culture media mineralization is strictly linked with the occurrence of factors determining activity of earthworms, including: oxygen content of the medium as well as its humidity and degree of compression.

4. An increase in culture medium humidity results in less active penetration of the culture medium by the earthworms.

5. An increase in the bulk density of the medium is accompanied by decreased intensity of pore boring by the earthworms.

6. In the case of a lack of inspections (variant III), galleries bored by the earthworms failed to provide a sufficient quantity of oxygen to them.

Translated by JOANNA MOLGA

Accepted for print 21.12.2006

References

KALISZ L., KAŹMIERCZUK M., SAŁBUT J., NECHAY A., SZYPROWSKA E. 2000. *Wykorzystanie dżdżownic do przetwarzania osadów stabilizowanych tlenowo*. Mon. Inst. Ochr. Środowiska, Warszawa.

KOSTECKA J. 1995. *Wermikultura jako sposób na rozwiązanie problemu zagospodarowania osadów ściekowych w oczyszczalniach ścieków*. I Międz. Konf. Nauk.-Tech., Częstochowa, ss. 293-303.

KOSTECKA J. 2000. *Wermikompostowanie osadów z komunalnej oczyszczalni ścieków w Ustroniu*. IV Kraj. Konf. *Ekologiczne i gospodarcze znaczenie dżdżownic.* Rzeszów, ss. 47-55.

PIEKARZ J., LIPIEC J. 1996. *Wstępne badania nad wpływem gęstości i wilgotności gleby na aktywność dżdżownic*. Zesz. Nauk. AR w Krakowie, 310(47): 111-115.

STALIŃSKI J., LITWA M. 2000. *Akumulacja kadmu i ołowiu w ciele dżdżownicy w środowisku podlegającym wysokiemu skażeniu komunikacyjnemu*. Zesz. Nauk. AR, 372, IV Konf. *Ekologiczne i gospodarcze znaczenie dżdżownic*, Rzeszów, ss. 117-124.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 92-106, Y. 2007

DOI 10.2478/v10020-007-0010-y

SIMULTANEOUS NITRIFICATION AND DENITRIFICATION IN IMMOBILISED BIOMASS

Magdalena Zielińska, Irena Wojnowska-Baryła

Chair of Environmental Biotechnology
University of Warmia and Mazury in Olsztyn

A b s t r a c t

Nitrogen removal from communal wastewater by activated sludge immobilised in ceramic carriers with various internal structures was attributed to simultaneous nitrification and denitrification (SND). The experiment was performed with a hydraulic retention time (HRT) ranging from 70 to 15 minutes, under aeration conditions. The effectiveness of SND with aeration was as follows: in the 7-channelled carrier – from 59.2 to 91.0%, in the 3-channelled carrier – from 36.7 to 84.4%, in the 8-channelled carrier – from 46.2 to 73.0%. For a short HRT (30 and 15 minutes) the peak effectiveness of nitrogen removal (as a result of SND) per unit area of the carrier's active surface was achieved in the 3-channelled carrier with the largest, 50% portion of free spaces.

K e y w o r d s: immobilised activated sludge, simultaneous nitrification and denitrification (SND).

SYMULTANICZNA NITRYFIKACJA I DENITRYFIKACJA W UNIERUCHOMIONEJ BIOMASIE

Magdalena Zielińska, Irena Wojnowska-Baryła

Katedra Biotechnologii w Ochronie Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

A b s t r a k t

Usuwanie azotu ze ścieków komunalnych przez osad czynny unieruchomiony w nośnikach ceramicznych, różniących się strukturą wewnętrzną, następowało w wyniku symultanicznej nitryfikacji i denitryfikacji (SND). Eksperyment prowadzono w hydraulicznym czasie zatrzymania ścieków (HRT) od 70 do 15 min w warunkach napowietrzania. Efektywność SND w nośnikach wynosiła odpowiednio: w 7-kanałowym – od 59.2 do 91.0%, w 3-kanałowym – od 36.7 do 84.4%, w 8-kanałowym od 46.2 do 73.0%. W warunkach krótkiego HRT (30 i 15 min) najwyższą sprawność usuwania azotu w wyniku SND, w przeliczeniu na jednostkę powierzchni aktywnej nośnika, uzyskano w nośniku 3-kanałowym o największym 50% udziale wolnych przestrzeni.

S ł o w a k l u c z o w e: unieruchomiony osad czynny, symultaniczna nitryfikacja i denitryfikacja (SND).

Address: Magdalena Zielińska, University of Warmia and Mazury, 10-709 Olsztyn, Słoneczna St. 45G, phone: 48 (089) 523 41 85; e-mail: magdalena.zielinska@uwm.edu.pl)

Introduction

In conventional biological wastewater treatment systems, nitrogen is removed in different bioreactors or at different times, because nitrification takes place in an aerobic zone, while denitrification occurs in an anoxic zone. Nitrification and denitrification can take place simultaneously, because they are complementary. The products of nitrification (nitrates and nitrites) are substrates for denitrification, while denitrification generates alkalinity that is required in nitrification (MENOUD et al. 1999).

Numerous papers have described nitrification and denitrification, taking place at the same time, in one reactor, under the same conditions (MÜNCH et al. 1996). This phenomenon is called simultaneous nitrification and denitrification (SND). The SND process running in continuous reactors is more cost efficient because of the possibility of decreasing of the size of a reactor. The advantages of the SND process in periodical reactors include a reduction of time needed for the total nitrification and denitrification.

KELLER et al. (1997), quoting TURK and MAVINIC (1986, 1989), noted that the demand of organic matter in simultaneous denitrification is 40% lower than in conventional wastewater treatment plants, the denitrification rate is 63% higher and the biomass increase in anaerobic conditions is 300% lower. BERTANZA (1997) showed that SND can help to achieve the same effectiveness of nitrogen removal in a chamber of 20% smaller volume than a typical system of wastewater treatment with pre-denitrification. In addition, 50% less energy is used for aeration.

While studying the mechanism of simultaneous nitrification and denitrification, MÜNCH et al. (1996) showed that SND takes place under the conditions of oxygen concentration gradients in a floc or in a biofilm. Nitrifiers occupy areas with high oxygen concentrations, while denitrifiers are active in zones with low oxygen concentrations. Researchers have also observed the presence of aerobic denitrifiers and heterotrophic nitrifiers in the activated sludge. POCHANA, KELLER (1999) and POCHANA et al. (1999) have corroborated a hypothesis according to which SND is connected with limiting oxygen diffusion to an activated sludge flocs, which generates anoxic areas within the floc. In the study by POCHANA, KELLER (1999), experiments were conducted with flocs of two sizes: 80 and 40-50 μm, and the concentration of dissolved oxygen between 0.3 and 2.5 mg $O_2 \cdot dm^{-3}$. Reducing the size of a floc did not speed up the nitrification that indicated that it was not limited by oxygen supply. After grinding the biomass, the concentration of nitrates in the treated waste was much higher. When the flocs were larger, the effectiveness of SND reached 52%, when they were smaller the SND efficiency was 21%. In the study conducted by POCHANA et al. (1999) with flocs diameters of 382 and 155 μm, 98.5% and 26.3% effectiveness of simultaneous nitrification and denitrification

was achieved, respectively, while with a 77 μm floccula, the process effectiveness was 4.3%. Using a reactor with a biomass immobilised in Siporax™ ring shaped glass carriers, MENOUD et al. (1999), observed that aerobic nitrifiers present in the outer layers of the carrier used up oxygen and shielded the anoxic denitrifiers, which populated the inner parts of the carrier. WARTCHOW (1990) compared the transformations of nitrogen compounds in systems with free and settled biomass. He found out that the oxygen diffusion reduced the effectiveness of nitrification in the settled biomass by 20-30% as compared to the free biomass. According to WATANABE et al. (1995), if nitrifiers and denitrifiers are present in a biofilm and if the amount of oxygen is sufficient for nitrification and organics oxidation and low enough for denitrification, these processes can run simultaneously. In addition, WISTROM, SCHROEDER (1996) explained the simultaneous nitrification and denitrification by the presence of aerobic areas and those with insufficient supply of oxygen in a floc, with a limited concentration of dissolved oxygen in a reactor.

According to KELLER et al. (1997), the factors that affect simultaneous nitrification and denitrification include: a concentration of dissolved oxygen, free ammonia, red-ox potential and the floc characteristic.

The literature describes the course of simultaneous nitrification and denitrification in continuous or intermittent aeration in sequencing batch reactors SBR (MÜNCH et al. 1996), in SBR reactors with submerged membranes (CHOO, STENSEL 2000), in fluidised-bed reactors (SCHRAMM et al. 1999, XING et al. 2000), in airlift reactors (BEUN et al. 2001), in reactors with biomass immobilised on different carriers (MENOUD et al. 1999, CHUI et al. 1996, WOODBURY et al. 1998), in a biological aerated filter (PUZNAVA et al. 2001), in rotating bed reactors (WATANABE et al. 1992), in membrane reactors (CHIEMCHAISRI et al. 1992, CHIEMCHAISRI, YAMAMOTO 1993, SUZUKI et al. 1993).

The aim of this paper is to show that the nitrogen removal from communal wastewater in reactors with biomass immobilised in ceramic porous carriers takes place as a result of simultaneous nitrification and denitrification. The experiment was conducted when the air was supplied into a reactor with immobilised biomass at variable hydraulic retention time (HRT).

Methods

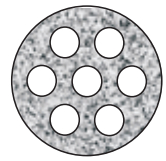
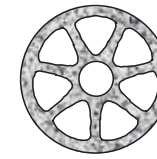
Characteristics of carriers

Activated sludge was immobilized in three porous ceramic cylinder-shaped carriers. The carriers differed in internal structure, number of internal channels, the size of internal surface and total volume (Table 1). The pore diameters of the carriers ranged from 4 to 6 μm and the material porosity was

35-40%. The carriers were made from the mixture of aluminium oxide (Al_2O_3), titanium oxide (TiO_2) and zirconium oxide (ZrO_2).

Table 1

Characteristics of porous carriers

Parameters	7-channelled carrier	3-channelled carrier	8-channelled carrier
Cross-section			
External diameter (mm)	25	10	25
Length (mm)	1178	1200	1178
Internal surface (m^2)	0.16	0.04	0.20
Total volume (dm^3)	0.578	0.094	0.578

Colonization procedure

Activated sludge, derived from a sequencing batch reactor from a full scale wastewater treatment plant with nitrification, was the source of inoculum. It was thickened to the concentration of about 23 g TSS · dm^{-3}. The immobilisation was made by circulating the activated sludge in the reactors for 24 h. The initial carriers loading reached 15.9 g TSS · dm^{-3} (7-channelled carrier), 24.5 g TSS · dm^{-3} (3-channelled carrier) and 18.2 g TSS · dm^{-3} (8-channelled carrier). The carrier loading was calculated on the total volume of a carrier.

Characteristics of reactor

Each carrier with immobilised biomass made the stationary filling of a reactor. The volume of the reactor with the 3-channelled carrier was 0.7 dm^3 and reactors with the 7-channelled and 8-channelled carriers – 1.2 dm^3. The bioreactors worked under aerobic conditions. In order to have dissolved oxygen (DO) concentration in the reactors of 2 mg O_2 · dm^{-3} it was necessary to supply about 50 $dm^3 \cdot h^{-1}$ of air to reactor with the 3-channelled carrier and about 120 $dm^3 \cdot h^{-1}$ to reactors with the 7-channelled and 8-channelled carriers. DO was measured in the upper part of the reactor in the effluent. The experiment was carried out at the temperature of 20°C. The reactor scheme is shown in Figure 1.

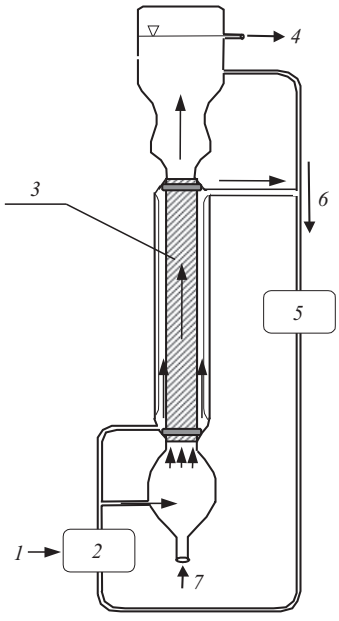


Fig. 1. A diagram of a reactor: *1* – influent, *2, 5* – pumps, *3* – porous carrier with immobilized biomass, *4* – effluent, *6* – circulation stream, *7* – air

The carrier was fixed into the bioreactor using O-rings. Two spaces were created: the internal channels and the external space. Raw wastewater flux and the circulating stream were mixed before they flew into the reactor. The influent was divided into two streams flowing parallel through the external space and internal channels. This allowed keeping the equal pressure on the internal and external surfaces. The internal circulation is advantageous because it dilutes the wastewater, prevents the excessive sludge growth and serves as the source of carbon needed to denitrification. Long solids residence time caused the strong lysis processes. Lysis products present in the internal circulation stream can serve as the endogenous source of carbon.

Characteristics of wastewater

During experimental period municipal wastewater was taken directly from an inspection chamber of a sewer pipe each day. Average content of organic compounds, nitrogen compounds and total suspended solids is presented in Table 2.

Table 2

Chemical characteristics of wastewater

Specification	Unit	Minimum value	Maximum value	Mean value
COD	(mg COD · dm^{-3})	183.5	593.3	337.6
COD soluble	(mg COD · dm^{-3})	49.4	200.5	118.0
Volatile acids	(mg CH_3COOH · dm^{-3})	47.9	105.6	85.4
Total Kjeldahl nitrogen	(mg N · dm^{-3})	30.9	62.0	47.4
Ammonia nitrogen	(mg N · dm^{-3})	14.6	38.2	27.0
Total suspended solids	(mg TSS · dm^{-3})	97.0	637.0	236.0

Experimental set-up

The studies were carried out for hydraulic retention times (HRT) from 70 to 30 min. Additionally, a HRT of 15 min was realised for the reactor with the 3-channelled carrier, as a result of still high efficiency of nitrification (over 85%). HRT was altered by changing the wastewater feed rate. The internal circulation was maintained on the level of 40 $dm^3 \cdot h^{-1}$ and it was constant throughout the various experimental runs. In Table 3 the volumetric loading rates for all the carriers are shown.

At every volumetric loading rate, after biomass adaptation for the experimental conditions, the research was carried out for about 2 weeks. The adaptation period was regarded to be finished when the range of changes of

Table 3

Technological parameters in reactors

Reactor	HRT (min)	Volumetric loading rate VLR_C (g COD · dm^{-3} · d^{-1})	Volumetric loading rate VLR_N (g N · dm^{-3} · d^{-1})
7-channelled carrier	70	5.9	0.9
	60	8.2	1.1
	40	17.2	2.0
	30	24.4	2.4
3-channelled carrier	70	6.5	0.9
	60	8.0	1.4
	30	20.8	2.4
	15	48.8	4.9
8-channelled carrier	70	5.4	0.9
	60	5.8	1.0
	30	16.6	2.5

particular parameters in the effluent within 7 days; time did not exceed 5-10%. During every 2 weeks of the experiment, samples were collected twice a day. Presented data are the arithmetic mean of the experimental results.

Analytical methods

Wastewater was assayed according to Polish Standards for the concentration of organic compounds expressed as COD (PN 74/C-04578.03), volatile fatty acids (PN 75/C-4616.04), Kjeldahl nitrogen (PN 81/C-04527), ammonia (PN-C--04576-4:1994; PN-ISO 5664:2002), nitrite nitrogen (PN 73/C-04576.06), nitrate nitrogen (PN 73/C-04576.08) and total suspended solids (PN-EN--12879:2004).

Calculation methods

The oxidised ammonia nitrogen (N_{ox}) was calculated with the following equation:

$$N_{ox} = TKN_i - NH_{4e} - N_{syn} \text{ (mg N} \cdot \text{dm}^{-3}) \quad (1)$$

TKN_i – concentration of total Kjeldahl nitrogen in influent (mg N · dm^{-3}),
NH_{4e} – concentration of ammonia nitrogen in effluent (mg N · dm^{-3}),
$N_{org\ e}$ – concentration of organic nitrogen in effluent (mg N · dm^{-3}),
N_{syn} – concentration of nitrogen used for biomass synthesis (mg N · dm^{-3}).

Concentration of nitrogen used for biomass synthesis (N_{syn}) was calculated as follows:

$$N_{syn} = Y \cdot (COD_i - COD_e) \cdot F_N \text{ (mg N} \cdot \text{dm}^{-3}) \quad (2)$$

Y – sludge yield coefficient (g TSS g^{-1} COD),
COD_i – concentration of organic compounds in influent (mg COD · dm^{-3}),
COD_e – concentration of organic compounds in effluent (mg COD · dm^{-3}),
F_N – nitrogen amount in immobilised biomass (equals to 6.5% of TSS).

The efficiency of simultaneous nitrification and denitrification (SND) was calculated according to ZENG et al. (2003):

$$SND = \frac{N_{ox} - (NO_2 + NO_3)}{N_{ox}} \cdot 100\ (\%) \qquad (3)$$

$NO_2 + NO_3$ – concentration of nitrite and nitrate in effluent (mg $N \cdot dm^{-3}$).

Results

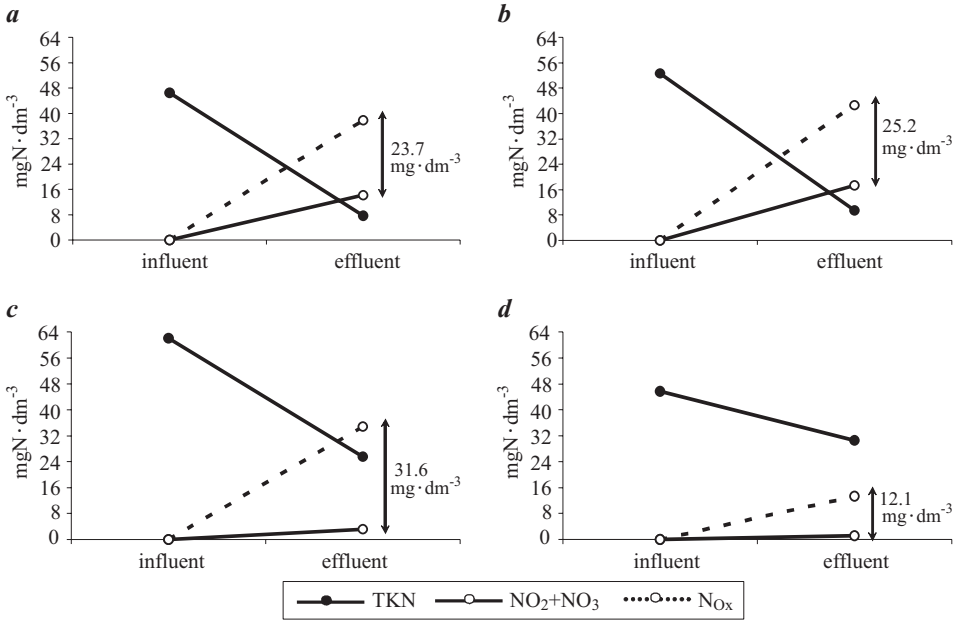
The process of nitrogen removal from communal wastewater employed an activated sludge immobilised in ceramic porous carriers that provided a stationary filling of the reactors. The air was continuously supplied into the reactors in amounts that maintained the oxygen concentration at 2 mg $O_2 \cdot dm^{-3}$. The experiment was conducted at a hydraulic retention time in the reactor of 70 min to 15 min, with the assumption that the effectiveness of nitrification determines the applied values of HRT.

In the experiment, the total Kjeldahl nitrogen (TKN) was determined in the influent and effluent. The difference between the TKN in communal wastewater inflowing to the reactor and the concentration in the treated wastewater indicates that nitrification takes place in the immobilised sludge at given HRT.

Based on the experimental results, the amount of the oxidised nitrogen (N_{ox}) was calculated taking into account the amount of ammonia nitrogen used for biomass growth, which is not oxidised, and the amount of organic nitrogen which was not amonified – Formula (1). The concentration of ammonia nitrogen used for biomass synthesis was low and ranged from 0.7 to 2.7 mg $N \cdot dm^{-3}$, which was a result of a low increase in the amount of immobilised biomass (ZIELIŃSKA 2002). The mean concentration of organic nitrogen in the treated wastewater was 4.5 mg $N \cdot dm^{-3}$, and that of ammonia nitrogen ranged from 0.4 to 17.2 mg $N \cdot dm^{-3}$, depending on the HRT. The concentrations of nitrites and nitrates ($NO_2 + NO_3$) in the effluent were lower than the calculated value of *Nox*. The difference between the calculated concentration of oxidised nitrogen and the concentration of nitrates and nitrites in the treated wastewater is equivalent to the amount of nitrogen removed as a result of simultaneous nitrification and denitrification (N_{rem}).

In the reactor with the 7-channeled carrier, with a hydraulic retention time of 40 minutes and the nitrogen load (VLRN) 2.0 g $N \cdot dm^{-3} \cdot d^{-1}$, the concentration of nitrogen removed as a result of simultaneous nitrification and denitrification was the highest and reached 31.6 mg $N \cdot dm^{-3}$ (Figure 2). The SND effectiveness equalled 90.8%. With the shortest HRT of 30 minutes, the SND effectiveness reached 91%, with the difference between Nox and ($NO_2 + NO_3$) in the outflow of 12.1 mg $N \cdot dm^{-3}$. Nitrogen removal at VLR_N of

2.4 g N · dm^3 · d^{-1} and at VLR_C of 24.4 g COD · dm^{-3} · d^{-1} was restricted by the effectiveness of nitrification in the immobilised activated sludge, which provided a confirmation of the presence of TKN in the treated wastewater at 30.6 mg N · dm^{-3}.

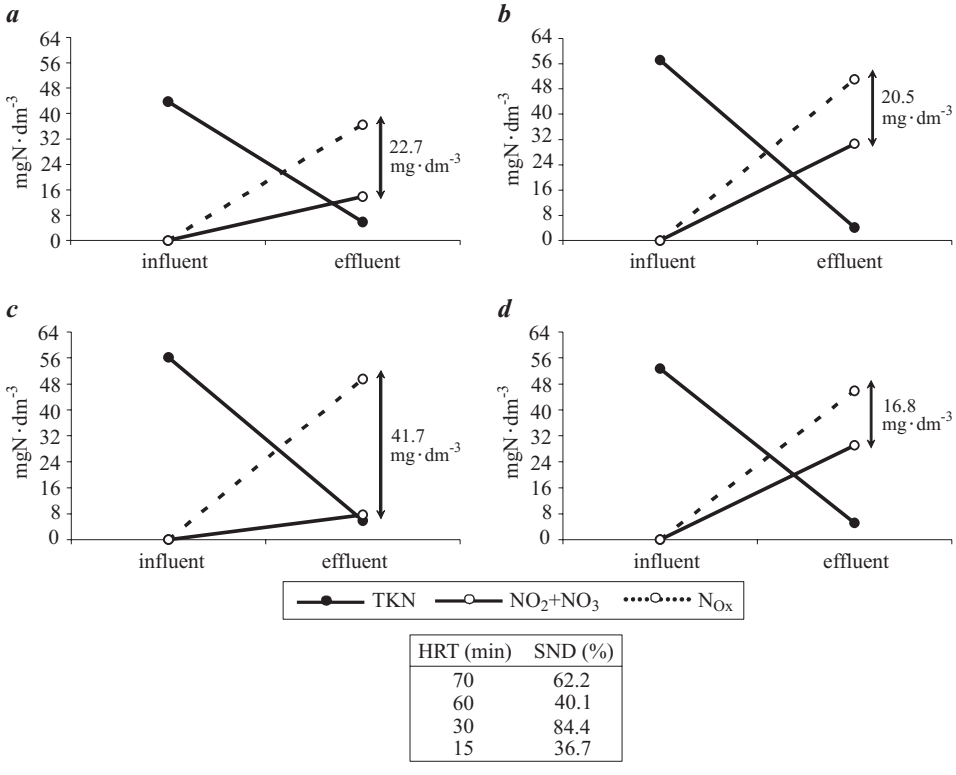


HRT (min)	SND (%)
70	62.7
60	59.2
40	90.8
30	91.0

Fig. 2. Concentration of total Kjeldahl nitrogen (TKN), nitrite and nitrate (NO_2 + NO_3) in influent and effluent and concentration of oxidized ammonium nitrogen (N_{ox}) at varied HRT in the reactor with the 7-channelled carrier: *a* – HRT 70 min, *b* – HRT 60 min, *c* – HRT 40 min, *d* – HRT 30 min. In a table below the figures there is the efficiency of simultaneous nitrification and denitrification (SND)

In the reactor with the 3-channelled carrier, at HRT of 30 min and VLR_N of 2.4 g N · dm^{-3} · d^{-1}, the concentration of nitrogen removed as a result of simultaneous nitrification and denitrification was about twice as high as at other HRT values and equalled 41.7 mg N · dm^{-3}. The effectiveness of SND was 84.4% (Figure 3), the concentration of TKN in the treated wastewater equalled to 5.9 mg N · dm^{-3}. The lowest value of the process effectiveness – 36.7% – was achieved at HRT of 15 min and VLR_N of 4.9 g N · dm^{-3} · d^{-1} – 36.7%. Supposedly,

the denitrification process limited the degree of nitrogen removal as the nitrate nitrogen concentration in the treated wastewater was 30 mg N · dm^{-3}, and the TKN concentration was 5.2 mg N · dm^{-3}.



HRT (min)	SND (%)
70	62.2
60	40.1
30	84.4
15	36.7

Fig. 3. Concentration of total Kjeldahl nitrogen (TKN), nitrite and nitrate (NO_2 + NO_3) in influent and effluent and concentration of oxidized ammonium nitrogen (N_{ox}) at varied HRT in the reactor with the 3-channelled carrier: *a* – HRT 70 min, *b* – HRT 60 min, *c* – HRT 30 min, *d* – HRT 15 min. In a table below the figures there is the efficiency of simultaneous nitrification and denitrification (SND)

The difference between the theoretical concentration of oxidised nitrogen and the determined concentration of nitrites and nitrates in the treated wastewater in the reactor with the 8-channelled carrier was similar at HRT of 70, 60 and 30 min, and averaged 14.8 mg N · dm^{-3}. However, the effectiveness of SND increased from 46.2% at HRT of 70 min and VLR_N of 0.9 g N · dm^{-3} · d^{-1} to 73% at HRT of 30 min and VLR_N of 2.5 g N · dm^{-3} · d^{-1} (Figure 4). At HRT of 30 min, as in the 7-channelled reactor, the nitrification restricted the removal of nitrogen and the TKN concentration in the treated wastewater was 22.3 mg N · dm^{-3}.

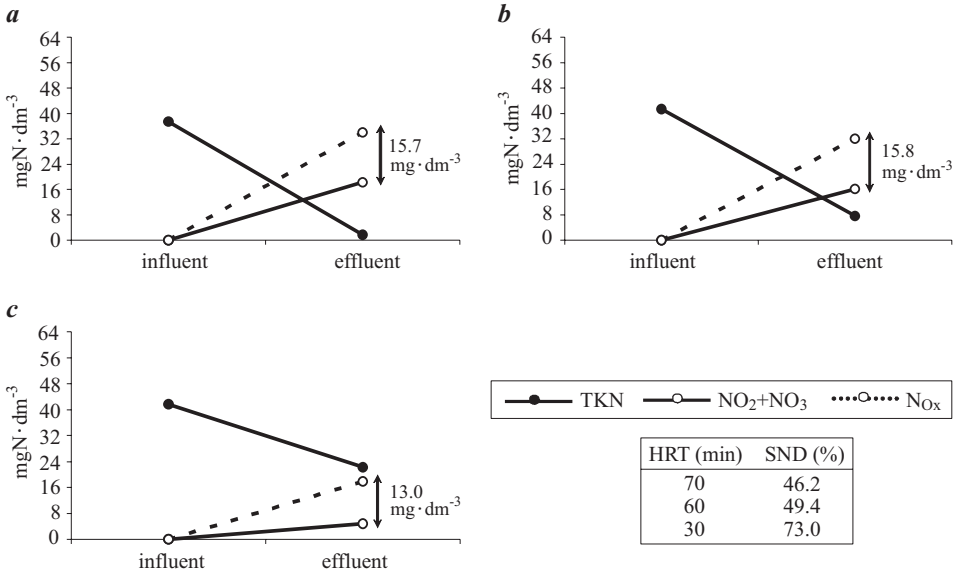


Fig. 4. Concentration of total Kjeldahl nitrogen (TKN), nitrite and nitrate (NO_2 + NO_3) in influent and effluent and concentration of oxidized ammonium nitrogen (N_{ox}) at varied HRT in the reactor with the 8-channelled carrier: *a* – HRT 70 min, *b* – HRT 60 min, *c* – HRT 30 min. In a table below the figures there is the efficiency of simultaneous nitrification and denitrification (SND)

Despite the differences in the surface size available for microorganisms (Table 1), a characteristic feature of the 7-, 3- and 8-channelled carriers was the different portion of free space in the total volume of the carrier, which was: 28%, 50% and 44%, respectively. In order to compare the effectiveness of simultaneous nitrification and denitrification in biomass immobilised in carriers differing in terms of their internal structure, Table 4 shows the concentration of nitrogen removed per unit area of the carrier active surface.

The concentration of nitrogen removed by immobilised biomass in the 7-channelled carrier per carrier unit surface (N_{rem-A}) increased up to 4.0 g N · m^{-2} · d^{-1} at HRT of 40 min, while at HRT of 30 min N_{rem-A} decreased to 2.3 g N · m^{-2} · d^{-1}. In the 3-channelled carrier, the highest values of N_{rem-A} were

Table 4
Concentration of nitrogen removed by immobilized biomass counted for the unit of the carriers surface (N_{rem-A})

Reactor	7-channelled carrier				3-channelled carrier				8-channelled carrier		
HRT (min)	70	60	40	30	70	60	30	15	70	60	30
N_{rem-A} (g N · m^{-2} · d^{-1})	1.6	1.9	4.0	2.3	1.0	1.2	4.2	3.7	0.9	1.1	2.0

achieved at the shortest retention times: 4.2 and 3.7 g $N \cdot m^{-2} \cdot d^{-1}$. In the 8-channelled carrier, the concentration of nitrogen removed per unit surface of the carrier increased from 0.9 to 2.0 g $N \cdot m^{-2} \cdot d^{-1}$ with a decrease in HRT from 70 to 30 min.

Discussion

Nitrogen removal from communal wastewater was conducted in aeration condition. The oxygen concentration in the treated wastewater was controlled and maintained at 2 mg $O_2 \cdot dm^{-3}$. In these conditions, the ammonia nitrogen was oxidised and the oxidised forms of nitrogen reduced. The total concentration of nitrites and nitrates in the treated wastewater was lower than would result from the nitrification effectiveness, which indicates the simultaneous nitrification and denitrification in the range of nitrogen loading 0.9-2.4 g $N \cdot dm^{-3} \cdot d^{-1}$ (7-channelled carrier), 0.9-4.9 g $N \cdot dm^{-3} \cdot d^{-1}$ (3-channelled carrier), 0.9-2.5 g $N \cdot dm^{-3} \cdot d^{-1}$ (8-channelled carrier). The effectiveness of simultaneous nitrification and denitrification by immobilised biomass was: 59.2-91.0%, 36.7-84.4%, 46.2-73.0%, respectively. In the reactors with the 7- and 8-channelled carriers at HRT 30 min, the nitrification restricted the removal of nitrogen from wastewater as a result of restricting the oxidising of ammonia nitrogen because of a high load of contaminants in a reactor. In the reactor with the 3-channelled carrier, nitrification was not limited at the shortest HRT of 15 minutes, while the removal of nitrogen was restricted by low effectiveness of denitrification.

CHIEMCHAISRI et al. (1992) and CHIEMCHAISRI, YAMAMOTO (1993) showed that nitrogen removal from communal wastewater in a reactor with biomass immobilised on a hollow-fibre membrane was only possible with intermittent aeration, when the anoxic conditions favoured denitrification. Simultaneous nitrification and denitrification allowed achieving over 80% reduction of nitrogen compounds. MÜNCH et al. (1996) also conducted alternate aeration in an SBR reactor and determined the oxygen concentration which resulted in the total SND to be equal 0.5 mg $O_2 \cdot dm^{-3}$. The rate of aerobic denitrification was the highest at the beginning of the aeration phase and then decreased. An SBR reactor in the study conducted by ZENG et al. (2003) worked in anaerobic-aerobic conditions. In the aeration phase, the concentration of dissolved oxygen was maintained at a low level of 0.5 mg $O_2 \cdot dm^{-3}$. In the aerobic conditions the ammonia nitrogen was oxidised, however, no accumulation of nitrites or nitrates was observed, which indicates that SND took place. When the oxygen concentration equalled 0.5, 1.5 and 2.5 mg $O_2 \cdot dm^{-3}$, the effectiveness of simultaneous nitrification and denitrification was 98, 62 and 51%,

respectively. In an SBR reactor with a membrane for immobilising biomass, Choo, STENSEL (2000) observed the simultaneous nitrification and denitrification with the effectiveness of 27% at the concentration of dissolved oxygen of 2.8 mg $O_2 \cdot dm^{-3}$, while at the oxygen concentration less than 0.6 mg $O_2 \cdot dm^{-3}$, the process effectiveness was 47%. In a fluidised-bed reactor, the maximum SND effectiveness was 85% in aerobic conditions. Air was fed into the reactor filled with polyurethane cubes in order to achieve the dissolved oxygen concentration in the reactor of 5-7 mg $O_2 \cdot dm^{-3}$ (XING et al. 2000). MENOUD et al. (1999) observed SND at a high concentration of oxygen of 2.0 mg $O_2 \cdot dm^{-3}$ in the outflow from the reactor. This concentration of oxygen, which is considered to inhibit denitrification, suggests that denitrification took place inside the porous structure of the carrier, and not in the anoxic zone close to the outflow from the reactor. PUZNAVA et al. (2001) achieved simultaneous nitrification and denitrification in a biological aerated filter. At oxygen concentrations ranging from 0.5 to 3.0 mg $O_2 \cdot dm^{-3}$ with ammonium load up to 1.8 kg $N\text{-}NH_4 \cdot m^{-3} \cdot d^{-1}$, the effectiveness of nitrogen removal was 60-70%. SUWA et al. (1992) observed simultaneous nitrification and denitrification under aerobic conditions in a reactor with high biomass concentration, with organics loading of 0.33-1.66 g $BOD_5 \cdot dm^{-3} \cdot d^{-1}$ and total nitrogen loading of 0.032-0.268 g $N \cdot dm^{-3} \cdot d^{-1}$. Denitrification was found to had taken place in aerobic conditions in anoxic microzones when the rate of oxygen consumption by microorganisms which populate the external layers of the membrane was higher than the oxygen diffusion to the deeper layers.

In analysing the concentration of nitrogen removed during the simultaneous nitrification and denitrification calculated per unit of active surface of the carrier, it can be assumed that the carrier structure influenced the SND effectiveness. Under experimental conditions, at HRT of 70 and 60 minutes, biomass immobilised per one square metre of the surface of porous carriers removed a similar amount of nitrogen from wastewater. However, it was found that in the reactor with the 3-channelled carrier, with the smallest surface of 0.04 m^2 and the largest portion of free space (50%) in the whole volume of the carrier, at low hydraulic retention times of 30 and 15 min, the unit effectiveness of nitrogen removal was much higher than in the other reactors. These results may corroborate the hypothesis by MATSUMURA et al. (1997), that it is very important to achieve a balance between the active surface of a carrier and the surface open to a free flow, in order to speed up the process of mass transfer.

Conclusions

Simultaneous nitrification and denitrification was observed in reactors with biomass immobilised in ceramic porous carriers, with air supplied to the

reactor in a continuous manner. The SND effectiveness was 59.2-91.0% in the 7-channelled carrier reactor, 36.7-84.4% in the 3-channelled carrier reactor, 46.2-73.0% in the 8-channelled carrier reactor.

The efficiency of SND depended on organics and nitrogen loading. In order to maintain the highest effectiveness of SND, the organics loading should not exceed the values: 17.2, 20.8 and 16.6 g COD $\cdot$ $dm^{-3} \cdot d^{-1}$, respectively in the reactors with 7-, 3- and 8-channelled carriers.

The use of the carrier with the smallest active surface (0.04 m^2) and the highest (50%) portion of free spaces in the total volume, at HRT of 30 and 15 minutes, proved to be the most effective measure. However, further research is needed to define the effect of the internal structure of carriers on the effectiveness of contamination removal.

Translated by JOANNA JENSEN

Accepted for print 21.11.2006

References

BERTANZA G. 1997. *Simultaneous nitrification-denitrification process in extended aeration plants: pilot and real scale experiences*. Wat. Sci. Tech., 35: 53-61.

BEUN J.J., HEIJNEN J.J., VAN LOOSDRECHT M.C.M. 2001. *N-removal in a granular sludge sequencing batch airlift reactor*. Biotechnol. Bioeng., 75: 82-92.

CHIEMCHAISRI C., WONG Y.K., URASE T., YAMAMOTO K. 1992. *Organic stabilization and nitrogen removal in membrane separation bioreactor for domestic wastewater treatment*. Wat. Sci. Tech., 25: 231-240.

CHIEMCHAISRI C., YAMAMOTO K. 1993. *Biological nitrogen removal under low temperature in a membrane separation bioreactor*. Wat. Sci. Tech., 28: 325-333.

CHOO K.H., STENSEL H.D. 2000. *Sequencing batch membrane reactor treatment: nitrogen removal and membrane fouling evaluation*. Wat. Environ. Res., 72: 490-498.

CHUI P.C., TERASHIMA Y., TAY J.H., OZAKI H. 1996. *Performance of a partly aerated biofilter in the removal of nitrogen*. Wat. Sci. Tech., 34: 187-194.

KELLER J., SUBRAMANIAM K., GÖSSWEIN J., GREENFIELD P.F. 1997. *Nutrient removal from industrial wastewater using single tank sequencing batch reactors*. Wat. Sci. Tech., 35: 137-144.

MATSUMURA M., YAMAMOTO T., WANG P., SHINABE K., YASUDA K. 1997. *Rapid nitrification with immobilized cell using macro-porous cellulose carrier*. Wat. Res., 31: 1027-1034.

MENOUD P., WONG C.H., ROBINSON H.A., FARQUHAR A., BARFORD J.P., BARTON G.W. 1999. *Simultaneous nitrification and denitrification using Siporax™ packing*. Wat. Sci. Tech., 40: 153-160.

MÜNCH E.V., LANT P., KELLER J. 1996. *Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors*. Wat. Res., 30: 277-284.

POCHANA K., KELLER J. 1999. *Study of factors affecting simultaneous nitrification and denitrification (SND)*. Wat. Sci. Tech., 39: 61-68.

POCHANA K., KELLER J., LANT P. 1999. *Model development for simultaneous nitrification and denitrification*. Wat. Sci. Tech., 39: 235-243.

Polish Standards: Polish Committee for Standardization (http://www.pkn.pl).

PUZNAVA N., PAYRAUDEAU M., THORNBERG D. 2001. *Simultaneous nitrification and denitrification in biofilters with real time aeration control*. Wat. Sci. Tech., 43: 269-276.

SCHRAMM A., DE BEER D., VAN DEN HEUVEL J.C., OTTENGRAF S., AMANN R. 1999. *Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors*. Appl. Environ. Microbiol., 65: 3690-3696.

SUWA Y., SUZUKI T., TOYOHARA H., YAMAGISHI T., URUSHIGAWA Y. 1992. *Single-stage, single-sludge nitrogen removal by an activated sludge process with cross-flow filtration.* Wat. Res., 26: 1149-1157.

SUZUKI Y., MIYAHARA S., TAKEISHI K. 1993. *Oxygen supply method using gas-permeable film for wastewater treatment.* Wat. Sci. Tech., 28: 243-250.

WARTCHOW D. 1990. *Nitrification and denitrification in combined activated sludge systems*. Wat. Sci. Tech., 22: 199-206.

WATANABE Y., MASUDA S., ISHIGURO M. 1992. *Simultaneous nitrification and denitrification in micro-aerobic biofilms*. Wat. Sci. Tech., 26: 511-522.

WATANABE Y., OKABE S., HIRATA K., MASUDA S. 1995. *Simultaneous removal of organic materials and nitrogen by micro-aerobic biofilms*. Wat. Sci. Tech., 31: 195-203.

WISTROM A.O., SCHROEDER E.D. 1996. *Enhanced nutrient removal by limiting dissolved oxygen concentration in a continuously fed, intermittently decanted, activated sludge plant*. Environ. Technol., 17: 371-380.

WOODBURY B.L., DAHAB M.F., MIHALTZ P., CSIKOR Z. 1998. *Evaluation of reversible fixed-film static-bed bio-denitrification reactors.* Wat. Sci. Tech., 38: 311-318.

XING X.H., JUN B.H., YANAGIDA M., TANJI Y., UNNO H. 2000. *Effect of C/N values on microbial simultaneous removal of carbonaceous and nitrogenous substances in wastewater by single continuous-flow fluidized-bed bioreactor containing porous carrier particles*. Biochem. Eng. J., 5: 29-37.

ZENG R.J., LEMAIRE R., YUAN Z., KELLER J. 2003. *Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor.* Biotechnol. Bioeng., 84: 170-178.

ZIELIŃSKA M. 2002. *Efficiency of nitrogen removal by immobilised activated sludge.* Warsaw University of Technology, Warsaw (PhD thesis).

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 107-115, Y. 2007

DOI 10.2478/v10020-007-0011-x

ARTIFICIAL SPAWNING OF COMMON TENCH (*TINCA TINCA* L.) COLLECTED FROM WILD POPULATIONS*

***Dariusz Kucharczyk*[1], *Roman Kujawa*[1], *Andrzej Mamcarz*[1], *Katarzyna Targońska*[1], *Sławomir Krejszeff*[1], *Elżbieta Wyszomirska*[2]**

[1] Chair of Lake and River Fisheries
University of Warmia and Mazury in Olsztyn
[2] Department of Chemical Analysis
National Institute of Public Health

K e y w o r d s: artificial spawning, fish culture, ovulation, tench, wild population.

A b s t r a c t

Artificial spawning of tench collected from wild populations was studied under controlled conditions. In first part of present work, the accurate moment of applying hormonal injection was tested. The best results were obtained, when fish were stimulated between 1st and 3rd day after their collection. From tested hormonal combination (second part of work) ovopel containing GnRHa and dopamine antagonist: metoclopramide, produced the best results. All tested hormones influenced in high quality of oocytes. The differences were observed in the latency time. Fish stimulated by carp pituitary extract (CPE) ovulated much earlier than those stimulated by ovopel. Spawning techniques described in this paper were successful and provided consistent results in reproduction of tench collected from wild populations.

KONTROLOWANY ROZRÓD LINA (*TINCA TINCA* L.) POZYSKANEGO Z NATURALNYCH POPULACJI

***Dariusz Kucharczyk*[1], *Roman Kujawa*[1], *Andrzej Mamcarz*[1], *Katarzyna Targońska*[1], *Sławomir Krejszeff*[1], *Elżbieta Wyszomirska*[2]**

[1] Katedra Rybactwa Jeziornego i Rzecznego
Uniwersytet Warmińsko-Mazurski w Olsztynie
[2] Zakład Analizy Chemicznej
Narodowy Instytut Zdrowia Publicznego, Warszawa

S ł o w a k l u c z o w e: rozród kontrolowany, akwakultura, owulacja, lin, naturalne populacje.

Address: Dariusz Kucharczyk, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 5, 10-718 Olsztyn, Poland, phone: 48 (089) 523 42 15, e-mail: darekk@uwm.edu.pl
* This study was supported by Project No 080600.202 financed by University of Warmia and Mazury in Olsztyn, Poland.

Abstrakt

W warunkach kontrolowanych przeprowadzono rozród lina pochodzącego z naturalnych populacji. Opisano wpływ czasu iniekcji (liczony od momentu pozyskania ryb) na efekty rozrodu. Wykazano, że najlepsze rezultaty osiąga się, gdy iniekcje hormonalne zostaną wykonane między 1. a 3. dniem od pozyskania tarlaków. W pracy testowano efekty podania rybom różnych środków hormonalnych, stosowanych w krajowym wylęgarnictwie. Wszystkie działały bardzo dobrze, czego wyrazem była wysoka przeżywalność embrionów (ponad 80%) do stadium zaoczkowania. Różnice między preparatami hormonalnymi obserwowano w czasie wystąpienia owulacji. Wykazano, że w warunkach kontrolowanych możliwe jest skuteczne przeprowadzenie rozrodu lina pochodzącego z naturalnych populacji. Najlepsze rezultaty otrzymano po zastosowaniu ovopel.

Introduction

The one of the most important problem in cyprinid aquaculture is obtaining good quality gametes (KULIKOVSKY et al. 1996, HORVATH et al. 1997). For this reason many hormonal treatments were used for stimulation gametes maturation in commercial cyprinid culture. One of the most commonly applied spawning agents is carp pituitary extract (CPE) (YARON et al. 1984, THALATHIAH et al. 1988), in same cases with addition of human chorionic gonadotropin – hCG (KUCHARCZYK et al. 1997a). The good results in induced ovulation in cyprinid fish were also obtained after hormonal stimulation with synthetic analogue of gonadotropin releasing hormone (GnRH), frequently with strong dopamine antagonists (YARON 1995, BARTH et al. 1997, HORVATH et al. 1997).

Since many cyprinids wild stocks become extinct, there is a need to fast develop techniques of controlled propagation of these fish. Generally, the papers presented methods of artificial spawning of wild cyprinids are very limited, as well as data of reproductive biology of wild cyprinid and about hatchery techniques (PENAZ, GAJDUSEK 1979, KUCHARCZYK et al. 1997c, BABIAK et al. 1998). Some of experiments on artificial reproduction were finished only at the moment of ovulation (GLUBOKOV et al. 1991). Our previous study on wild cyprinids showed that biological quality of gametes should be considered in artificial spawning research (KUCHARCZYK et al. 1997b, 2005). On the other hand the development of controlled reproduction of wild cyprinids is still needed as an integral component of ongoing conservation efforts (WILDT et al. 1993).

Tench (*Tinca tinca* L.) is one of the most important freshwater species in Poland and some other European countries. Many of the published papers about tench artificial propagation focused on pond cultured stocks (BARTH et al. 1997). The aim of present study was to applicate some spawning agents in artificial propagation of wild matured tench, collected from natural populations.

Material and Methods

Broodstock collection

Tench spawners were collected in middle June from some Mazurian lakes, north Poland. Fish were selected according to the following criteria: the belly of females had to be fully distended and bulging, soft and resilient to touch; males had to be little spermiating. The size of spawners ranged from 0.8 to 1.2 kg. The selected males and females were kept in separate 1000 l tanks in a hatchery with controlled temperature (22°C) and photoperiod – 18 hrs light and 6 hrs dark (KUJAWA et al. 1999). Water temperature in the hatchery unit was gradually raised from 18 to 22°C.

Checking the oocytes maturation

All fish were individually marked using floy tags and weighted. Oocytes were taken from females using the method described by KUJAWA and KUCHARCZYK (1996), were sampled *in vivo* and placed in Serra's solution for clarification of the cytoplasm. After 5 minutes, the position of oocyte nucleus was determined using a 4-stages scale:

– stage 1 – germinal vesicle in central position,
– stage 2 – early migration of germinal vesicle (less than half of radius),
– stage 3 – late migration of germinal vesicle (more than half of radius),
– stage 4 – periphery germinal vesicle or germinal vesicle breakdown (GVBD).

In all cases, the sampled oocytes were in different maturity stage and size (diameter). For this reason the best criteria to select tench female for reproduction was visible one: the belly of females had to be fully distended and bulging, soft and resilient to touch.

Hormonal treatment

Two experiments were performed. During first experiment, the optimum time of hormonal treatment application was recognized. In second one, the different kinds of hormonal stimulation were tested.

First experiment

Collected fish (ten females in each subgroup) were immediately transported to the hatchery. The females (10 specimens each day) were hormonally stimulated on the day of collection (day 0) and later on days: 1, 2, 3, 5, 8 and 10. Fish received a single injection of GnRHa containing pellet (ovopel) at dose 2 pellets kg^{-1}. One ovopel pellet (average weight about 25 mg) contains a mammalian GnRH analogue (D-Ala6, Pro9Net-mGnRH at dose 18-20 μg) and dopamine antagonist: metoclopramide (dose 8-10 mg) (HORVATH et al. 1997). Experiment was carried out two times during two spawning season.

Second experiment

Fish were divided into four groups: three experimental and control one. After two days of acclimation to the 22°C, the fish were treated with respective single hormonal injection of common carp pituitary (CPE, Argent, USA) extract (group 1), human chorionic gonadotropin (hCG, Biomed, Poland) or GnRHa containing pellet (ovopel, HORVATH et al. 1997) – Table 1. All spawning agents were prepared with 0.9% NaCl: pituitary extract was homogenised, hCG dissolved and ovopel pellets were pulverised in a mortar and than dissolved. Injections of hCG were intramuscularly in the dorsal area of the body (THALATHIAH et al. 1988). Injections of pituitary (KUCHARCZYK et al. 1997a) and ovopel (HORVATH et al. 1997) extracts were intraperitoneal at the base of the pelvic fin. Before manipulations fish were anaesthetised with 2-phenoxyethanol (0.2 ml l^{-1}). Experiment was carried out two times during two spawning season.

Table 1

Hormonal treatment applied to induce artificial spawning in common tench (*Tinca tinca*)

Group of fish	Type of hormones	Dose of hormones
1	CPE	3 mg kg^{-1}
2	hCG	1000 IU kg^{-1}
3	Ovopel	2 pellet kg^{-1}
4 (control)	0.9% NaCl	0.5 ml kg^{-1}

Explanations:
CPE – carp pituitary extract, hCG – human chorionic ągonadotropin, ovopel – GnRHa containing pellet, N/group = 20 females

Collection of gametes and incubation

Ripe gamete donors were anaesthetized in a solution of 2-phenoxyethanol (0.2 ml per 1l). Milt was collected with plastic syringes and kept at 4°C. Females were checked every one hour between 8 and 16 hours after injection. Eggs were stripped into a plastic vessel. Eggs were fertilized using "dry method" (KUCHARCZYK et al. 1997a). For eggs fertilization only those sperm were taken which showed the motility of more than 80% of spermatozoa. The sperm motility was recognized subjectively under light microscope (500 x). Two egg samples (250-300 eggs each) from each female were mixed with 0.05 ml of pooled milt sample. Eggs were incubated at 24°C. During eyed-egg-stage, the survival of embryos was noted.

Statistical analysis

Statistical differences between groups (incubation success) were analysed with Duncan's multiple range test $P < 0.05$ (PLATT 1977).

Results

Experiment 1

The highest percentage of ovulated tench females was observed when hormonal stimulation was applied during first three days after sampling (Figure 1a). After this time, the number of ovulated females decreased. In the day of sampling (day 0) only 60% of female ovulated. The highest survival of embryos to the eyed-egg-stage was noted in the same groups, in which the highest percentages of ovulated females were observed (Figure 1b). The latency time (time between hormonal injection and ovulation) in each group was similar: between 12 and 15 hrs.

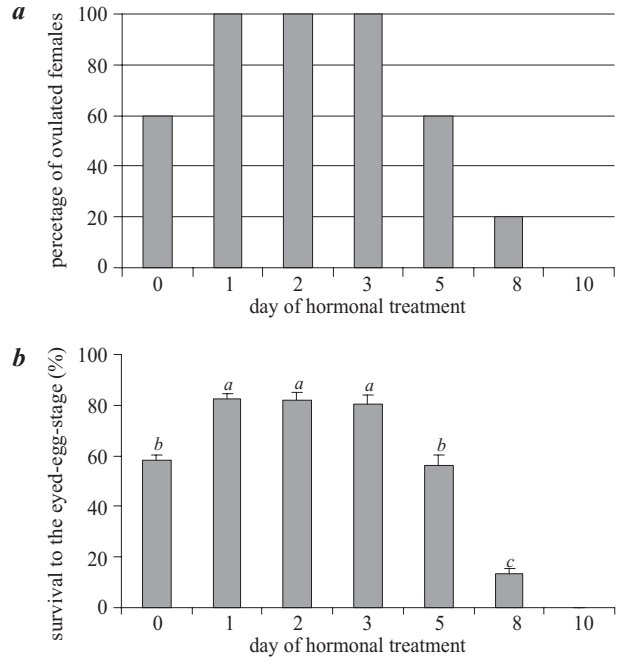


Fig. 1. The results of artificial spawning of tench in depending on time of hormonal injection with ovopel: *a* – percentage of ovulation in wild matured tench, *b* – embryo survival (±SD). Data marked with the same letter did not differ statistically ($P < 0.05$)

Experiment 2

From tested different spawning agents: hCG, CPE and ovopel (GnRHa), the last was recognized as the best (Figure 2a). Females from control group, injected with saline solution, did not ovulate. All females stimulated with ovopel ovulated. The differences in biological quality of eggs, expressed as embryo survival to the eyed-egg-stage were small (Figure 2b), however the lowest survival was observed in CPE-treated group. In all treated groups, the embryo survival was about 80%. The shortest latency time (8-11 hrs) was noted in group 2 (hCG-treated), then in group 1 (10-12 hrs) (CPE-treated). The longest time was noted in group 3 (ovopel-treated): 12-16 hrs. This last result was significantly different from latency time from other groups.

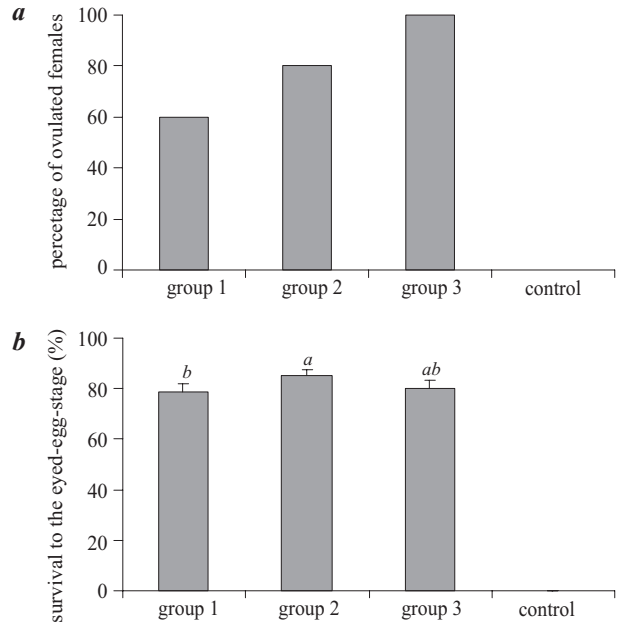


Fig. 2. The results of artifical spawning of tench: *a* – influence of hormonal treatment on ovulation, *b* – embryo survival (±SD). Data marked with the same letter did not differ statistically ($P < 0.05$). Groups are described in Table 1

Discussion

The recognition of the best moment for applying hormonal induction in cyprinid artificial spawning is very important. In the most species, the best method is examining the oocyte maturity stage (KOZLOWSKI 1994). But in fish, which have more than one ovulation during spawning season, these criteria do not work well. In such case, the best method to find accurate moment for hormonal stimulation is experimental way. In present work, the hormonal stimulation was applied in different time after fish collection (first experiment). For this purpose, the ovopel, GnRHa containing pellets with metoclopramide were used. This hormonal combination was worked very well in tench collected from pond culture (Z. OKONIEWSKI – personal communication). The best time for performing artificial propagation of wild tench was first few days after collection and transport fish to a hatchery. Later in time, the results were much poorer. Similar situation was reported for rudd (*Scardinius erythrophthalmus*), where stimulation after five days of acclimation gave poor results (KUCHARCZYK et al. 1997c).

The satisfactory results in artificial reproduction of cyprinids were

obtained using the acetone dried common carp pituitary extract in cyprinid aquaculture, what was reported by YARON et al. (1984), DRORI et al. (1994), KOZLOWSKI (1994), and YARON (1995). The hCG alone was generally used for multi-ovulated marine species, i.e. for snapper (*Pagrus auratus*) (BATTAGLENE and TALBOT 1992), mulloway (*Argyrosomus hololepidotus*) (BATTAGLENE and TALBOT 1994) and sand whiting (*Sillago ciliata*) (BATTAGLENE 1996). In cyprinids, hCG usually was not effective (KUCHARCZYK et al. 1997a,b); only in limited number of species, i.e. in rudd, the obtained results were satisfactory (KUCHARCZYK et al. 1997c). The range of dose of this hormone was in wide range: from 300 IU kg^{-1} (BATTAGLENE 1996) up to 4000 IU kg^{-1} (BATTAGLENE, SELOSSE 1996, KUCHARCZYK et al. 1997c). Moreover, in many other cyprinid species combined injections of hCG and carp pituitary was also effective. The GnRHa analogues, combined with strong dopamine antagonists, generally were very effective in wild and cultured species stimulation (GLUBOKOV et al. 1991, KULIKOVSKY et al. 1996, BARTH et al. 1997, HORVATH et al. 1997). However, the discussion with these data is rather difficult, because different forms of GnRHa analogues have been usually used. Different forms of GnRHa, sometimes from different sources, i.e. mammalian, fish, chicken, etc., have different activity. The spawning success, expressed as a percentage of ovulated females and of embryo survival, was high in all groups.

Important differences were observed in the latency time. Shortest time between injections and ovulation was noted when CPE was used as a spawning agent, in contrast to those fish stimulated using ovopel. The latency of the response on artificial propagation in cyprinids is only reported in general terms (GLUBOKOV et al. 1991, DRORI et al. 1994, YARON 1995). Therefore, before any spawning recommendations for fish breeders, the dose of hormone and the precise timing in relation to water temperature had to be studied under local conditions. The differences in latency in females treated with CPE and GnRH were reported in many papers. DRORI et al. (1994) and YARON (1995) suggest that latency was always shorter in fish treated with carp pituitary extract than in fish treated with other hormones and drugs. It may be explained by fact that GnRH release from the pituitary and the ovarian response to the released for hormones is a sequential process, while in fish injected with carp pituitary extract, ovarian response to the exogenous GtH was a single process (DRORI et al. 1994).

In conclusion, spawning techniques described in this paper were successful and provided consistent results in reproduction of tench collected from wild populations.

Translated by DARIUSZ KUCHARCZYK

Accepted for print 28.11.2006

References

BABIAK I., GŁOGOWSKI J., KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1998. *Cryopreservation of asp. Aspius aspius* (L.) *sperm*. Prog. Fish-Cult., 60: 146-148.

BARTH T., KOURIL J., HAMACKOVA J., VELEK J., BARTHOVA J., HULOWA I., JEZEK J., POSPISEK J. 1997. *Induced ovulation and artificial stripping in tench (Tinca tinca L.) and other freshwater fish species by means of GnRH analogues. Czech experiences 1980-1996. A minireview*. Pol. Arch. Hydrobiol., 44: 183-190.

BATTAGLENE S.C. 1996. *Hormone-induced ovulation of sand whiting* (*Sillago ciliata*). Asian Fisheries Sci., 9: 169-176.

BATTAGLENE S.C., TALBOT R.B. 1992. *Induced spawning and larval rearing of snapper, Pagrus auratus* (*Pisces, Sparidae*), *from Australian waters*. New Zealand, J. Marine Freshwater Res., 26: 179-183.

BATTAGLENE S.C., TALBOT R.B. 1994. *Hormone induction and larval rearing of mulloway. Argyrosomus hololepidotus* (*Pisces, Sciaenidae*). Aquaculture, 126: 73-81.

BATTAGLENE S.C., SELOSSE P.M. 1996. *Hormone-induced ovulation and spawning of captive and wild broodfish of the catadromous Australian bass*, *Macquaria novemaculeata* (*Steindachner*), (*Per cichthyidae*). Aquacult. Res., 27: 191-204.

DRORI S., OFIR M., LEVAVI-SIVAN B., YARON Z. 1994. *Spawning induction in common carp (Cyprinus carpio) using pituitary extract or GnRH superactive analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature*. Aquaculture, 119: 393-407.

GLUBOKOV A.I., MOTLOCH N.N., SEDOVA M.A. 1991. *Effect of a synthetic LHRH analogue and dopamine antagonists on the maturation of bream. Abramis brama* L. Aquaculture, 95: 373-377.

HORVATH L., SZABO T., BURKE J. 1997. *Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species*. Pol. Arch. Hydrobiol., 44: 221-226.

KOZLOWSKI B. 1994. *Practise of hormonal stimulation of artificial spawning in cyprinid fishes*. (in Polish). Broszura IRŚ Olsztyn, 162, 41 pp.

KUCHARCZYK D., KUJAWA R., LUCZYNSKI M., GLOGOWSKI J., BABIAK I., WYSZOMIRSKA E. 1997a. *Induced spawning in bream. Abramis brama* (L.), *using carp pituitary extract and hCG*. Aquacult. Res., 28: 139-144.

KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E. 1997b. *Artificial spawning in bream* (*Abramis brama* L.). Pol. Arch. Hydrobiol., 44: 201-205.

KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E. 1997c. *Induced spawning in rudd* (*Scardinius erythrophthalmus* L.). Pol. Arch. Hydrobiol, 44: 207-211.

KUCHARCZYK D., KUJAWA R., MAMCARZ A., TARGOŃSKA-DIETRICH K., WYSZOMIRSKA E., GLOGOWSKI J., BABIAK I., SZABÓ T. 2005. *Induced spawning in bream* (*Abramis brama* L.) *using GnRH-containing pellets*. Czech J. Anim. Sci, 50:89-95.

KUJAWA R., KUCHARCZYK D. 1996. *A method of in vivo designating oocytes from cyprinid and percid fish using catheter*. Kom. Ryb., Olsztyn, 4: 20-21. (in Polish).

KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1999. *A model system for keeping spawners of wild and domestic fish before artificial spawning*. Aquacult. Eng., 20: 85-89.

KULIKOVSKY Z., MARTIN F.J.B., YARON Z. 1996. *A comparison of two spawning-inducing agents for common carp*. Isr. J. Aquacult. – Bamidgeh, 48: 108-111.

PENAZ M, GAJDUSEK J. 1979. *Early development of bream*, *Abramis brama from the water reservoir Mostiste*. CSSR Fol. Zool., 28: 347-360.

PLATT C. 1977. *Problems with calculus of probability and statistical mathematics*. PWN, Warszawa (in Polish).

THALATHIAH S., AHMAD A.O., ZAINI M.S. 1988. *Induced spawning techniques practised at Batu Berendam*. Melaka, Malaysia. Aquacult., 74: 23-33.

WILDT D.E., SEAL U.S., RALL W.F. 1993. *Genetic resource banks and reproductive technology for wildlife*. In: *Genetic Conservation of Salmonid Fishes*. Eds. J.G. CLOUD, G.H. THORGAARD. Plenum Press, New York, 159-174 pp.

YARON Z. 1995. *Endocrine control of gametogenesis and spawning induction in the carp*. Aquaculture, 129: 49-73.

YARON Z., BOGOMOLNAYA A., LEVAVI B. 1984. *A calibrated carp pituitary extract as a spawning – inducing agent*. H. ROSENTAL & S. SARIG Eds. Res. Aquacult., 8: 151-168.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 116-125, Y. 2007

DOI 10.2478/v10020-007-0012-9

EFFECT OF THE CHEMICAL COMPOSITION OF SELECTED COMMERCIAL JUICES ON THEIR RHEOLOGICAL PROPERTIES

***Elżbieta Haponiuk*[1], *Halina Pieńkowska*[2], *Ryszard Zadernowski*[3]**

[1] Chair of Processing Engineering and Equipment
[2] Chair of Agricultural Engineering and Natural Raw Materials
[3] Chair of Plant Processing and Food Chemistry
University of Warmia and Mazury in Olsztyn

K e y w o r d s: rheology of juices, apparent viscosity, yield stress.

A b s t r a c t

A study was undertaken to analyze the effect of the chemical composition of selected fruit-vegetable juices with the following trade names: "prof. Zdrówko", "Kubuś", "Sonda", "TIP", "Karotka-Fortuna", "Smakuś", "Pysio" and "Junior Frut", on their rheological properties. Investigations demonstrated that the juices were non-Newtonian fluids, strongly thinned out by shearing, whose flow curves was approximated with both the Ostwald de Waele and the Casson models. In all cases examined, the determined contents of dry matter, pectins, extract and vitamins were found to exert a considerable effect on the consistency and viscosity of the juices analyzed, whereas the content of dietary fibre appeared not the affect the rheological parameters under study. The highest value of the consistency coefficient K, and consequently the highest viscosity η, were reported for Prof. Zdrówko 1Z, 2Z, 3Z as well as Kubuś 1K – 3K juices that were characterized by the highest content of extract and dry matter.

WPŁYW SKŁADU CHEMICZNEGO WYBRANYCH SOKÓW SPOŻYWCZYCH NA ICH WŁAŚCIWOŚCI REOLOGICZNE

***Elżbieta Haponiuk*[1], *Halina Pieńkowska*[2], *Ryszard Zadernowski*[3]**

[1] Katedra Inżynierii i Aparatury Procesowej
[2] Katedra Inżynierii Rolnej i Surowców Naturalnych
[3] Katedra Przetwórstwa i Chemii Surowców Roślinnych
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: reologia soków, lepkość pozorna, granica płynięcia.

Address: Elżbieta Haponiuk, University of Warmia and Mazury, Michała Oczapowskiego 7, 10-719 Olsztyn, Poland, phone: 48 (089) 523 47 82, e-mail: elzbieta.haponiuk@uwm.edu.pl

A b s t r a k t

Badano wpływ składu chemicznego na właściwości reologiczne wybranych soków owocowo-warzywnych o następujących nazwach handlowych: „prof. Zdrówko", „Kubuś", „Sonda", „TIP", „Karotka-Fortuna", „Smakuś", „Pysio" i „Junior Frut". Badania wykazały, że soki spożywcze są cieczami nienewtonowskimi, silnie rozrzedzanymi ścinaniem, których charakterystyki płynięcia aproksymowano modelami Ostwalda-de Waele i Cassona. Stwierdzono, że we wszystkich przypadkach zawartość suchej masy, pektyn, ekstraktu oraz witamin miała znaczący wpływ na konsystencję i lepkość badanych soków, natomiast zawartość błonnika pokarmowego nie wpływała na badane parametry reologiczne. Najwyższą wartość współczynnika konsystencji *K*, a tym samym największą lepkość η, miały soki spożywcze o nazwie handlowej Prof. Zdrówko 1Z, 2Z, 3Z i Kubuś 1K – 3K, gdyż zawierały najwięcej ekstraktu i suchej substancji.

Introduction

Producers of fruit juices, pomace and concentrates used in the food industry are paying increasing attention to the rheological properties of their products. The production of fresh fruit juices and pomace requires maintenance of their stable organoleptic traits since they serve as the basis for the production of mono- or multi-compound commercial juices. This, in turn, entails providing reproducible rheological properties, such as consistency, apparent viscosity and yield stress, that are aimed at preserving specified taste attributes, thus a high quality of the product expected by a consumer (LEE et al, 2002).

Therefore, the rheology of the ready-to-serve juice is affected by physicochemical changes in the chemical composition of this product under the influence of multiple technological treatments.

The study was aimed at determining the impact of the chemical composition of commercial juices on their rheological properties.

Material and Methods

Rheological analyses were carried out on selected juices appearing under the following trade names: "prof. Zdrówko", "Kubuś", "Sonda", "TIP", "Karotka-Fortuna", "Smakuś", "Pysio" and "Junior Frut". Table 1 presents the characteristics of the selected commercial juices.

Physicochemical properties of the selected commercial juices:

The selected commercial juices were analyzed for their physicochemical properties of nutritional and technological significance, including:

– the content of dry matter was determined with the gravimetric method according to Polish Standard PN-90/A75101/03,
– the content of total extract was determined with the refractometric method according to Polish Standard PN-90/A-75101/02,

Table 1

Characteristics of juices examined

Trade name of juice	No of juice	Composition of juice
prof. Zdrówko	1Z	carrot
	2Z	carrot, apple and banana
	3Z	carrot, pine apple and celery
Kubuś	1K	carrot
	2K	carrot and wild strawberry
	3K	carrot, raspberry and apple
	4K	banana, apple and peach
Sonda	1S	carrot and strawberries
	2S	carrot and orange
Tip	1T	carrot and banana
	2T	carrot and orange
Karotka-Fortuna	1K – F	carrot, apple and strawberry
	2K – F	carrot, apple and banana
Smakuś	1S	carrot
Pysio	1P	carrot, apple and peach
Junior-Frut	1J – F	carrot

– the content of total sugars and reducing sugars was assayed with the modified method of Lane-Eynon according to Polish Standard PN-90/A--75101/07,
– dietary fibre was assayed following the NDF method (modification of the method developed by ASP et al. 1986),
– total pectins were determined following the modified method of SABIR et al. of 1976,
– the sum of carotenoids and the content of β-carotene were determined according to Polish Standard PN-00/EN-12136,
– the concentration of vitamin C was assayed according to Polish Standard PN-90/A-75101/11.

Data referring to the assays of the above-mentioned physicochemical characteristics in the commercial juices examined are collated in Table 2.

Empirical flow curves of the products examined were plotted with the use of a rotary rheometer Rheotest 2 (VEB MLW Prufgerate – Werk Medingen, Sitz Freital, Germany) equipped with a set of S/S3 cylinders. The shear rate $\dot{\gamma}$ used in this study ranged from 0.166 s^{-1} to 72.9 s^{-1}. Measurements were carried out at a temperature of 20 ± 0.1°C and recorded after 60 s of sample shearing at each level of shear rate gradient.

Calculations were made by means of Statistika 6 Pl software (Statsoft inc, Tulska, Usa).

Table 2

The chemical composition of selected commercial juices

Trade name of juice	No of juice	Chemical composition of selected commercial juices							
		dry matter (%)	extract (%)	total sugars (%)	reducing sugars (%)	dietary fibre (g 100 g^{-1})	pectins (g of galacturonic acid 100 g^{-1})	β-carotene (mg 100 g^{-1})	vitamin C (mg 100 g^{-1})
Prof. Zdrówko	1Z	13.44±0.01	13.0±0.0	13.28±0.02	8.65±0.01	1.51±0.05	0.30±0.03	3.19±0.02	39.7±0.7
	2Z	12.16±0.03	12.9±0.1	11.55±0.03	5.99±0.03	1.67±0.07	0.44±0.03	3.02±0.01	37.5±0.7
	3Z	13.20±0.13	11.7±0.1	12.43±0.02	5.41±0.03	1.58±0.04	0.42±0.01	3.70±0.00	41.4±0.7
Kubuś	1K	12.05±0.09	12.0±0.0	10.86±0.06	5.78±0.03	1.58±0.07	0.37±0.02	3.27±0.01	54.5±0.7
	2K	11.38±0.07	12.5±0.1	10.80±0.02	5.42±0.00	1.60±0.03	0.40±0.06	3.02±0.05	54.9±0.7
	3K	11.34±0.07	12.6±0.0	11.12±0.02	5.77±0.00	1.64±0.05	0.26±0.03	2.94±0.01	53.3±0.7
	4K	11.57±0.03	12.5±0.1	11.22±0.06	5.58±0.26	1.42±0.05	0.26±0.01	traces	55.3±0.7
Sonda	1S	11.72±0.03	11.5±0.0	9.22±0.05	4.91±0.01	1.55±0.09	0.46±0.04	3.89±0.03	34.0±0.7
	2S	11.65±0.11	11.1±0.1	9.98±0.04	6.25±0.01	1.53±0.06	0.44±0.02	3.06±0.04	44.6±0.7
Tip	1T	12.21±0.13	10.5±0.0	10.01±0.01	6.53±0.12	1.37±0.04	0.44±0.04	2.60±0.01	37.1±0.7
	2T	11.59±0.05	10.4±0.1	10.84±0.02	6.78±0.06	1.47±0.05	0.43±0.04	3.07±0.00	43.5±0.7
Karotka-Fortuna	1K – F	11.59±0.02	11.3±0.3	11.19±0.04	4.19±0.15	1.53±0.06	0.51±0.03	3.02±0.01	31.2±0.7
	2K – F	11.14±0.09	10.9±0.1	10.87±0.02	3.32±0.08	1.43±0.07	0.43±0.03	2.63±0.09	25.1±0.3
Smakuś	1Sm	10.01±0.14	10.2±0.1	9.87±0.05	5.50±0.01	1.60±0.06	0.36±0.01	3.19±0.04	43.9±0.7
Pysio	1P	11.50±0.04	12.5±0.1	10.65±0.02	4.90±0.02	1.46±0.03	0.38±0.03	2.73±0.03	36.3±0.7
Junior – Frut	1J – F	11.87±0.02	12.2±0.1	11.65±0.06	4.99±0.04	1.87±0.04	0.65±0.03	4.55±0.07	14.1±0.3

Results and Discussion

It was found that the character of the flow curves of the commercial juices was typical of fluids thinned out by shearing and possessing the yield stress. Changes in the shear stress p caused by the increased shear rate proceeded in a non-linear mode, whereas the apparent viscosity c was observed to decrease rapidly in the range of 0.166 s^{-1} to 13.5 s^{-1} (FERGUSON et al. 1995). The dependency of shear stress τ and apparent viscosity η on the shear rate $\dot{\gamma}$ determined experimentally was approximated with the rheological models of: Ostwald – de Waele and Casson (BARNES et al. 1989, RAMASWAMY et al. 1991, LIMANOWSKI et al. 2000).

Ostwald-de Waele: $$\tau = K \cdot \dot{\gamma}^{n} \qquad \eta = K \cdot \dot{\gamma}^{n-1} \tag{1}$$

Casson: $$\sqrt{\tau} = \sqrt{\tau_c} + \sqrt{\eta_c \cdot \dot{\gamma}} \qquad \sqrt{\eta} = \sqrt{\tau_c / \dot{\gamma}} + \sqrt{\eta_c} \tag{2}$$

where:
τ – shear stress (Pa),
K – coefficient of consistency (Pa · s^n),
n – flow index,
$\dot{\gamma}$ – shear rate (s^{-1}),
η_c – Casson's viscosity (Pa · s),
τ_c – Casson's yield stress (Pa).

The flow curves and curves of apparent viscosity changes as a function of shear rate for the selected juices are shown in Figures 1-4, whereas the parameters of the rheological models together with the degree of equation fitting based on the coefficients of correlation R are presented in Tables 3-4.

On the basis of the results obtained, it was found that the highest values of consistency coefficients and, consequently, the highest viscosity were displayed by the juices under the trade name prof. Zdrówko (1Z, 2Z, 3Z), whereas the lowest values of consistency coefficients and apparent viscosity were exhibited by juices: Sonda 1S, Kubuś 4K and Tip 2T. The values of consistency coefficients reached: K = 11.567 (Pa · s^n) at n = 0.148 for juice 1Z, K = 11.004 (Pa · s^n) at n = 0.167 for juice 2Z, K = 10.715 (Pa · s^n) at n = 0.177 for juice 3Z, K = 2.653 (Pa · s^n) at n = 0.262 for juices available under the trade name Sonda, K = 2.683 (Pa · s^n) at n = 0.317 for the juice Kubuś 4K, and K = 2.509 (Pa · s^n) at n = 0.297 for the juice Tip 2T. It is common knowledge that, apart from temperature, the rheological parameters are affected, to a great extent, by the physicochemical properties of the products examined, namely, the contents of dry matter, extract and proximate chemical compounds, including:

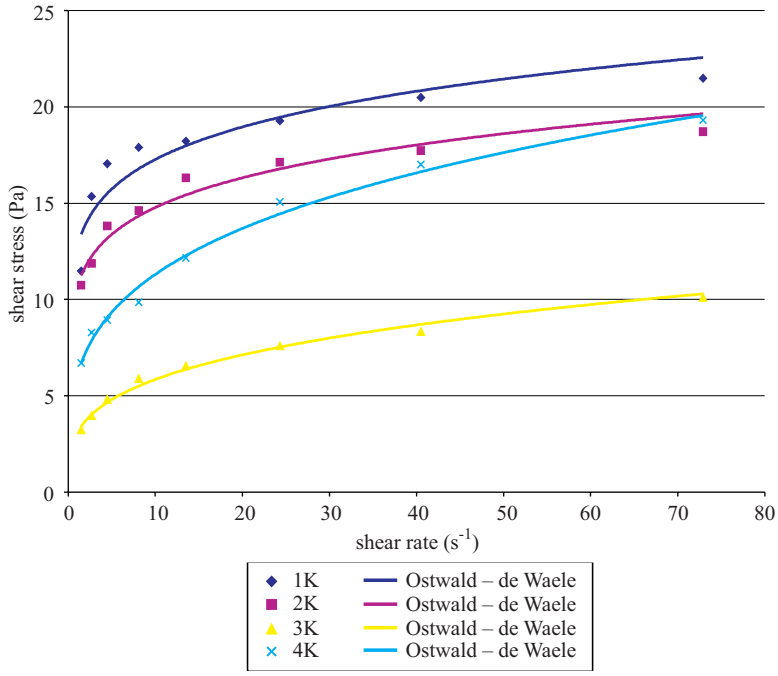


Fig. 1. Flow curves with the fitting of the Ostwald-de Waele's model for commercial juices "Kubuś" – 1K, 2K, 3K, 4K

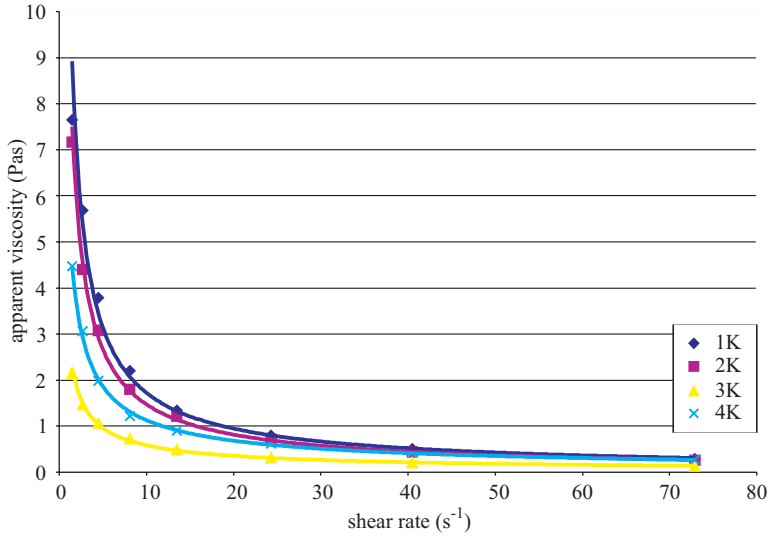


Fig. 2. Viscosity curves with the fitting of the Ostwald-de Waele's model for commercial juices "Kubuś" – 1K, 2K, 3K, 4K

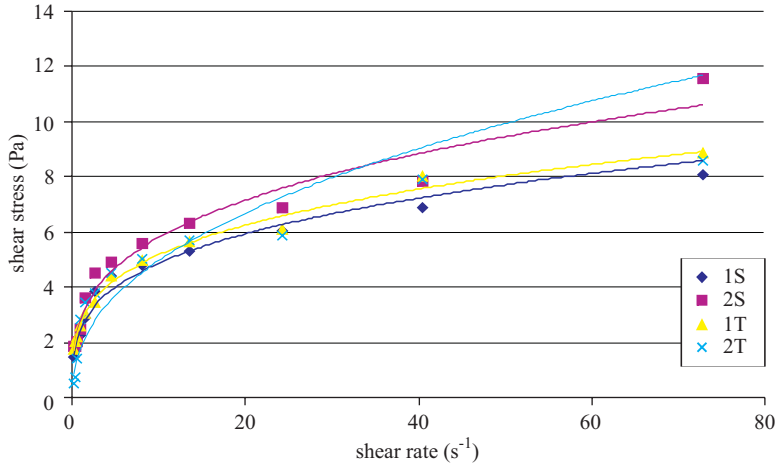


Fig. 3. Flow curves with the fitting of the Ostwald-de Waele's model for commercial juices: "Sonda" – 1S , 2S and "Tip" – 1T, 2T

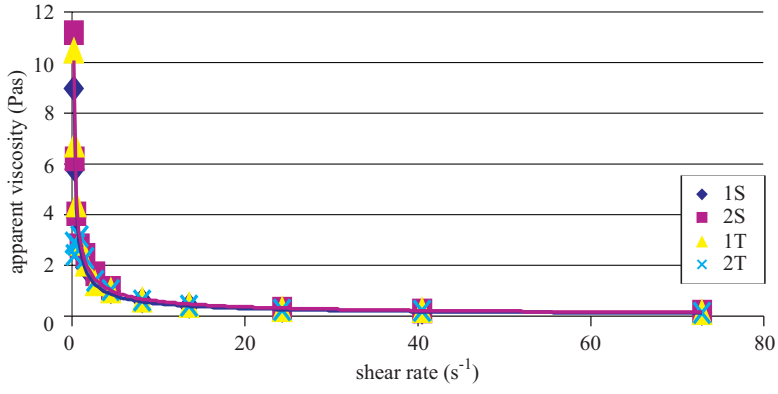


Fig. 4. Viscosity curves with the fitting of the Ostwald-de Waele's model for commercial juices: "Sonda" – 1S , 2S and "Tip" – 1T, 2T

total saccharides, dietary fibre, pectins and vitamins (RAO et al. 1986). Commercial drinking juices are produced by mixing concentrated fruit juices and pomace (from 20% to 50%) with water. The substantial difference in the rheological properties of the above-mentioned fruit-vegetable juices was most likely due to the differentiated physicochemical composition of the produces examined. Hence, the juices containing carrot pomace, e.g. "Prof. Zdrówko",

Kubuś 1K, 2K, 3K, were characterized by a better viscosity and, consequently, a better consistency and a good quality of the finished product since the carrot pomace contributed to the increase in the contents of extract, dry matter, total sugars and pectins (Table 2) which, in turn, has a considerable impact on the rheology of juice under scrutiny (SCHRAMM 1998). The commercial juices (4K, 1S, 2T) that were produced on the basis of fruit pomace exhibited a remarkably lower content of extract, dry matter and proximate physicochemical compounds, thus were characterized by nearly twofold lower viscosity and, consequently, worse consistency and quality of the ready-to-serve juice. The parameters of the Casson's model reflected the changes in the coefficients of consistency (Table 4). The highest yield stress τ_c i.e. τ_c = 10.131 (Pa), was reported for the juice under the trade name "Prof. Zdrówko" 2Z, whereas the lowest, i.e. τ_c = 2.084 (Pa), was for the Tip – 1T juice.

Table 3

Rheological parameters of commercial juices

Trade name of juice	No of juice	Ostwald-de Waele's model		Apparent viscosity at $\dot{\gamma}$ = 10, 50, 100 s^{-1}			Coefficient of correlation
		K (Pa s^n)	n (–)	η_{10} (Pa s)	η_{50} (Pa s)	η_{100} (Pa s)	R
Prof. Zdrówko	1Z	11.567	0.148	1.626	0.412	0.228	0.932
	2Z	11.004	0.167	1.616	0.422	0.237	0.933
	3Z	10.715	0.177	1.610	0.428	0.242	0.939
Kubuś	1K	10.846	0.180	1.641	0.438	0.248	0.948
	2K	8.685	0.203	1.386	0.384	0.221	0.951
	3K	5.611	0.295	1.106	0.355	0.218	0.995
	4K	2.683	0.317	0.556	0.185	0.115	0.985
Sonda	1S	2.653	0.262	0.484	0.147	0.088	0.992
	2S	2.889	0.303	0.580	0.189	0.116	0.981
Tip	1T	2.736	0.276	0.516	0.161	0.097	0.995
	2T	2.509	0.297	0.497	0.160	0.098	0.971
Karotka-Fortuna	1K – F	5.648	0.180	0.854	0.228	0.129	0.973
	2 K – F	5.732	0.143	0.796	0.200	0.110	0.983
Smakuś	1S	3.699	0.215	0.606	0.171	0.099	0.958
Pysio	1P	3.154	0.242	0.550	0.162	0.096	0.986
Junior-Frut	1 J – F	3.996	0.183	0.609	0.163	0.093	0.994

Table 4

Rheological parameters of commercial juices

Trade name of juice	No of juice	Casson's model		Coefficient of correlation
		τ_c (Pa)	η_c (Pas)	R
Prof. Zdrówko	1Z	9.796	0.043	0.900
	2Z	10.131	0.038	0.900
	3Z	6.154	0.467	0.917
Kubuś	1K	2.211	0.048	0.927
	2K	5.809	0.523	0.940
	3K	4.731	0.083	0.953
	4K	5.102	0.297	0.914
Sonda	1S	2.395	0.047	0.965
	2S	2.273	0.029	0.942
Tip	1T	2.084	0.038	0.908
	2T	2.330	0.035	0.964
Karotka-Fortuna	1K – F	5.230	0.016	0.914
	2 K – F	3.035	0.316	0.903
Smakuś	1S	3.931	0.028	0.968
Pysio	1P	2.707	0.030	0.950
Junior-Frut	1 J – F	3.541	0.020	0.964

Conclusions

On the basis of the results obtained, the following conclusions can be drawn:

1. The best fitting to the empirical flow curves was demonstrated by the power model of Ostwald-de Waele for commercial juices under the trade name Sonda (1S, 2S) and Tip (1T,2T) in the entire range of shear rate, whereas for the other commercial juices – at shear rates ranging from 1.5 to 72.9 s^{-1}.

2. The dietary fibre content of the commercial juices examined did not affect their rheological properties.

3. The highest values of the coefficients of consistency, apparent viscosity and yield stress were reported for the juices under the trade name prof. Zdrówko 1Z, 2Z, 3Z, as they were characterized by the highest contents of dry matter, extract, pectin and vitamins.

4. The consistency coefficients and apparent viscosity of the selected commercial juices were found to be determined, to a great extent, by the chemical composition of juice, i.e. by the contents of dry matter, extract, pectins and vitamins.

Translated by JOANNA JENSEN

Accepted for print 6.01.2007

References

FERGUSON J., KEMBŁOWSKI Z. 1995. *Reologia stosowana płynów*. Wyd. Marcus, Łódź.

RAO M.A., COOLEY H.J., NOGUEIRA J.N., MC LELLAN M.R. 1986.R*heology of apple sauce: Effect of apple cultivar, frimness and processing parameters.* J. Food. Sci., 51(1): 176-179,

Handbook of food engineering. Eds. HELDMAN D.R., LUND D.B., 1992. Marcel Dekker Inc. New York.

LEE Y., BOBROFF S., MC CARTHY L. 2002. *Rheological characterization of tomato concentrates and the effect on uniformity of processing.* Chem. Eng.Comm., 189(3): 339-351.

BARNES H.A., HUTTON J. F., WALTERS K. 1989. *An introduction to rheology.* Elsevier, Amsterdam.

RAMASWAMY H.S., BASAK S. 1991. *Rheology of stirred yogurts.* J. Text. Stud., 22(2): 231-241.

O'CONNORS R.T. 1960. *Fats, oil, fatty acid and glycerides-UV spectroskopy*. *The Encyclopedia of Spectroscopy*. Reinhold Publishing Co., New York, pp. 102-129.

BARTOSZ G. 1995. *Druga twarz tlenu*. PWN, Warszawa, pp. 94-99.

CHEŁKOWSKI A. 1993. *Fizyka dielektryków*. PWN, Warszawa, pp. 87-89.

SCHRAMM G. 1998. *Reologia. Podstawy i zastosowanie*. Ośrodek Wyd. Nauk., Poznań.

LIMANOWSKI J., E. HAPONIUK, A. GŁOWACKI 2000. *Badania lepkości i granicy płynięcia wybranych koncentratów owocowych*. Biul. Nauk. UWM, 8: 215-223.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 126-136, Y. 2007

DOI 10.2478/v10020-007-0013-8

THE QUALITY OF WHEAT BREAD SUPPLEMENTED WITH DRIED CARROT POMACE

Małgorzata Tańska, Ryszard Zadernowski, Iwona Konopka

Chair of Food Plant Chemistry and Processing
University of Warmia and Mazury in Olsztyn

K e y w o r d s: wheat bread, dried carrot pomace, nutrition value, rheological properties.

A b s t r a c t

The influence of 5, 7.5 and 10% additions of dried carrot pomace on selected wheat dough and bread features was tested. It was stated that all used additives can be with good results utilized in bakery. Dried carrot pomace supplemented bread in carotenoids, fiber, mineral components. At the same time properties of bread were not adversely changed. The best from rheological and organoleptic point of view was 5% addition of carrot pomace. It increased dough water absorption of 8% and bread volume of 10%. Crumb of this bread was the most elastic and generally this bread attained the highest number of points in organoleptic evaluation.

JAKOŚĆ PIECZYWA PSZENNEGO SUPLEMENTOWANEGO SUSZEM Z WYTŁOKÓW MARCHWIOWYCH

Małgorzata Tańska, Ryszard Zadernowski, Iwona Konopka
Katedra Przetwórstwa i Chemii Surowców Roślinnych
Uniwersytet Warmińsko Mazurski w Olsztynie

S ł o w a k l u c z o w e: pieczywo pszenne, susz z wytłoków marchwi, wartość odżywcza, cechy reologiczne.

A b s t r a k t

W pracy badano wpływ 5; 7,5 i 10% dodatków zmielonego suszu z marchwi na wybrane cechy jakościowe ciasta i pieczywa pszennego. Stwierdzono, że wszystkie dodatki mogą być z dobrym skutkiem stosowane w piekarstwie. Susz z marchwi wzbogacił pieczywo w składniki odżywcze, takie jak m.in.: karotenoidy, błonnik i składniki mineralne. Jednocześnie nie pogarszał cech jakościowych pieczywa w stosunku do pieczywa kontrolnego. Najlepszy okazał się 5% dodatek zmielonego suszu z marchwi, przyczynił się bowiem do zwiększenia wodochłonności ciasta o 8%. Gotowe pieczywo miało lepszą elastyczność miękiszu, większą o ok. 10% objętość i uzyskało najwyższą ocenę organoleptyczną.

Address: Małgorzata Tańska, University of Warmia and Mazury, pl. Cieszyński 1, 10-726 Olsztyn, Poland, phone: 48 (089) 523 41 13, e-mail: m.tanska@uwm.edu.pl

Introduction

Bakery products constitute an important element of the human diet, since people, on average, consume about 250 g of bread a day (SKIBNIEWSKA et al. 2004). Consumer preferences have forced the producers to modify bread recipes. Potential possibility of changing the quality of bread consists in introducing various types of additives (AMBROZIAK 1998, GĄSIOROWSKI 2000). Natural additives are preferable, not only in order to improve the nutritional quality of bread, but also to influence its organoleptic qualities, such as: appearance, taste, smell, crumb elasticity and crispness of the crust. Increasing often, the market offers bread with the addition of sunflower, soya bean, sesame, poppy and evening primrose seeds, linseed, non-bread cereal grains (e.g. barley and oat), plums, olives and onion. Additionally, certain attempts have been made to assess the effects of using additives on selected qualities of wheat dough and bread. The subjects of these studies were linseeds (GAMBUŚ et al. 1999a,b, ROTKIEWICZ et al. 2003), flaxseeds (ROTKIEWICZ et al. 2003), evening primrose and borage seeds as well as the oil pressed from those seeds (KONOPKA et al. 1999) and lupin seeds (SKIBNIEWSKA et al. 2003).

Obesity is regarded as a plague of the societies in highly developed countries. The awareness of dangers related to excessive weight (among others, a high risk of cardiovascular diseases and diabetes), along with the need to change one's own image, are inducing more and more consumers to search for products with low calorific values (BIAŁKOWSKA, SZOSTAK 2000). However, such food must meet the criterion of organoleptic attractiveness. It is also required to contain amounts of ballast compounds, such as fibre, which supports the functions of the gastrointestinal tract (BIAŁKOWSKA, SZOSTAK 2000, GĄSIOROWSKI 2000) as well as other biologically active ingredients, among others, vitamins, antioxidants as well as micro- and macroelements (ZIEMLAŃSKI 2000). One of the potential ways of increasing the sensory attractiveness of bread while at the same time reducing its calorific value, might be the use of fruit or vegetable additives, which are naturally rich in biologically active, non-nutritional components. Such a component could be, e.g. carrot, which is a rich source of fibre, pectins, phenolic compounds, vitamin C, carotenoids and mineral components (ZADERNOWSKI et al. 2003). Many of those precious components remain in pomace, a by-product of the juice manufacturing industry.

Bearing in mind the potential advantages resulting from an increase in the supply of nutrients and a reduction, at the same time, of the calorific value of bread due to the addition of dried carrot pomace, a study was carried out in order to determine its optimal addition to bread. The organoleptic qualities of bread, as well as the rheological characteristics of dough and selected physical

and chemical features of bread were used as the criteria determining the choice of the amount of the additive.

Material and Methods

The experimental material was wheat flour 550 type as well as dried and milled carrot pomace.

Flour was tested for: moisture content (PN-91/A-74010), falling number value (PN-ISO-3093:1996), Zeleny test (PN-ISO-5529:1998) as well as wet gluten content (PN-A-74043-3). In dried carrot pomace were analysed: dry mass (PN-90/A-75101/03), total and reducing sugars according to Lane-Eynon method (PN-90/A-75101/07), starch (AOAC 1975), carotenoids (PN-90/A--75101), pectins according to method described by SABIR et al. (1976), total dietary fibre, in this insoluble, accord to modified method of ASP et al. (1983) and acid detergent fibre (ADF) (AOAC 1990). Water absorption of dried carrot pomace was determined as described by REYES-MORENO et al. (2002). Mineral composition of dried carrot pomace was analyzed according to PN-90/A-75101. Water absorption by flour as well as it mixtures with dried carrot pomace were determined using of Brabender farinograph (PN-ISO 5530-1).

Rheological properties of dough were tested for dough yield 150% in relation to moisture of flour equal 15%. Dough of initial temperature 20°C was made in laboratory mixer GM type. Time of mixing was 5 min. Four variants of dough were prepared:

1) control sample (flour with only distilled water);

2-4) mixtures of flour with 5, 7.5 and 10% of dried carrot pomace plus distilled water.

Extrusion properties were tested immediately after dough preparation and were done in OTMS cell installed in UTM INSTRON 4301. Details of investigation methodology are presented in work of KONOPKA et al. (2004).

Baking value of flour with carrot additives was tested for their optimal farinograph water absorption. Baking mixtures contained also 3% of yeast and 1% of salt. Quality of bread was estimated 24 hours after baking. Bread volume was analyzed in SA-Wy apparatus. Bread crumb acidity was determined according to PN-A-74108:1996. Crumb elasticity was measured for 25x25x25 mm cubes, sliced from central part of loaf. Rheological properties of crumb were tested in UTM Intron 4301. Parameters of test were following: capacity of head – 1000 N, compressing element – flat pin about diameter 35 mm, speed of compression – 50 $mm \cdot min^{-1}$ and range of deformation 25%. Bread organoleptic analysis was done pointwise according to PN-A-74108:1996. All analyses were done in duplicate. The experimental results were analyzed using the Statistica 6.0 PL software.

Results and Discussion

Characteristics of flour and dried carrot pomace

Flour used for baking bread was characterized by a good technological quality, which was shown by the values of discriminants (Table 1) within limits specified by the Polish Standard (PN-91/A-74022). The falling number value, determining the level of starch damage during kernel sprouting, was within the optimal range of 250-400 s (AMBROZIAK 1998). Wet gluten content complied with the quality requirements for wheat flour type 550 (PN-91/A-74022), as it considerably exceeded 25%. The results of a Zeleny test, indicating the content of high molecular weight prolamins, the main indicator of the baking value of flour, reached the value of 32 cm^3. According to the classification provided by HABER and HORUBAŁOWA (1992), this indicated only a satisfactory quality of wheat from which the flour was obtained.

Table 1

Characteristic of wheat flour and dried carrot pomace

Feature		Value
Wheat flour		
Moisture content (%)		12.5 ± 0.07
Falling number value (s)		381.0 ± 2.80
Zeleny's test (cm^3)		32.0 ± 0.71
Wet gluten content (%)		34.8 ± 0.03
Dried carrot pomace		
Dry mass (%)		93.7 ± 1.45
Sugars (% of d.b.), in this:		50.8 ± 3.61
	reducing	18.0 ± 1.20
Starch (%)		6.6 ± 0.78
Total fibre (% of d.b.), in this:		37.5 ± 2.50
	insoluble	25.0 ± 2.97
Fibre ADF (% of d.b.), in this:		17.5 ± 2.50
	lignin	8.9 ± 0.50
	cellulose	8.5 ± 0.40
Total pectins (% of d.b.)		6.9 ± 0.90
Ash (% of d.b.)		5.5 ± 0.26
Lipids (% of d.b.)		6.0 ± 0.43
Carotenoids (mg 100 g^{-1} of d.b.)		45.6 ± 2.33
Water absorption (%)		551.1 ± 0.68

The dried carrot pomace used in the research was a rich mixture of organic components, mainly polysaccharides (Table 1). Total sugar content exceeded 50% of dry matter and reducing sugars constituted 36% of their total quantity. A high amount of monosaccharides is potentially beneficial for baking bread of

flour with low amylase activity, since they constitute the main source of carbon for multiplying baker's yeast (AMBROZIAK 1998). The content of starch, insoluble in cell sap and therefore remaining in pomace, was determined at the level of 6.6%. Non-starch polysaccharides present in pomace included fibre (17.5%) and pectin compounds (6.9%). According to BAO and CHANG (1994) arabinose and galactose are main monosaccharides of non-starch polysaccharides. Other biologically important components of dry pomace were lipids (6.0%), natural solvent for carotenoids and the source of many valuable unsaturated fatty acids. Dried carrot also contained 5.5% of mineral components, with the prevalence of iron, zinc, potassium and manganese (Table 2). They can enrich and supplement wheat bread mineral composition because it is a poor source of microelements (e.g. 100 g supplies only 1.4 mg of iron) (AMBROZIAK 1998).

Moisture content of dried carrot pomace was 6.3%, while its capacity to absorb water was determined at the level of 551.1% (Table 1). The water absorption of commercial flour amounted to 60.1% (Table 3). Replacing 5% of the flour weight with the same amount of dried carrot pomace resulted in an increase in water absorption by 8%, while 7.5 and 10% of dried additives increased the capacity to absorb water by 12 and 16%, respectively. Therefore, it has been proven that each percentage of dried additives below 10% corresponds to 1.6% increase in water absorbability of the baking mix.

Table 2

Mineral composition of dried carrot pomace

Microelement	(mg · g^{-1} of d.b.)
Na	3.2 ± 0.08
K	18.6 ± 0.10
P	1.8 ± 0.04
Ca	3.0 ± 0.06
Mg	1.1 ± 0.05
Cu	4.0 ± 0.07
Mn	10.8 ± 0.12
Fe	30.5 ± 0.14
Zn	29.4 ± 0.16

Table 3

Water absorption of mixture wheat flour with dried carrot pomace

Sample	(%)
Control (wheat flour type 550)	60.1 ± 0.46^a
Wheat flour type 550 with 5% addition of dried carrot pomace	67.8 ± 0.68^b
Wheat flour type 550 with 7.5% addition of dried carrot pomace	71.7 ± 0.56^c
Wheat flour type 550 with 10% addition of dried carrot pomace	75.6 ± 0.72^d

Rheological characteristics of wheat dough

Rheological characteristic of dough was determined with the use of compression test in an OTMS extrusion chamber. The results of this test highly correlate with an alveographic evaluation (KONOPKA et al. 2003), which is preferred in industrial practice in European Union countries (ABRAMCZYK 1997) and is very often used in research (RASPER et al. 1986, SCHÖGGL 1998).

The maximal force of compression for the control dough was 385 N (Figure 1). The dough with the addition of dried carrot pomace required increased force for extrusion in OTMS cell. It was observed that this trend can be described by a parabolic curve. The highest value (almost two-fold in relation to the control variant) was observed in the case of the dough with 10% of dried pomace. The increase in the value of the maximal force of compression indicates strengthening of dough structure. It can be explained by the high ability to water absorption by dried carrot pomace (Table 2). The values of compression energy of the dough were similar to values of maximal force of compression (Figure 2). The highest energy, over 19 J, was characteristic for the dough with addition of 10% dried carrot pomace. The analyzed dough samples also featured a varied hardness. Replacing 5; 7.5 and 10% of flour with the same amount of dried carrot pomace progressively increased the hardness of dough, and the course of the changes observed may be described by the exponential curve $y = 39.596e^{0.3836x}$ (Figure 3).

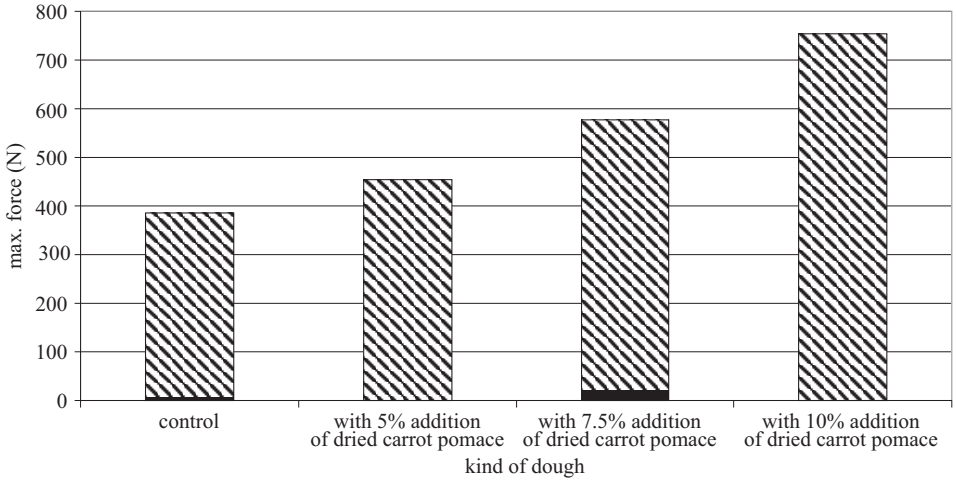


Fig. 1. Maximal force of dough compression

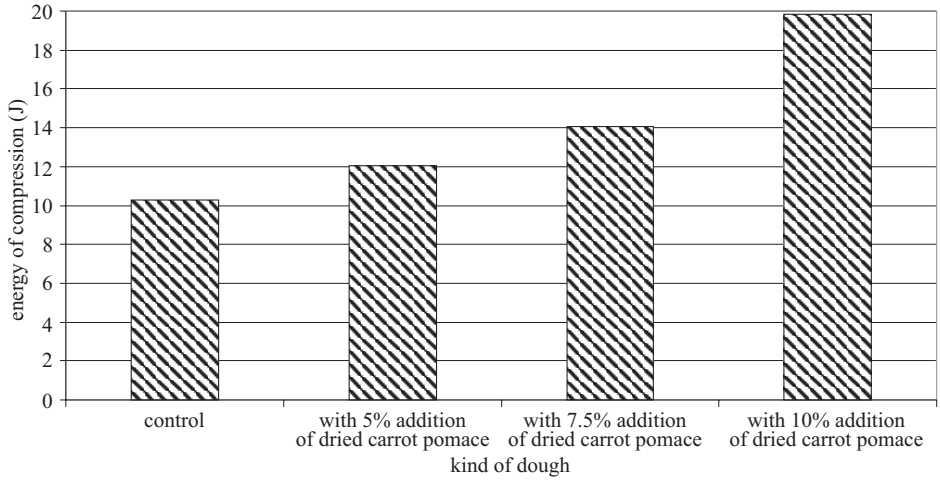


Fig. 2. Energy of dough compression

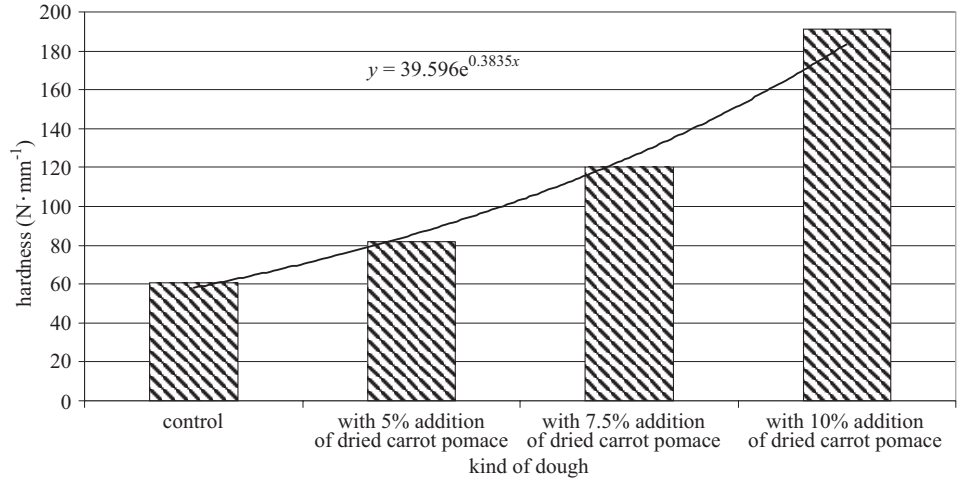


Fig. 3. Hardness of wheat dough

Physical qualities of experimental bread

The highest and the lowest volume of 100 g of bread, amounting to 318 and 283 cm^3 respectively, were found for the samples with a 5% and 10% of dried carrot pomace. The lowest volume points to a 10% reduction in comparison with the control sample (Figure 4). According to Polish Standard PN-92/A-74105, the volume of 100 g of bread made of type 550 flour should not be lower than 280 cm^3, therefore all amounts of additives used did not disqualify the bread.

The quality standard of PN-92/A-74105 determines the upper acidity level for the bread made of type 550 flour at 3°. The acidity of crumb of control bread was 1.15°. Addition of dried carrot pomace resulted in gradually increase of bread acidity to the value of 1.65° for variant with 10% addition of dried carrot pomace (Figure 5). The increase in the acidity of bread with the addition of dried carrot resulted from introducing organic acids, among others, in the form of malic and citric acid salt (the presence of which in carrot amounts to about 1%) (ZADERNOWSKI, OSZMIAŃSKI 1994) and the possible exchange of some reducing sugars into the form of organic acids.

The elasticity of the bread crumb was determined on the basis of the value of maximal compression force and its increase at unit strain. The maximal value of compression force for the control bread was equal 6.7 N. Addition of 5% of dried carrot pomace resulted in more elastic bread crumb, contributing to an over two-fold reduction in maximal compression force (Figure 6) and unit-increase in compressive force (Figure 7). The elasticity of the bread crumb with higher amounts of additives resembled that of the control bread.

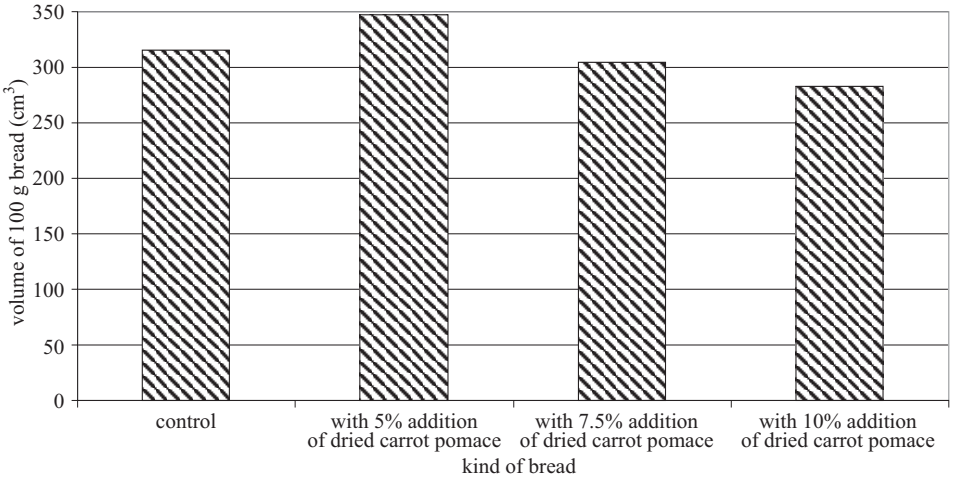


Fig. 4. Volume of wheat bread

The organoleptic analysis confirmed the best physical properties of bread with 5% addition of dried carrot pomace (Table 4). On the basis of PN--A-74108:1996 it was classified to the first class of quality. Other breads, also control one, were classified to second class. Generally, breads with dried carrot pomace were darker and similar in colour and properties to wholemeal bread.

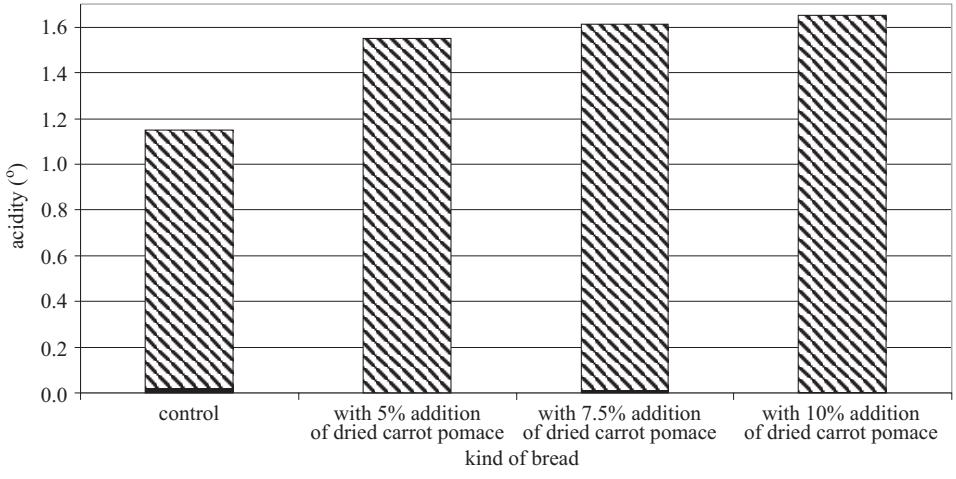


Fig. 5. Acidity of bread crumb

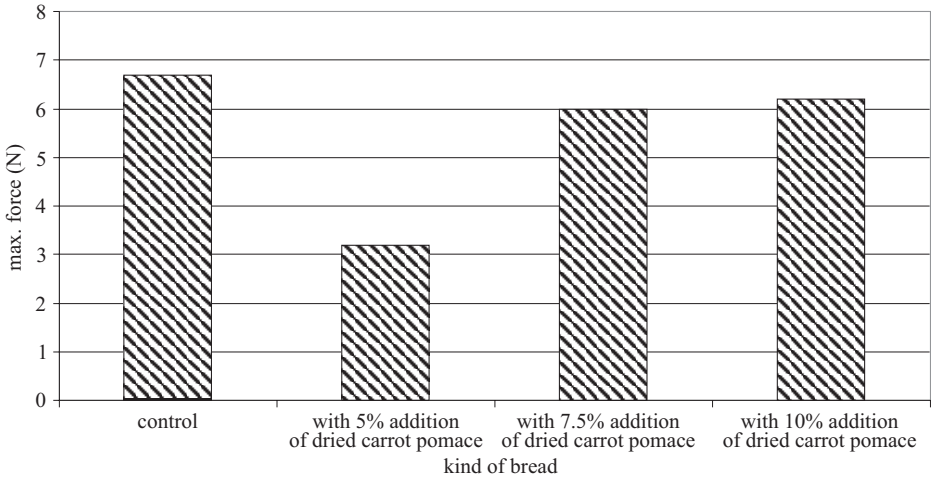


Fig. 6. Maximal force of crumb bread compression

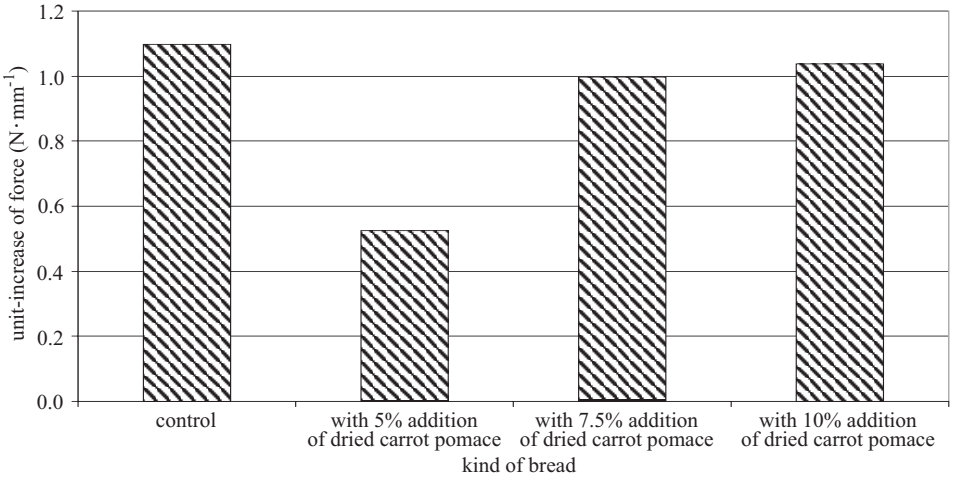


Fig. 7. Elasticity of bread crumb measured as force increase in time of sample deformation

Table 4

Organoleptic characteristic of bread

Sample	Number of points
Control bread	33 ± 2.46^{a}
Bread with 5% addition of dried carrot pomace	38 ± 1.68^{b}
Bread with 7.5% addition of dried carrot pomace	35 ± 1.56^{c}
Bread with 10% addition of dried carrot pomace	32 ± 1.72^{a}

Conclusions

The results of our work indicated that the optimal amount of dried carrot pomace used in the baking mix was 5%. It resulted in an increase in the water absorption of the bread by 8%, and at the same time, the ready-made product was characterized by better elasticity of the bread crumb and 10% larger volume. It was confirmed by it the highest organoleptic quality. Bread with greater (7.5 and 10%) addition of dried carrot pomace is richer in biologically active compounds and less calorific but it worse rheological and organoleptic properties can be a less accepted by consumers.

Acknowledgements

The authors thank mgr Jadwiga Rothkaehl from the Institute of Agricultural and Food Biotechnology in Warsaw for analyses of flour water absorption in farinograph.

Translated by JOANNA JENSEN

Accepted for print 11.12.2006

References

AMBROZIAK Z. 1998. *Technologia piekarstwa*. WSiP, Warszawa.

ABRAMCZYK D. 1997. *Klasyfikacja jakościowa ziarna pszenicy w oparciu o parametry oceny alweograficznej*. Prz. Zboż.-Młyn., 4: 8-12.

AOAC 1990. *Association of the Official Analytical Chemists*, 973.18. Washington, DC.

ASP N.G., JOHANSSON C.G., HELLMER H., SILJESTROM M. 1983. *Rapid enzymatic assay insoluble and soluble dietary fiber*. J. Agric. Food Chem., 31: 476-482.

BIAŁKOWSKA M., SZOSTAK W.B. 2000. *Żywienie w otyłości*. W: *Żywienie człowieka zdrowego i chorego*. Cz. 2. Red. J. HASIK i J. GAWĘCKI. PWN SA, Warszawa, 154-164.

BAO B., CHANG K.C. 1994. *Carrot pulp chemical composition, color, and water-holding capacity as affected by blanching*. J. Food Sci., 59 (6): 1159-1161.

GAMBUŚ H., BOROWIEC F., GAMBUŚ F., ZAJĄC T. 1999a. *Zdrowotne aspekty chleba z dodatkiem nasion lnu oleistego*. Żywność, 4(21): 185-193.

GAMBUŚ H., GAMBUŚ F., BOROWIEC F., ZAJĄC T. 1999b. *Możliwość zastosowania nasion lnu oleistego w piekarstwie*. Zesz. Nauk. AR w Krakowie, 360: 83-94.

GĄSIOROWSKI H. 2000. *Chleb wczoraj, dziś i jutro*. Cz. 3. *Jutro*. Prz. Zboż.-Młyn., 2: 2-6.

HABER T., HORUBAŁOWA A. 1992. *Analiza techniczna w przetwórstwie zbóż*. WSiP, Warszawa.

KONOPKA I., FORNAL Ł., ABRAMCZYK D., ROTHKAEHL J., ROTKIEWICZ D. 2003. *Statistical evaluation of different technological and rheological tests of Polish wheat varieties for bread volume prediction*. Int. J. Food Sci. Techn., 38: 1-10.

KONOPKA I., ZADERNOWSKI R., ROTKIEWICZ D., RASHED A.A. 1999. *Badania nad możliwością poprawy jakości pieczywa pszennego dodatkiem oleju i nasion wiesiołka i ogórecznika*. Zesz. Prob. Post. Nauk Rol., 468: 349-357.

REYES-MORENO C., MILAN-CARRILLO J., ROUAUD-SANDEZ O., GARZON-TIZNADO J., MORA-ESCOBEDO R. 2002. *Dehulling/softening/extrusion (DSE): technological alternative to improve nutritional quality of chickpea*. Agrociencia, 36: 181-189.

RASPER V.F., PICO M.-L., FULCHER R. 1986. *Alveography in quality assessment of soft white winter wheat cultivars*. Cereal Chem., 63: 395-400.

ROTKIEWICZ D., KONOPKA I., TAŃSKA M. 2003. *Comparison of the influence of two forms (oil – and fibre – type) of flaxseeds on selected wheat dough and bread properties*. Pol. J. Natur. Sc., 14 (2): 545-555.

SABIR M.A., SOSULSKI F., CAMPBELL S.J. 1976. *Polymetaphosphate and oxalate extraction on sunflower pectins*. J. Agric. Food Chem., 24 (2): 348-350.

SCHÖGGL G. 1998. *Überprüfung der technologischen Aussagefähigkeit von Alveogrammwerten zur Weizenbeurteilung*. Getreide Mehl und Brot, 52: 218-223.

SKIBNIEWSKA K.A., ZAKRZEWSKI J., BIENIASZEWSKI T. 2003. *Uwalnianie żelaza i manganu z chleba w procesie trawienia in vitro*. Rocz. PZH, 55 (Supl.): 55-60.

SKIBNIEWSKA K.A., FORNAL Ł., KONOPKA I. 2004. *Uwalnianie magnezu, wapnia i fosforu z chleba wypieczonego z dodatkiem mąki pięciu odmian łubinu*. J. Element., 8 (Supl.): 28-29.

ZADERNOWSKI R., BOROWSKA J., KOWALSKA M., BUDREWICZ G., SZAJDEK A. 2003. *Quality of carrot juice conditioned by raw material and technology*. Fruit Proc., 5/6: 183-192.

ZIEMLAŃSKI S. 2000. *Żywienie osób dorosłych z uwzględnieniem zróżnicowanej aktywności fizycznej*. W: *Żywienie człowieka zdrowego i chorego*. Cz. 2. Red. J. HASIK, J. GAWĘCKI. PWN SA, Warszawa, ss. 154-164.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 137-149, Y. 2007

DOI 10.2478/v10020-007-0014-7

THE INFLUENCE OF MERCURY AND CADMIUM ON THE ACTIVITIES OF SOME ENZYMES FROM SIBERIAN STURGEON (*ACIPENSER BAERI* BRANDT 1869) SEMEN

***Beata Sarosiek*[1], *Grzegorz Zdanio*[2], *Radosław Kajetan Kowalski*[1], *Jan Glogowski*[1]**

[1] Institute of Animal Reproduction and Food Research
Polish Academy of Sciences, Olsztyn
[2] Chair of Ichthyology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: acid phosphatase, lactic dehydrogenase, arylsulfatase, β-N-acetylglucosaminidase, spermatozoa, Siberian sturgeon.

A b s t r a c t

The effects of mercury and cadmium ions, at the concentrations of 100, 10 and 1 mg · dm^{-3}, on the activity of enzymes contained in the sperm of Siberian sturgeon were determined. Whole semen was frozen without a cryoprotector. Acid phosphatase (AcP) activity decreased significantly following the application of mercury and cadmium ions at the highest concentration. Lactic dehydrogenase (LDH) was found to be the most sensitive to metal ions of all enzymes examined in the study. The concentration of 10 mg · dm^{-3} almost completely inhibited the activity of this enzyme. It was also found that mercury and cadmium ions slightly stimulated the activity of arylsulfatase (AS). The activity of β-N-acetylglucosaminidase (β-NAG) decreased considerably when mercury ions were added to the reaction mixture, in contrast to cadmium ions.

The effects of Hg and Cd ions on the affinity of AcP and AS for substrates were also determined. It was found that these metal ions decreased the affinity of the above enzymes for p-nitrophenylphosphate and p-nitrocatechol sulfate respectively.

Address: Beata Sarosiek, Polish Academy of Sciences, ul. Bydgoska1/8, 10-243 Olsztyn, Poland, pirosia@pan.olsztyn.pl

WPŁYW JONÓW RTĘCI I KADMU NA AKTYWNOŚĆ WYBRANYCH ENZYMÓW W NASIENIU JESIOTRA SYBERYJSKIEGO (*ACIPENSER BAERI* BRANDT 1869)

***Beata Sarosiek*[1], *Grzegorz Zdanio*[2], *Radosław Kajetan Kowalski*[1], *Jan Glogowski*[1]**
[1] Instytut Rozrodu Zwierząt i Badań nad Żywnością
Polska Akademia Nauk, Olsztyn
[2] Katedra Ichtiologii
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: kwaśna fosfataza, dehydrogenaza mleczanowa, arylosulfataza, β-N-acetyloglukozoaminidaza, plemniki, jesiotr syberyjski.

A b s t r a k t

W pracy określono wpływ jonów rtęci i kadmu o stężeniach 100, 10 i 1 mg · dm^{-3} na aktywność enzymów obecnych w nasieniu jesiotra syberyjskiego. Pełne nasienie zamrożono bez dodatku krioprotektora. Aktywność fosfatazy kwaśnej (AcP) statystycznie istotnie malała jedynie przy najwyższym zastosowanym stężeniu jonów rtęci i kadmu. Bardziej wrażliwa na obecność metali ciężkich okazała się dehydrogenaza mleczanowa (LDH). Rtęć o stężeniu 10 mg · dm^{-3} powodowała prawie całkowite zahamowanie aktywności tego enzymu. Wykazano także, że wymienione metale powodują nieznaczne (statystycznie nieistotne) podwyższenie aktywności arylosulfatazy (AS). Jony rtęci znacząco obniżały aktywność β-N-acetyloglukozoaminidazy (β-NAG), natomiast jony kadmu nie wykazywały hamujacego wpływu na aktywność tego enzymu.

Określono również wpływ jonów rtęci i kadmu na powinowactwo fosfatazy kwaśnej i arylosulfatazy do substratu. Stwierdzono, że jony metali ciężkich zmniejszają powinowactwo wymienionych enzymów odpowiednio do *p*-nitrofenylofosforanu i *p*-nitrokatecholu.

Introduction

Acipenserids (*Acipenseridae*) belong to the oldest living vertebrates. They do not spawn every year – females lay spawn once in 2-11 years, and males have their spawning season every 1-6 years (BEMIS, KYNARD 1997). That is why all acipenserid species are threatened with extinction (KOLMAN 1999), and studies on the biology of their gametes and fertilization are of greater and greater significance. Sexual reproduction of living organisms depends on the production of high-quality semen. Sperm damage caused by pollutants found in the aquatic environment and cryopreservation may result in tail shortening and head deformation, thus affecting the fertilization ability of spermatozoa (VAN LOOK, KIME 2003).

The spermatozoa of acipenserid species differ considerably from the spermatozoa of the teleosts. These differences concern sperm morphology (the presence of the acrosome), physiology (long-term motility and acrosomal reaction) and biochemistry (the presence of acrosine, arylsulfatase, GLOGOWSKI et al. 2002). The enzymology of fish gametes has been poorly investigated, as compared to the mammals. Professional literature provides

only fragmentary information on the biochemical parameters of the sperm acrosome in the sturgeon, and focuses on acrosine-like activity (CIERESZKO et al. 1994, 1996, 2000).

The studies on the effects of heavy metal pollution on gamete quality, conducted so far, have been based exclusively on sperm motion analysis (RURANGWA et al. 1998, VAN LOOK, KIME 2003). However, damage to the motor apparatus of the sperm is not necessarily accompanied by damage to the structures responsible for gamete fusion. It follows that sperm motility analysis should be supplemented by the determination of the pollutant effect on acrosomal integrity and enzymatic activity. Mercury affects the viability and motility of spermatozoa, as well as the final stages of reproduction, since it reduces the efficiency of fertilization (KHAN, WEIS 1987a,b) and hatching (HEISINGER, GREEN 1975). Fish show different sensitivity to mercury, and the physiological mechanism underlying the effects of mercury on spermatozoa arouses controversy (RURANGWA et al. 1998). Most fish respond by reproduction disorders to mercury concentration of about 10 $\mu g \cdot dm^{-3}$, but in sensitive species (e.g. trout) such a response is observed at lower concentrations, i.e. 0.2 to 0.7 $\mu g \cdot dm^{-3}$ (WEIS, WEIS 1991). Cadmium also disturbs the reproduction process in fish. This element reduces sperm viability and fertilization ability (LAHNSTEINER et al. 2004), disturbs spermatogenesis and seasonal cycles at the androgen level, and causes testicle degeneration (KIME 1999, MOUSA, MOUSA 1999).

Materials and Methods

Fish, semen collection and preparation of seminal plasma and supernatants Siberian sturgeon (age 7+) were maintained in Dolna Odra power plant (Department of General Zoology, Szczecin University, Poland). Siberian sturgeons ($n = 5$) were stimulated hormonally in April. All males were injected with 1 pellet of Ovopel kg^{-1} of fish weight (Interfish Ltd, Hungary; HORVATH et al. 1997), containing of 18-20 μg of GnRH analog and 8-10 mg of metoclopramide. Spermiation occurred within 24 h after hormonal stimulation and milt was collected using a syringe with attached rigid tubing inserted into urogenital opening.

Before being stripped fishes were anesthetized in a 1 ml per 10 l water solution of clove oil (Aetheroleum cariophylli, Fuzio-Pharma Ltd. Szekszard, Hungary). The belly of anesthetized fishes was wiped dry and semen was collected into dry syringes attached to the genital aperture of the fishes by a gentle pressure on the abdomen. Samples contaminated with excrement or urine were excluded from further work.

Milt of Siberian sturgeons was stored on ice (+4°C) for 8 hours. Milt was frozen at -79°C and was design to evaluate an extent of leakage of enzymes after damage to sperm, to determined the total enzymatic activity observed in spermatozoa and seminal plasma together. Milt was thawed at room temperature, centrifuged (10 min, 8000 x g) and supernatant obtained was used for biochemical analysis.

Analytical procedures

Acid phosphatase activity was measured using 5 mM p-nitrophenylphosphate (disodium salt) in 20 mM citrate buffer (GLOGOWSKI et al. 1996). After 30 min incubation at 37°C, the reaction was stopped with 0.1 M NaOH and absorbance at 410 nm was measured. LDH activity was measured with UV-method with pyruvate and NADH (VASSAULT 1983). The reaction mixture consisted of 1.6 mM sodium pyruvate and 0.2 mM NADH in 80 mM Tris-HCl buffer containing 200 mM NaCl. A decrease of absorbance was measured at 339 nm (30°C, 5 min). Activity of arylsulfatase was measured using *p*-nitrocatechol sulphate as a substrate (20 mM) in 0.5 M sodium acetate, pH 4.9 (GADELLA et al. 1992). After 30 min of incubation at 37°C, the reaction was stopped with 1 M NaOH and absorbance at 515 nm was measured. Activity of β-N-acetylglucosaminidase was measured using 0.5 mM *p*-nitrophenyl β-N--glucosaminide as a substrate in 0.1 M citrate buffer, pH 5.0 (JAUHIAINEN, VANHA-PERTTULA 1986). After 60 min of incubation at 37°C, the reaction was stopped with 0.1 M NaOH and absorbance at 410 nm was measured. Protein was measured by the LOWRY method (1951).

We checked the influence of heavy metals using Hg^{2+} and Cd^{2+} dissolved in distilled water, at 1, 10, 100 $mg \cdot dm^{-3}$ final concentration in the incubation mixture, which corresponded with 5-500 μM of Hg and 8.9-890 μM of Cd ions. These heavy metals concentrations were used to obtain significant effect on the milt supernatants enzymatic activities determined after 0, 4, 24 hours incubation at +4°C.

Statistical analysis

Data were analyzed using GraphPad PRISM package. Significance of differences were inferred at $P < 0.05$.

Results

Acid phosphatase activity

The effects of mercury ions on the activity of acid phosphatase are presented in Figure 1a. Only the highest concentration of this metal (100 mg · dm^{-3}) caused a significant decrease in the activity of this enzyme within all exposure time limits analyzed. A similar tendency was observed in the case of cadmium, whose highest concentration substantially decreased acid phosphatase activity (Figure 1b).

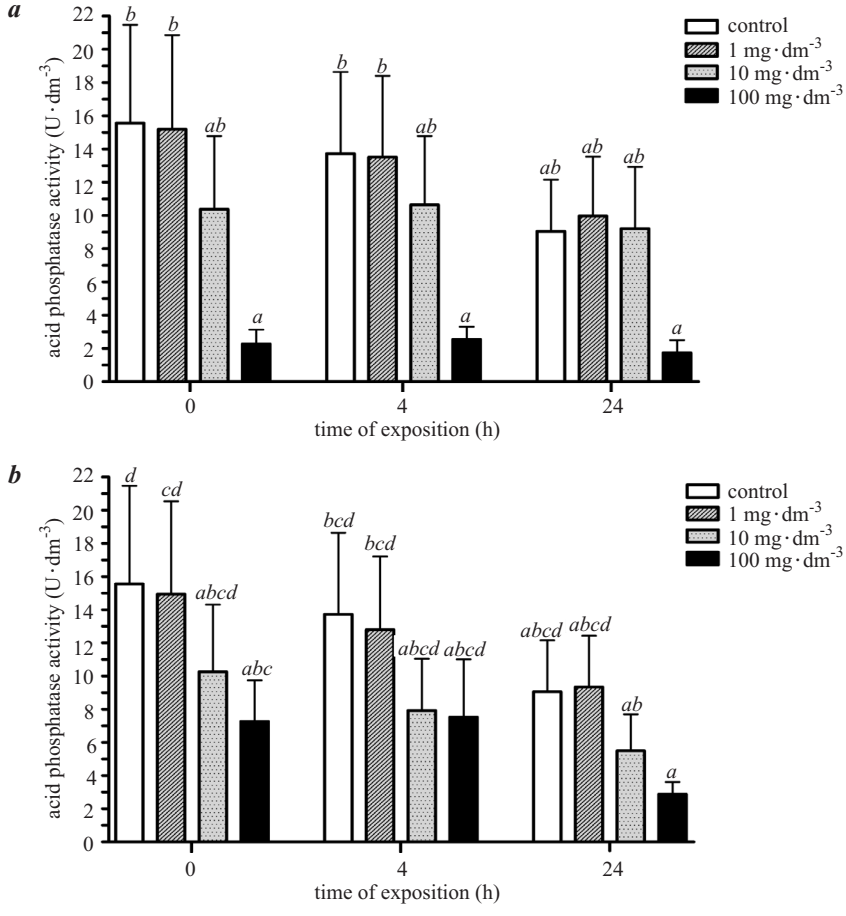


Fig. 1. The influence of mercury (*a*) and cadmium (*b*) ions on the acid phosphatase activity. Vertical bars are standard errors (S.E.). The value having different letters are significantly different ($p < 0.05$)

LDH activity

The addition of mercury ions to the reaction mixture, at a concentration of 2.5 mg · dm^{-3}, caused a 25% decrease in the activity of lactic dehydrogenase (Figure 2a). The highest decrease in LDH activity was recorded at a concentration of 10 Hg^{2+}mg · dm^{-3}, when LDH activity accounted for only 0.5% of the initial activity. After 24-hour incubation the activity of this enzyme decreased by 44% and 50% in the control sample and in the sample containing 1 mg Hg^{2+} · dm^{-3} respectively. The highest concentration of mercury resulted in complete inactivation of LDH. Cadmium did not cause such drastic changes in

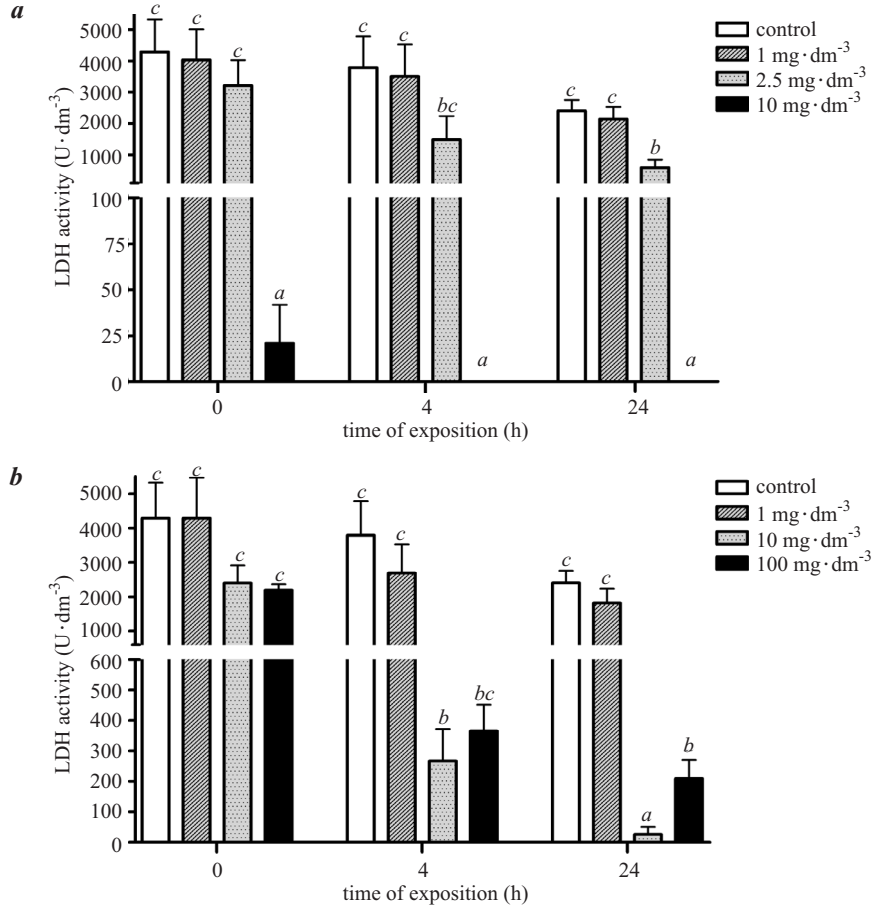


Fig. 2. The influence of mercury (*a*) and cadmium (*b*) ions on the lactic dehydrogenase activity. Vertical bars are standard errors (S.E.). The value having different letters are significantly different ($p < 0.05$)

LDH activity. None of the concentrations applied caused a statistically significant decrease in the activity of this enzyme, determined immediately after adding the metal to the reaction mixture (Figure 2b). Changes in LDH activity were observed after 4-hour incubation, and were the most noticeable at a concentration of 10 and 100 Cd^{2+} $mg \cdot dm^{-3}$.

Arylsulfatase activity

No decrease in arylsulfatase activity was recorded at the lowest concentration of mercury ions – 1 $mg \cdot dm^{-3}$. The level of this activity was the same as in the control sample (Figure 3a). Mercury ions applied at a concentration of

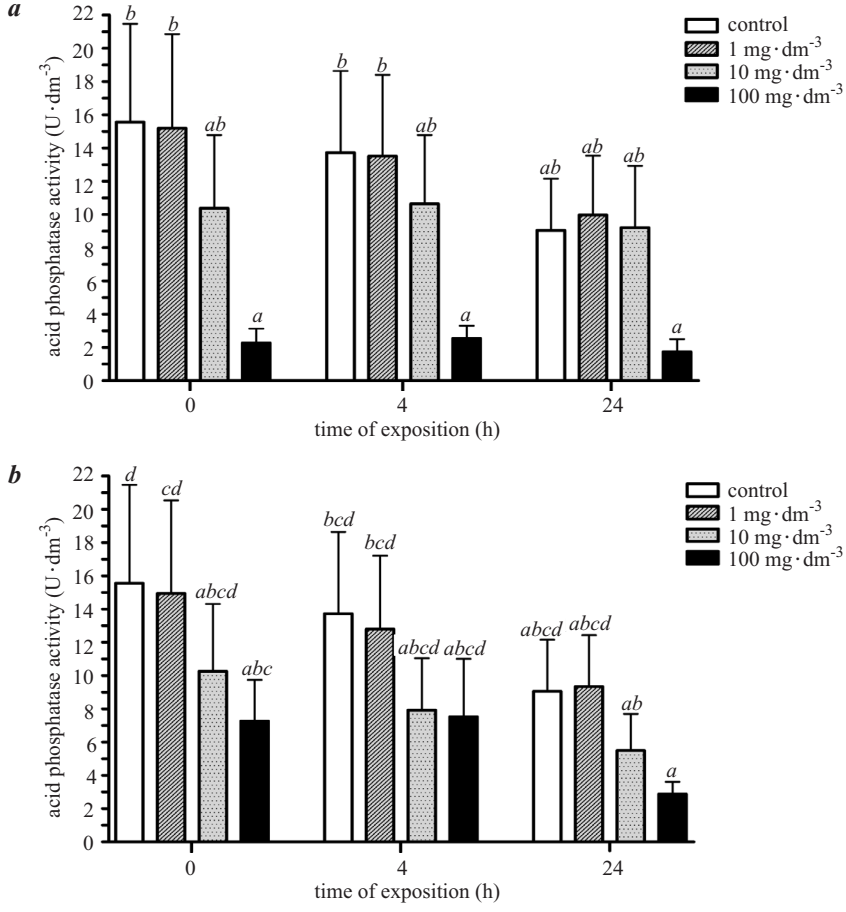


Fig. 3. The influence of mercury (*a*) and cadmium (*b*) ions on the arylsulfatase activity. Vertical bars are standard errors (S.E.). The value having different letters are significantly different ($p < 0.05$)

10 and 100 mg · dm^{-3} caused a distinct and statistically significant decrease in arylsulfatase activity. At 100 mg Hg^{2+} · dm^{-3} the activity of this enzyme decreased by 59%, compared with the initial activity. After four hours of exposure these relationships did not change, whereas 24-hour incubation resulted in a slight increase in arylsulfatase activity in the control sample and in the sample containing 1 mg Hg^{2+} · dm^{-3}, even in comparison with activity measured at zero time (Figure 3a). As regards the effects of cadmium ions on arylsulfatase activity in the sperm extract of Siberian sturgeon, a slight decrease in this activity was recorded in the control sample after four hours of incubation. After 24-hour incubation the activity of arylsulfatase increased in all samples (Figure 3b).

β-N- acetylglucosaminidase activity

The activity of β-N-acetylglucosaminidase did not decrease after the addition of 1 mg Hg^{2+} · dm^{-3} (Figure 4a). Mercury ions at a concentration of 10 mg · dm^{-3} caused a 60% decrease in the activity of this enzyme, but this difference was statistically non-significant. At 100 mg Hg^{2+} · dm^{-3} β-N-acetylglucosaminidase activity decreased 80-fold. Similar tendencies were observed after 4 and 24 hours of incubation. Cadmium, in contrast to mercury, did not show an inhibitory effect on the activity of β-N- acetylglucosaminidase (Figure 4b).

Effects of mercury and cadmium ions on enzyme affinity for substrates

The effects of mercury and cadmium ions on enzyme affinity for substrates were determined for acid phosphatase and arylsulfatase, at the concentrations of both metals sufficient to inhibit enzymatic activity in the 30-70% range. In the case of acid phosphatase these concentrations were 100 mg Cd^{2+} · dm^{-3} and 10 mg Hg^{2+} · dm^{-3}. As for arylsulfatase, only the influence of mercury ions, applied at 100 mg · dm^{-3}, was estimated, since cadmium ions had no inhibitory effect on the activity of this enzyme.

The affinity of acid phosphatase for *p*-nitrophenylphosphate was determined at $K_m = 1.21 \cdot 10^{-3}$M in a sample that contained no metal ions, and at $K_m = 1.72 \cdot 10^{-3}$ M in the presence of 10 mg Hg^{2+} · dm^{-3}. The value of the K_m, calculated for acid phosphatase in the presence of cadmium ions (100 mg · dm^{-3}), was $2.21 \cdot 10^{-3}$ M. These results show that acid phosphatase affinity for the substrate decreased in the presence of metal ions. It was demonstrated that

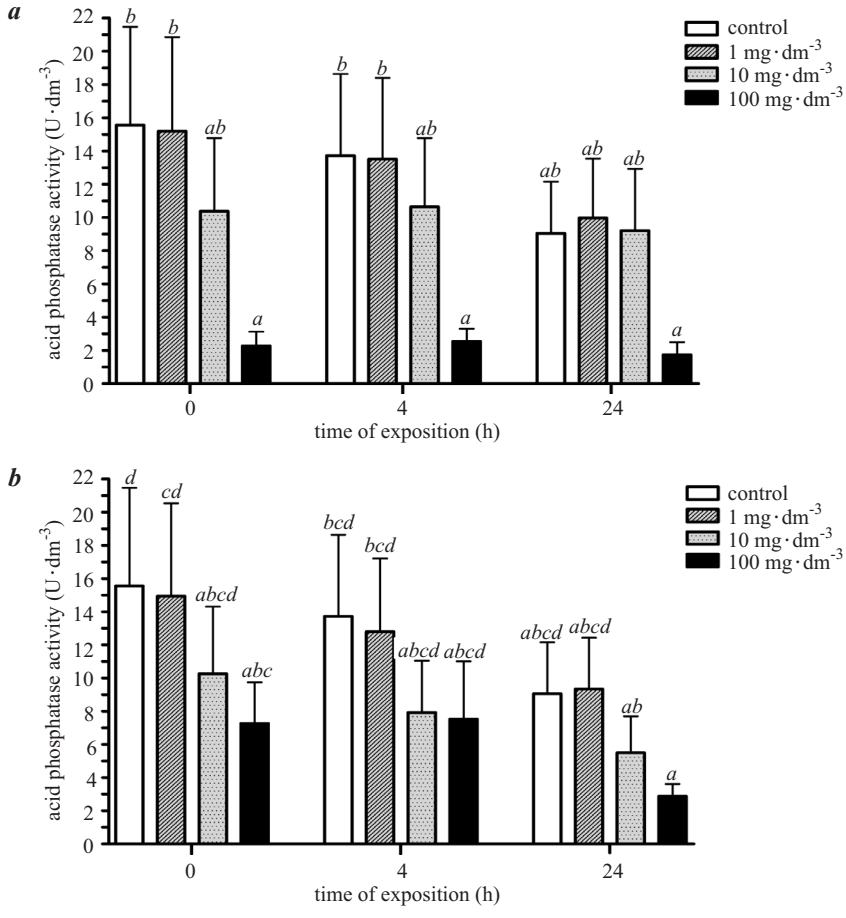


Fig. 4. The influence of mercury (*a*) and cadmium (*b*) ions on the β-N-acetylglucosaminidase activity. Vertical bars are standard errors (S.E.). The value having different letters are significantly different ($p < 0.05$)

mercury ions at a concentration of 100 mg · dm^{-3} caused a decrease in the affinity of arylsulfatase to the substrate, p-nitrocatechol sulfate, by nearly 30%, at $K_m = 6.8 \cdot 10^{-4}$ M without the metal, and at $K_m = 8.7 \cdot 10^{-4}$ M in the presence of mercury.

Discussion

In the present study we determined the enzymatic activity of acid phosphatase, lactic dehydrogenase, arylsulfatase and β-N-acetylglucosaminidase

obtained from the semen of Siberian sturgeon, subjected to short-term exposure to mercury and cadmium ions at three concentrations. The results were compared with the control sample, in which – similarly as in the experimental samples – we tried to achieve the maximum enzymatic activity by means of freezing and thawing whole semen without a cryoprotector, to liberate the enzymes contained in spermatozoa. This enabled to measure the total enzymatic activity as a sum of activities observed in the seminal plasma and spermatozoa.

LAHNSTEINER et al. (2004) observed that heavy metals had different influence on fish spermatozoa motility, for ex. *Salmo trutta fario* spermatozoa motility was highly inhibited by Hg ions (0.5 $mg \cdot dm^{-3}$) but effective cadmium doses was 25 $mg \cdot dm^{-3}$. In the other hand, *Leuciscus cephalus* spermatozoa motility was inhibited by 50 $mg \cdot dm^{-3}$ Hg and 2.5 $mg \cdot dm^{-3}$ Cd ions. So the heavy metals doses depended on the chosen fish species and analyzed parameters (used cadmium concentration was not effective to decrease the arylsulfatase activity in this paper).

The mechanism of the negative effect of mercury and cadmium on acid phosphatase has not been investigated in detail yet. According to JACKIM et al. (1970), the toxic effect of metals on enzymes consists in metal ion displacement from the active center. The influence of mercury on the activity of phosphatases and other enzymes in fish was studied by GILL et al. (1990). The results of in vivo examinations showed a decrease in AcP activity in all tissues except for the testicles. These authors also demonstrated that cadmium reduced the activity of this enzyme in all tissues except for the ovaries.

In this study an attempt was made, for the first time, to determine in vitro the effects of heavy metals on the activity of lactic dehydrogenase from the semen of Siberian sturgeon. Lactic dehydrogenase was found to be the most sensitive to mercury ions of all enzymes examined in the study, so this metal was applied at lower concentrations, to observe the decrease in enzymatic activity, and not only its complete inhibition.

RURANGWA et al. (2002) studied the effect of tributyltin (TBT) on the metabolism and sperm motility in African catfish and carp. Among six enzymes analyzed, only the activity of LDH in catfish semen responded significantly to treatment with 27 mg TBT $\cdot dm^{-3}$. The decrease in LDH activity was about 75% in the catfish and 41% in the carp, which indicated considerable interspecific differences. TBT was used as a model toxicant because it substantially reduced the amount of semen and sperm motility at concentrations naturally found in the environment (HAUBRUGE et al. 2000, KIME et al. 2001), and because it is still present in aquatic ecosystems, just like heavy metals. However, the molecular mechanism of the inhibition of LDH activity remains unknown (RURANGWA et al. 2002). SCHEMEL et al. (1987) used lactic dehydrogenase as an indirect indicator of sperm damage in the trout. Also PIROS et al. (2002) suggested the

possibility of applying LDH activity as a marker of plasma membrane damage in the semen of Siberian sturgeon and sterlet.

The studies on the effects of heavy metal ions on arylsulfatase activity, presented in this paper, seem to be of pioneer character, since professional literature provides no information on that topic. The results of arylsulfatase treatment with cadmium ions are an untypical manifestation of the impact of heavy metals. The presence of cadmium in the sample caused a slight, but statistically significant, increase in the activity of this enzyme as the time of incubation was prolonged. Heavy metal ions generally bind to the thiol groups in proteins. For instance, silver ions not only block the SH groups of the active center of the enzyme, but at higher concentrations they cause irreversible denaturation of the protein component, or change its conformation. Arylsulfatase, as an acrosomal enzyme, may significantly contribute to sperm-zona pellucida binding (SAROSIEK et al. 2004), and in this way to successful egg fertilization. It follows that studies on environmental pollutants, such as heavy metals, should be continued in order to thoroughly analyze their potentially negative effects on fertilization success.

Studies on the inhibition of the activity of β-N-acetylglucosaminidase usually focus on the effects of organic inhibitors, like N-acetylglucosaminolactone (JOYCE et al. 1986), thiol compounds (JAUHIAINEN, VANHA-PERTTULA 1986), N-acetylgalactosamine, N-acetylglucosamine (MARTINEZ et al. 2000). The results of these studies show that the inhibition of β-N-acetylglucosaminidase activity prevented gamete fusion, which indicates that this enzyme is crucial for fertilization success. The effects of metal ions on β-N-acetylglucosaminidase were analyzed by CALVO et al. (1978). These authors demonstrated that iron and mercury ions inhibit the activity of this enzyme. Similar results were obtained by SHIGETA et al. (1982), who treated the enzyme with ions of silver, iron and mercury. Also FAROOQUI, SRIVASTAVA (1980) and CHEN et al. (2004) proved the inhibitory effect of Ag^{+} and Hg^{2+} ions on β-N-acetylglucosaminidase. In our study it was confirmed that mercury ions are strong inhibitors of the enzyme, whereas cadmium ions cause a slight decrease in the activity of β-N-acetylglucosaminidase, but their effect increases in the case of prolonged exposure. We used cadmium chloride and mercury chloride as a source of metal ions, while in the natural environment mercury occurs primarily in the methyled form, whose toxicity is much higher (VAN LOOK, KIME 2003), so further investigations on this problem seem justified.

The knowledge about negative factors which might disturb the sturgeons fertilization may be useful in regard of endangered status of some genus from this order (BEAMESDERFER, FARR 1997). Herein we shown that heavy metal ions can disrupt biochemical activity of some enzymes from Siberian sturgeon milt. Our results indicate that monitoring of heavy metal ions water contamination should be conducted together with restitution process of that fish.

Acknowledgments

This study was supported by the State Committee for Scientific Research (KBN) as a solicited project No. PBZ-KBN-084/P06/2002/5.8 in years 2003-2005.

R. Kowalski was awarded the Domestic Grant for Young Scientists from the Foundation for Polish Sciences.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 21.11.2006

References

BEAMESDERFER R. C. P., FARR R. A. 1997. *Alternatives for the protection and restoration of sturgeons and their habitat.* Environ. Biol. Fish, 48:407-417.

BEMIS W. E., KYNARD B. 1997. *Sturgeon rivers: an introduction to acipenseriform biogeography and life history*. Environ. Biol. Fishes., 48: 167-183.

CALVO P., REGLETO A., CABEZAS J. A., 1978. *Purification and properties of beta-N -acetylhexosaminidase from the mollusca Helicella ericetorum Muller*. Biochem. J., 175(2): 743-750.

CHEN Y., LIU W., HSU H., LEE Y., CHEN C. 2004. *Purification and characterization of isoforms of β-N-acetylhexosaminidase from mungbean seedlings.* Bot. Bull. Acad. Sin., 45: 275-283.

CIERESZKO A., DĄBROWSKI K., LIN F., DOROSHOV S. I. 1994. *Identification of trypsin-like activity in sturgeon spermatozoa.* J. Exp. Zool., 268: 486-491.

CIERESZKO A., DĄBROWSKI K., OCHKUR S. I. 1996. *Characterization of acrosin-like activity of lake sturgeon (Acipenser fulvescens) spermatozoa*. Mol. Reprod. Dev., 45: 72-77.

CIERESZKO A., DĄBROWSKI K., MIMS S. D., GLOGOWSKI J. 2000. *Characteristics of sperm acrosin-like activity of paddlefish (Polyodon spathula Walbaum).* Comp. Biochem. Physiol., B 125: 197-203.

FAROOQUI A. A., SRIVASTAVA P. N. 1980. *Isolation of beta-N-acetylhexosaminidase from rabbit semen and its role in fertilization*. Biochem. J., 191(3): 827-834.

GADELLA B. M., COLENBRANDER B., VAN GOLDE L. M., LOPES-CARDOZO M. 1992. *Characterization of three arylsulfatases in semen: seminolipid sulfohydrolase activity is present in seminal plasma.* Biochim. Biophys. Act., 1128: 155-162.

GILL T. S., TEWARI H., PANDE J. 1990. *Use of the fish enzyme system in monitoring water quality: effects of mercury on tissue enzymes*. Comp. Biochem. Physiol., C 97: 287-292.

GLOGOWSKI J., BABIAK I., GORYCZKO K., DOBOSZ S., 1996. *Activity of aspartate aminotransferase and acid phosphatase in cryopreserved trout sperm.* Reprod. Fertil. Dev., 8: 1179-1184.

GLOGOWSKI J., KOLMAN R., SZCZEPKOWSKI M., HORVATH A., URBANYI B., SIECZYNSKI P., KRZEMIENIECKI A., DOMAGALA J., DEMIANOWICZ W., KOWALSKI R., CIERESZKO A. 2002. *Fertilization rate of Siberian sturgeon (Acipenser baeri Brandt) milt cryopreserved with methanol.* Aquaculture, 211: 367-373.

HAUBRUGE E., PETIT F., GAGE M. 2000. *Reduced sperm counts in guppies (Poecilia reticulata) following exposure to low levels of tributyltin and bisphenol A.* Proc. R. Biol., B 267: 2333-2337.

HEISINGER J. F., GREEN W. 1975. *Mercuric chloride uptake by eggs of the ricefish and resulting teratogenic effects*. Bull. Environ. Contam. Toxicol., 14: 665-673.

HORVATH L., SZABO T., BURKE J. 1997. *Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species*. Pol. Arch. Hydrobiol., 44: 221-226.

JACKIM E., HAMLIN J. M., SONIS S. 1970. *Effects of metal poisoning on five enzymes in the killifish (Fundulus heteroclitus).* J. Fish Res. Bd. Can., 27: 383-390.

JAUHIAINEN A., VANHA-PERTTULA T., 1986. *Beta-N-acetylglucosaminidase in the reproductive organs and seminal plasma of the bull*. J. Reprod. Fertil., 76(1): 239-250.

JOYCE C. L., MACK S. R., ANDERSON R. A., ZANEVELD L. J. 1986. *Effect of hyaluronidase, beta-glucuronidase and beta-N-acetylglucosaminidase inhibitors on sperm penetration of the mouse oocyte.* Biol. Reprod., 35(2): 336-346.

KHAN A. T., WEIS J. S. 1987a. *Toxic effects mercuric chloride on sperm and egg viability of two populations of mummichog (Fundulus heteroclitus).* Environ. Pollut., 48: 263-273.

KHAN A. T., WEIS J. S., 1987b. *Effects of methymercury on sperm and egg viability of two populations of killifish (Fundullus heteroclitus).* Arch. Environ. Contam. Toxicol., 16: 499-505.

KIME D. E. 1999. *A strategy for assessing the effects of xenobiotics on fish reproduction.* Sci. Total. Environ., 225: 3-11.

KIME D. E., VAN LOOK K. J. W., MCALLISTER B. G., HUYSKENS G., RURANGWA E., OLLEVIER F. 2001. *Computer assisted sperm analysis (CASA) as a tool for monitoring sperm quality in fish.* Comp. Biochem. Physiol., 130: 425-433.

KOLMAN R. 1999. *Jesiotry*. WIRŚ, Olsztyn, ss. 136.

LAHNSTEINER F., MANSOUR N., BERGER B. 2004. *The effect of inorganic and organic pollutants on sperm motility of aome freshwater teleosts*. J. Fish Biol., 65: 1283-1297.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J. 1951. *Protein measurement with the Folin phenol reagent*. J. Biol. Chem., 193: 265-275.

MARTINEZ M. L., MARTELOTTO L., CABADA M. O. 2000. *Purification and biological charactrization of N-acetyl-β*-D-glucosaminidase from Bufo arenarum spermatozoa. Mol. Reprod. Dev., 57: 194-203.

MOUSA S. A., MOUSA M. A., 1999. *Immunocytochemical and histological studies on the hypophyseal gonadal system in the freshwater Nile tilapia, Oreochromis niloticus (L.), during sexual maturation and spawning in different habitats*. J. Exp. Zool., 284: 343-354.

PIROS B., GLOGOWSKI J., KOLMAN R., KRZEMIENIECKI A., DOMAGALA J., HORVATH A., URBANYI B., CIERESZKO A. 2002. *Biochemical characterization of Siberian sturgeon Acipenser baeri and sterlet Acipenser ruthenus milt plasma and spermatozoa.* Fish Physiol. Biochem., 26: 289-295.

RURANGWA E., ROELANT I., HUYSKENS G., EBRAHIMI M., KIME D. E., OLLEVIER F., 1998. *The minimum effective spermatozoa: egg ratio for artificial insemination and the effects of mercury on sperm motility and fertilization ability in Clarias gariepinus*. J. Fish Biol., 53: 402-413.

RURANGWA E., BIEGNIEWSKA A., SLOMINSKA E., SKOKOWSKI E. F., OLLEVIER F. 2002. *Effect of tributyltin on adenylate content and enzyme activities of teleost sperm: a biochemical approach to study the mechanisms of toxicant reduced spermatozoa motility*. Comp. Biochem. Phys., C 131: 335-344.

SAROSIEK B., CIERESZKO A., KOLMAN R., GLOGOWSKI J. 2004. *Characteristics of arylsulfatase in Russian sturgeon (Acipenser gueldenstaedti) semen*. Comp. Biochem. Physiol., 139 (B): 571-579.

SCHEMEL M. K., GRAHAM E. F., ERDAHL D. A. 1987. *Chemical constituents of trout seminal plasma after minimal and maximal cell damage treatments with possible application to semen evaluation assays.* Aquaculture, 62: 311-318.

SHIGETA S., MATSUDA A., OKA S., 1982. *Purification and characterization of beta-N-acetylhexosaminidase of sea-squirt*. J. Biochem., 92(1): 163-172.

VAN LOOK K. J. W., KIME D. E., 2003. *Automated sperm morphology analysis in fishes: the effect of mercury on goldfish sperm*. J. Fish Biol., 63: 1020-1033.

VASSAULT A., 1983. *Lactate dehydrogenase. UV-method with pyruvate and NADH*. In: *Methods in Enzymatic Analysis*. Ed. by BERGMEYER H. U., Verlag Chemie, Weinheim, 3: 118-126.

WEIS P., WEIS J. 1991. *The development toxicity of metals and metalloids on fish*. In: *Metal ecotoxicology, concepts and applications*. Eds. M. C. NEWMAN A. W. MCINTOSH, Lewis Publ., Chelsea, Mich., pp. 145-169.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol. 22(1): 150-156, Y. 2007

DOI 10.2478/v10020-007-0015-6

THE INFLUENCE OF THE INVASION OF *NOSEMA APIS* ON THE NUMBER OF POLLEN SEEDS IN BEES' INTESTINES

***Rajmund Sokół*[1], *Dorota Molska*[1], *Maciej Siuda*[2]**

[1] Chair of Infectious and Invasive Diseases
[2] Chair of Apiculture
University of Warmia and Mazury in Olsztyn

K e y w o r d s: bees, *Nosema apis*, nosemosis, pollen.

A b s t r a c t

The dependence between the number of pollen seeds in the bee's intestine and the intensity of the parasite's invasion has been studied. Bees for the examination were collected in June, July and August. Group I was formed from young bees taken from combs with unsealed brood, group II – from hive bees taken from honeycombs with sealed brood, and group III – from foraging bees returning to a hive. The results of the examination of the bees indicate some dependence between the invasion of *Nosema apis* and the number of pollen seeds in their intestines. The hive bees suffering from the invasion of *Nosema apis* have had a larger amount of pollen seeds in their intestines than the bees free from nosemosis, because a larger amount of protein food is necessary for their organisms to function properly due to the damage to their intestine cells. The greatest demand for protein has been noticed for the bees of group II, both for free from invasion ones and for the ones infected by the bees' apicomplexon. The protein demand for the flying bees (group III) has been slightly less than for the hive bees. The largest amount of pollen seeds has been found in the testiness of the bees suffering tom the invasion of *Nosema apis* in June and July i.e. in the time of the intensive brood raising.

WPŁYW INWAZJI *NOSEMA APIS* NA LICZBĘ ZIAREN PYŁKU W JELICIE PSZCZÓŁ

***Rajmund Sokół*[1], *Dorota Molska*[1], *Maciej Siuda*[2]**

[1] Katedra Chorób Zakaźnych i Inwazyjnych
[2] Katedra Pszczelnictwa
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: pszczoły, *Nosema apis*, nosemoza, pyłek kwiatowy.

Address: Rajmund Sokół, University of Warmia and Mazury, Michała Oczapowskiego 13, 10-718 Olsztyn, phone: 48 (089) 523 47 63, e-mail: sokol@uwm.edu.pl

Abstrakt

Badano zależność liczby ziaren pyłku w jelicie pszczoły robotnicy od intensywności inwazji pasożyta. Robotnice do badań pobierano w czerwcu, lipcu i sierpniu. Grupę I stanowiły pszczoły młode, pobrane z plastrów z czerwiem otwartym, grupę II – pszczoły ulowe, pobrane z plastrów z czerwiem krytym, a grupę III – pszczoły powracające do ula, pozyskane z wylotka. Wyniki badań wskazują na pewną zależność inwazji *Nosema apis* od liczby ziaren pyłku w przewodzie pokarmowym. Pszczoły ulowe, dotknięte inwazją *Nosema apis*, miały w swoim jelicie znacznie większą liczbę ziaren pyłku niż wolne od nosemozy. Do prawidłowego funkcjonowania organizmu z powodu uszkodzeń komórek jelita potrzebowały większej ilości pokarmu białkowego. Największe zapotrzebowanie na białko występowało u robotnic z grupy II, i to zarówno u wolnych od inwazji, jak i zarażonych sporowcem pszczelim. U pszczół lotnych (grupa III) zapotrzebowanie na białko było nieznacznie mniejsze niż u pszczół ulowych. Największą liczbę ziaren pyłku stwierdzono w jelicie pszczół dotkniętych inwazją *Nosema apis* w czerwcu i lipcu, tj. w okresie intensywnego wychowu czerwiu.

Introduction

Nosemosis is a contagious and invasive bees' disease with chronic course that intensifies in spring and often leads to a significant weakening of the bees' family, or even to its dying out. The course and dissemination of the disease in an apiary is influenced by numerous factors, including: the climate, bad condition of wintered bees, their age, the amount of insects in a bee's colony, the quality and condition of their winter reserves, and disturbing the bees during the wintering (GLIŃSKI, RZEDZICKI 1993, SOKÓŁ, MOLSKA 2006).

The disease is triggered by a parasite – a protozoon *Nosema apis Zander*. The epithelium of the middle intestine is its habitat, or more seldom – the cells of the excretory tubules (HARTWIG 1970). The infection is transmitted by food when consuming the spores in the honey, water and faeces. In the bee's intestine, on transmission of the spore's content to the epithelium cell, the breeding process of the parasite starts, which entails numerous cell divisions leading to the breakdown of the cell. The damage to the intestine and its dysfunction is reflected in the bee's metabolism, including: changes in the composition of the hemolymph (it is poorer in amino acids, complete proteins and lipids), disturbances in proportions between saturated and unsaturated fatty acids, and also a number of building cells of the fatty body is decreased. The protozoon's toxins damage also the cells of the pharynx glands, and thus the feeder bees produce too little milk indispensable for keeping the mother in good condition, as well as for feeding the young brood. Also, a worker bees' metabolism is disturbed, which manifests in decreased intake of liquid and oxygen and increased demand for the bee bread and pollen. The infected bees live from 10 to 27 days shorter (HARTWIG, TOPOLSKA 2001, KRZYŻAŃSKA 2001).

Taking the destructive influence of *Nosema apis* on the epithelium of the bee's middle intestine and its demand for the protein food into consideration, it has been decided to estimate the dependence between the intensity of the invasion and the amount of the pollen seeds in the bee's intestine.

Material and Methods

The research has been carried out within 29 families, in which numerous spores of *Nosema apis* were spotted in the winter dust in March 2006. In the mid-June, mid-July and mid-August, out of each of those families circa 100 bees were taken: group I was made of young bees taken from the combs with unsealed brood, group II – hive bees from the combs with honey and sealed brood, and group III – foraging bees returning to the hive. On their putting down, they have been examined in a laboratory for the presence of *Nosema apis* spores using the method described by Hartwig (HARTWIG, TOPOLSKA 1995). Additionally, pollen seeds have been counted in 5 visual fields of a microscope. The obtained data have been used to calculate the intensity of the invasion of *Nosema apis* and the number of pollen seeds in one visual field of a microscope (magnification 800x).

Results and Discussion

In the group of families that suffered from the invasion of *Nosema apis* in the early spring, some families free from the invasion of that apicomplexon have been noticed. The largest percentage of the families free from the bees' apicomplexon has been spotted in group I (58.8% on average) and the smallest one in group III (28.1%). In the families infected with *Nosema apis*, the intensity of the invasion has varied significantly, for instance: in group I the smallest number of *Nosema apis* spores in one visual field of a microscope (marked as one plus) has been found in 17.1% of the families and in group II (27.4%), while in group III a significant percentage of the families (37.7%) has suffered from the invasion of *Nosema apis* to the largest extent (three pluses) – Table 1.

In the families of group I it has been noticed that the largest number of bees was infected by the bees' apicomplexon in June, which is connected with their infection in the early spring i.e. time when in the hive and on the honeycombs numerous spores of *Nosema apis* could be found. In the group II, a significant infection could be noticed in June, July and August, while the bees returning to the hive (group III) were the most often infected by the bees' apicomplexon

Table 1

The course of invasion extensiveness of *Nosema apis* in bees depending on the month and the place of taking a sample

Group	Types of the examined bees	Months of examination	Extensiveness of invasion of *N. apis* group (families)			
			free from the invasion of *N. apis*	suffering from the bees' apicomplexon		
				+	++	+++
I	bees from combs with unsealed brood	June	37.5	21.4	21.4	21.4
		July	60.0	20.0	10.0	10.0
		August	80.0	10.0	–	10.0
	average e.i.		58.8	17.1	15.7	13.8
II	bees from combs with sealed brood	June	40.0	40.0	10.0	10.0
		July	66.7	22.2	–	11.1
		August	30.0	20.0	10.0	40.0
	average e.i.		45.6	27.4	10.0	20.4
III	foraging bees' returning to the hive	June	10.0	10.0	30.0	50.0
		July	44.4	11.1	22.2	22.2
		August	30.0	20.0	10.0	40.0
	average e.i.		28.1	13.7	20.7	37.7

in June and August. Such infection of the bees by *Nosema apis* in that group was the result of the former invasion of the hive bees and huge effort when collecting nectar and pollen (WILDE, BRATKOWSKI 1995).

The discussed course of the intensity of the *Nosema apis* invasion in the examined families indicates some regularities in the development of the parasite. At first, the bees' apicomplexon attacks the youngest bees, feeding the brood with pollen and honey, as well as a little older bees that clean the cells and prepare food reserves. Later, the invasion develops in the flying bees (KRZYŻAŃSKA 2001). In spite of the significant invasion of *Nosema apis* in the examined families, no visible influence of the parasite on their development has been noticed. It seems that influx of fresh food, low air humidity in the hive, ongoing building of the honeycombs and intensified out-of-the-hive activity of the bees were in favour of the natural defence of the family against the invasion of *Nosema apis* (WILDE, GOGOLEWSKA 1993).

As for the bees taken from the honeycombs with closed brood (group II), the average amount of pollen seeds calculated for three intensities of the invasion of *Nosema apis* (+, ++, +++) has been the same both for the ones free from the parasite and for the ones suffering from the bees' apicomplexon. In that group, a particularly large number of pollen seeds has been found in worker bees with very low invasion intensity (+), mostly in July. However, as for the bees suffering from the bees' apicomplexon in group III, the amount of

the pollen seeds has been almost half the size as in the families free from the parasite, and in the group I it has been slightly higher for those infected by *Nosema apis* (Table 2).

Table 2

The amount of pollen seeds in one visual field of a microscope (magn. 800x) in bees free from invasion and in infected by apicomplexon *Nosema apis*

Group	Months of examination	The amount of pollen seeds			
		group			
		free from the invasion of *N. apis*	infected by *N. apis*		
			+	++	+++
I	June	1.4	3.8	1.7	2.2
	July	1.6	3.1	1	3.2
	August	1.5	2	–	1.2
	average	1.5	2.96	1.35	2.2
Average for the group infected by *N. apis*			2.17		
II	June	2.8	4.2	6.4	1.4
	July	4.7	9.1	0	4
	August	1.7	1.3	0.4	0.75
	average	3.06	4.86	2.26	2.05
Average for the group infected by *N. apis*			3.05		
III	June	2.2	0.8	1.4	0.7
	July	2.9	1	2.4	1.4
	August	0.9	1.3	0.4	0.75
	average	2	1.03	1.4	0.95
Average for the group infected by *N. apis*			1.12		

The amount of the pollen seeds in the bee's intestine maybe an indicator of her nutrition (condition). Thus, the bees free from the invasion, as the healthy ones, utilize the protein food in a better way, which results in the lower amount of pollen seeds in their intestines (KONOPACKA et al. 1987). This hypothesis may be confirmed by the bees of group I and II, where the bees infected by the bees' apicomplexon, in order to sufficiently feed the brood and produce appropriate amount of milk, ate more protein food that healthy ones because of the damage to their digestive tract done by the parasite (Figures 1, 2). At the same time, in the group of flying bees, taken from the eyelet and free from the invasion, the amount of the pollen seeds was almost twice as big as for the infected ones (Figure 3). This indicates the need for eating a larger amount of protein to take flights for nectar, pollen and water (HARTWIG, TOPOLSKA 2001).

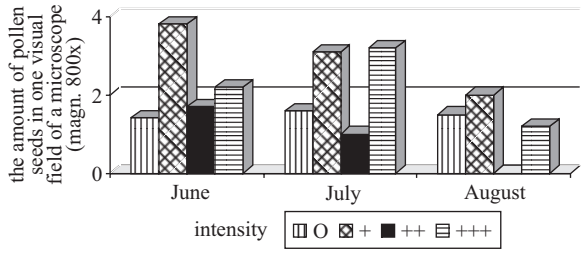


Fig. 1. The amount of pollen seeds in young bees depending on the intensity of the invasion of *N. apis*

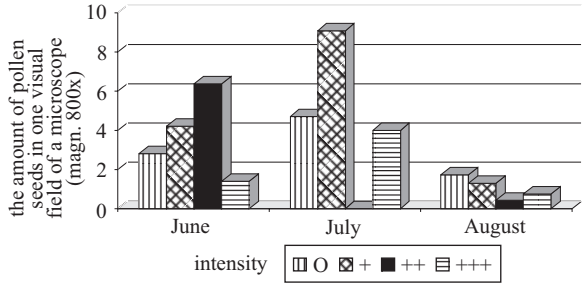


Fig. 2. The amount of pollen seeds in hive bees depending on the intensity of the invasion of *N. apis*

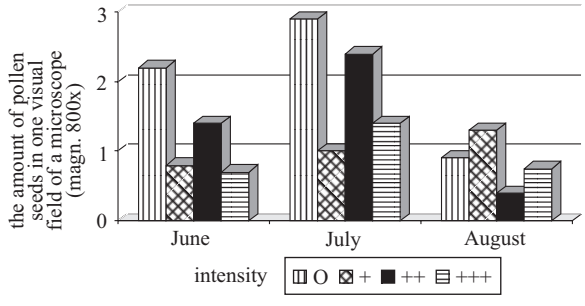


Fig. 3. The amount of pollen seeds in flying bees depending on the intensity of the invasion of *N. apis*

Conclusions

The results of the examinations show the influence of the invasion of *Nosema apis* and the age of the bees on the amount of the consumed protein food. The hive bees suffering from the invasion of *Nosema apis* in a small

degree require far more protein food for their organisms to function properly than the bees free from the infection because the protein in form of pollen of the entomophilous plants is necessary for brood raising, feeding the mother and for ensuring proper functions of the internal organs of the worker bee. As for the flying bees free from the invasion of *Nosema apis*, which fly long distances for their load, often in extremely changeable weather conditions, their demand for protein is slightly huger than that of the hive bees taken from the honeycombs with open brood. The hugest demand for protein is manifested by the hive bees free from the invasion, as well as the ones infected by the bees' apicomplexon that produce bees' milk (group II), fabricate wax and prepare the cells for the mother's breeding. A particularly large number of pollen seeds my be found in bees of every group in the time of intensive raising of the brood i.e. in June and July.

Translated by Maria Siemionek

Accepted for print 17.12.2006

References

Gliński Z., Rzedzicki J. 1993. *Choroby pszczół*. PWN, Warszawa.

Hartwig A. 1970. *Badania cytochemiczne nad jelitem środkowym pszczół robotnic zdrowych i zarażonych pasożytem Nosema apis Zander oraz zakażonych i poddanych leczeniu preparatem Fumagillin DCH*. Pszczel. Zesz. Nauk., 14 (1-3): 75-107.

Hartwig A., Topolska G. 1995. *Przydatność rutynowych badań pszczół z osypu zimowego*. Pszczel. Zesz. Nauk., 39: 71-77.

Hartwig A., Topolska G. 2001. *Choroby pszczół*. Wyd. SGGW, Warszawa.

Konopacka Z., Muszyńska J., Hartwig A. 1987. *Wpływ intensywności wychowu czerwiu i kondycji fizycznej pszczół robotnic na ich podatność na zakażenie sporowcem pszczelim (Nosema apis Zander)*. Pszczel. Zesz. Nauk., 31: 263-277.

Krzyżańska K. 2001. *Choroba sporowcowa pszczół (nosemoza).* Mag. Wet., 6: 63.

Sokół R., Molska D. 2006. *Nosemoza – choroba sporowcowa pszczół.* Weterynaria w praktyce, Suppl. Zwierzęta hodowlane, jesień 2006, pp. 65-67.

Wilde J., Gogolewska E. 1993. *Dieta pyłkowa a przebieg nosemozy w rodzinach pszczelich porażonych sporami Nosema apis*. Mat. XXX Nauk. Konf. Pszczel., Puławy 16-17 marca 1993, ss. 48-49.

Wilde J., Bratkowski J. 1995. *Nosemoza w rodzinach a pozyskiwanie pyłku*. Pszczel. Zesz. Nauk., 39(1): 99-105.