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**THE EFFECT OF INCREASING DOSES
OF MEAT-AND-BONE MEAL ON THE YIELD
AND MACRONUTRIENT CONTENT OF PERENNIAL
RYEGRASS (*LOLIUM PERENNE* L.)**

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Key words: perennial ryegrass, yield, macronutrients, meat-and-bone meal.

Abstract

The effect of three increasing doses of meat-and-bone meal on the yield and macronutrient content of perennial ryegrass (*Lolium perenne* L.) was studied during a two-year pot experiment (2005–2006). Meat-and-bone meal was applied in a single dose in 2005, before sowing. The meal was mixed with soil in the following dosage: 0.25, 0.5 and 1.0% per 10 kg soil per pot. The effect of meat-and-bone meal was compared with that of NPK fertilizers (control treatment) applied at the following rates: 1 g N, 0.5 g P and 1 g K per pot. Pots filled with soil mixed with meat-and-bone meal were also fertilized with 1 g potassium. The results were verified statistically by one-way analysis of variance in a completely randomized design.

The increasing doses of meat-and-bone meal had a significant effect on the yield and macronutrient content of perennial ryegrass. Meat-and-bone meal applied in a dose of 0.5% and 1% caused an 11% increase in the dry matter yield of perennial ryegrass over two years, compared with the control treatment (NPK). A beneficial residual effect of meat-and-bone meal was noted only for the highest dose, while the lowest dose (0.25%) caused a 3.5-fold yield decrease, which led to a 30% drop in the total yield of perennial ryegrass over two years. The above suggests that the dose of 0.25% was insufficient to meet the nutrient requirements of perennial ryegrass during two consecutive years. Meat-and-bone meal applied in a dose of 0.5% contributed to the most desirable mineral composition of perennial ryegrass, whereas the highest dose of meat-and-bone-meal resulted in excess accumulation of nitrogen and potassium in plants, thus inhibiting magnesium uptake.

WPLYW WZRASTAJĄCYCH DAWEK MĄCZKI MIĘSNO-KOSTNEJ NA PLON I ZAWARTOŚĆ MAKROSKŁADNIKÓW W ŻYCICY TRWAŁEJ (*LOLIUM PERENNE* L.)

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Słowa kluczowe: życica trwała, plon, makroskładniki, mączka mięsno-kostna.

Abstrakt

W dwuletnim doświadczeniu wazonowym (2005–2006) badano wpływ trzech wzrastających dawek mączki mięsno-kostnej na plon i zawartość makroskładników w życicy trwałej (*Lolium perenne* L.). Mączkę mięsno-kostną zastosowano jednorazowo, przedsiewnie w 2005 r., mieszając z glebą w dawkach: 0,25, 0,5 i 1,0% mączki w stosunku do masy (10 kg) gleby w wazonie. Działanie mączki porównywano z nawożeniem NPK (obiekt kontrolny) w ilości: 1 g N, 0,5 g P i 1 g K na wazon. Potas w ilości 1 g zastosowano również do wazonów z mączkami. Wyniki opracowano statystycznie metodą analizy wariancji dla doświadczeń jednoczynnikowych w układzie całkowicie losowym.

Z badań wynika, że zastosowanie wzrastających dawek mączki mięsno-kostnej istotnie różnicowało plon i zawartość badanych makroskładników w życicy trwałej. Pod wpływem średniej (0,5%) i najwyższej (1%) dawki mączki stwierdzono 11-procentowy wzrost plonu suchej masy życicy trwałej za dwa lata, w porównaniu z plonem obiektu kontrolnego (NPK), przy czym korzystne działanie następce mączki uwidoczniło się tylko w przypadku najwyższej dawki. Najmniejsza (0,25%) dawka mączki spowodowała natomiast w działaniu następczym średnio ponad 3,5-krotny spadek plonu, co skutkowało 30-procentowym spadkiem sumy plonu z dwóch lat. Można więc wnioskować, że jest to zbyt mała dawka dla badanej trawy na dwa lata wegetacji. Życica trwała uprawiana na średniej dawce mączki miała najkorzystniejszy skład mineralny, natomiast najwyższa dawka mączki powodowała nadmierną kumulację azotu i potasu w roślinie, co blokowało pobieranie magnezu.

Introduction

Meat-and-bone meal produced from the lowest-risk leftovers of the slaughtering process can be used as an organic fertilizer and a soil amendment (MAĆKOWIAK 2005). Animal meals have been increasingly applied to improve soil fertility in recent years, due to a considerable decrease in livestock population and the production of organic fertilizers accompanied by an increase in the prices of mineral fertilizers (SPYCHAJ-FABISIAK et al. 2007, KRZYWY et al. 2004). Meat-and-bone meals are a rich source of nutrients for plants, in particular nitrogen and phosphorus as well as magnesium, calcium and microelements. According to JENG et al. (2004), nitrogen supplied by meat-and-bone meal meets 80% of the nitrogen demand of grain crops. In comparison with manure, the dry matter of meat-and-bone meal contains approximately 4-fold more nitrogen, 10-fold more phosphorus and 8-fold more calcium, as well as over 4-fold less potassium and nearly 2-fold less magnesium.

Both fertilizers have a similar organic matter content. Owing to their beneficial influence on soil fertility and plant production, animal meals could be used on a larger scale in farms where no organic fertilizers are produced.

The aim of this study was to determine the direct and residual effect of increasing meat-and-bone meal doses on the yield and macronutrient content of perennial ryegrass.

Materials and Methods

A two-year (2005–2006) pot experiment involving perennial ryegrass (*Lolium perenne* L.) cv. Naki was conducted in the greenhouse of the University of Warmia and Mazury in Olsztyn. Modified Kick-Brauckmann pots were filled with slightly acidic (pH in 1 mol KCl dm⁻³ = 5.0) proper brown soil developed from loamy sand, with a high content of available phosphorus (85.0 mg P kg⁻¹) and potassium (116.2 mg K kg⁻¹) and an average content of available magnesium (28.0 mg Mg kg⁻¹). Meat-and-bone meal containing blood and feather hydrolyzate powder was applied in a single dose, in the first year of the study, before sowing. It was mixed with soil in the following dosage: 0.25, 0.5 and 1.0% per 10 kg soil per pot. The meal contained 96.05% dry matter, 71.42% organic matter, 27.64% crude ash, 13.69% crude fat, 7.88% total nitrogen, 4.67% phosphorus, 0.34% potassium, 10.03% calcium, 0.68% sodium and 0.20% magnesium. According to the classification of animal by-products, the meal used in the study was category 3 material which comprises animal by-products derived from the production of products intended for human consumption. Pots filled with soil mixed with meat-and-bone meal were also fertilized with 1.0 g K pot⁻¹ in the first and second year of the experiment, before sowing. The effect of meat-and-bone meal was compared with that of NPK fertilizers (control treatment): 1 g N (two doses of 0.5 g, before sowing and after the first harvest, in the form of CO(NH₂)₂), 0.5 g P (KH₂PO₄) and 1 g K (KH₂PO₄ and KCl) per pot applied pre-sowing in the first and second year of the study. Soil moisture in pots was maintained at a level of 60% maximum capillary water capacity.

Samples of three perennial ryegrass regrowths harvested in the first and second growing season were subjected to chemical analyses. The collected samples were mineralized in concentrated sulfuric acid with hydrogen peroxide as the oxidizing agent. Wet mineralized samples were assayed for the content of: total nitrogen – by the hypochlorite method, phosphorus – by the vanadium-molybdenum method, calcium, potassium and sodium – by atomic emission spectrometry (AES), and magnesium – by atomic absorption spectrometry (AAS). The results (the dry matter yield and the macronutrient

content of perennial ryegrass) were verified statistically by one-way analysis of variance in a completely randomized design, in four replications, at a significance level of $p = 0.01$.

Results and Discussion

The increasing doses of meat-and-bone meal containing blood and feather hydrolyzate powder, applied once during a two-year period, had a significant effect on perennial ryegrass yield (Table 1). In the first year of the study, meat-and-bone meal applied in the highest dose of 1% caused a significant, 3.5-fold decrease in the dry matter yield of the first perennial ryegrass regrowth compared with the control treatment, and an over 4-fold decrease relative to treatments fertilized with lower meal doses. Lower doses of meat-and-bone meal (0.25 and 0.5%) increased perennial ryegrass yield by around 30% (compared with the control treatment), but the noted differences were statistically non-significant. In the treatment fertilized with 0.5% meal, the yield of the second perennial ryegrass regrowth was significantly higher relative to the remaining treatments, and over 2-fold higher in comparison with the control treatment. The application of 0.5% meal contributed to the highest yield of three regrowths (74.8 g d.m.), which enabled to obtain a nearly

Table 1
The effect of meat and bone meal applied at different doses on the dry matter yield of perennial ryegrass [g pot⁻¹]

Year	Treatment [%]	Regrowth			Total yield of three regrowths
		I	II	III	
2005	NPK	17.6	17.0	5.8	40.4
	0.25	22.4	18.2	6.5	47.1
	0.5	23.1	39.6	12.1	74.8
	1	5.1	14.9	17.9	37.9
	Mean	17.0	22.4	10.6	50.0
NIR _{0.01}		9.5	20.0	r.n.	
2006	NPK [%]	18.2	18.7	7.5	44.4
	0.25	3.7	4.2	4.3	12.2
	0.5	5.6	6.1	5.6	17.3
	1	13.2	17.8	25.4	56.4
	Mean	10.2	11.7	10.7	32.6
NIR _{0.01}		4.8	12.7	r.n.	
Total yield for two years	NPK [%]	35.8	35.7	13.3	84.8
	0.25	26.1	22.4	10.8	59.3
	0.5	28.7	45.7	17.7	92.1
	1	18.3	32.7	43.3	94.3
	Mean	27.2	34.1	21.3	82.6

two-fold yield increment, compared with the control treatment. JENG et al. (2006) also applied increasing meat-and-bone meal doses and reported a significant increase in the dry matter yield of perennial ryegrass in both pot and field experiments.

In the second year of the experiment, the yield of the first perennial ryegrass regrowth was significantly lower than in the control treatment, regardless of the meal dose. The highest, approximately 5-fold, yield decrease was observed in treatments fertilized with the lowest dose (0.25%) of meat-and-bone meal. The yield of the second regrowth was approximately 4.5-fold lower in this treatment, relative to the control (significant difference). The highest annual and two-year yield (56.4 and 94.3 g d.m. per pot respectively) was obtained following the application of the highest (1%) meal dose, in both years of the study.

The results of the two-year experiment show that meat-and-bone meal applied pre-sowing in a single dose of 0.5% and 1% per pot caused an 11% increase in the dry matter yield of perennial ryegrass, compared with the control treatment. Similar data were reported for meadow fescue fertilized with animal meal (NOGALSKA, CZAPLA 2009). In experiments performed by STEPIEŃ and MERCIK (2002), and GÓRECKA et al. (2009), the yields of triticale, serradella and rapeseed fertilized with animal meals were similar to or higher than in the control treatment. In the present study, the lowest dose (0.25%) of meat-and-bone meal caused a 3.5-fold yield decrease, which led to a 30% drop in the total yield of perennial ryegrass over two years, relative to the control treatment. The above suggests that the dose of 0.25% was insufficient to meet the nutrient requirements of perennial ryegrass during two consecutive years.

The macronutrient content of perennial ryegrass dry matter changed substantially but irregularly under the influence of increasing animal meal doses. In the first year of the experiment, the nitrogen content of the studied grass increased significantly as a result of the application of the highest meal dose (1%) in three regrowths, the medium dose (0.5%) in the first and third regrowth and the lowest dose (0.25%) in the first regrowth (Table 2). Depending on fertilization levels, the average nitrogen content of perennial ryegrass ranged from 9.86 to 30.66 g N kg⁻¹ d.m., which corresponded to 6.16–19.16% protein. Protein content close to the optimum value of 16.46% determined for *Lolium perenne* (FALKOWSKI et al. 2000) was noted in all regrowths of plants fertilized with the highest meal dose and in the first regrowth of plants fertilized with 0.5% meat-and-bone meal. Relative to the control, the nitrogen content of perennial ryegrass increased almost 3-fold and 1.5-fold in the treatment fertilized with 1% and 0.5% meal, respectively.

Table 2

The effect of meat-and-bone meal dosage on the concentrations of N, P and K in perennial ryegrass biomass [g kg⁻¹ d.m.]

Year	Treat- ment	N				P				K			
		regrowth											
		I	II	III	weight- ed mean	I	II	III	weight- ed mean	I	II	III	weight- ed mean
2005	NPK [%]	11.25	11.71	5.95	10.68	5.64	5.80	8.19	6.07	47.13	26.50	27.88	35.68
	0.25	22.86	4.18	5.74	13.28	5.46	3.63	4.98	4.14	53.00	31.63	24.75	40.84
	0.5	29.68	11.20	9.15	16.57	5.89	4.44	4.51	4.90	57.25	31.25	23.75	38.07
	1	45.25	30.51	26.63	30.66	6.95	5.86	5.11	5.65	37.25	50.00	39.50	43.32
	Mean	27.26	14.40	11.87	17.83	5.98	4.93	5.70	5.53	48.66	34.84	28.97	37.49
Weighted mean		–	–	–	17.80	–	–	–	5.19	–	–	–	39.48
NIR _{0.01}		10.01	6.69	3.86	–	0.88	1.48	1.41	–	5.28	11.50	r.n.	–
2006	NPK [%]	14.40	12.70	4.60	12.03	5.20	3.98	5.35	4.71	26.75	21.45	23.05	23.89
	0.25	12.48	13.05	4.48	9.86	7.70	7.72	5.85	7.05	23.25	23.00	36.25	27.75
	0.5	11.70	17.03	7.50	12.22	6.43	7.90	5.65	6.70	19.70	20.85	31.88	24.02
	1	40.73	31.75	6.65	22.55	6.38	4.53	4.28	4.85	31.82	25.53	34.13	30.87
	Mean	19.83	18.63	5.81	14.76	6.43	6.03	5.28	5.91	25.38	22.67	31.33	26.47
Weighted mean		–	–	–	14.16	–	–	–	5.83	–	–	–	26.63
NIR ^{0.01}		6.05	15.52	r.n.	–	r.n.	2.34	r.n.	–	8.61	r.n.	10.06	–

In the second year of the experiment, a significant residual effect of the highest meat-and-bone meal dose was noted in the first and second perennial ryegrass regrowth which contained 40.73 and 31.75 g N kg⁻¹ d.m. on average, respectively, resulting in an over 2.5-fold increase in nitrogen content, compared with control plants. The average nitrogen content of perennial ryegrass increased along with an increase in meal dose in both years of the study. Similar changes in nitrogen concentrations were observed in meadow fescue fertilized with increasing doses of meat-and-bone meal (NOGALSKA, CZAPLA 2009).

In the first and second year of the study, the average phosphorus content of perennial ryegrass ranged from 4.14 to 7.05 g P kg⁻¹ d.m. According to FALKOWSKI et al. (2000), grassland vegetation should contain 2.8 to 3.6 g P kg⁻¹ d.m. The phosphorus content of the studied grass was over 1.5-fold higher than the above optimum values. NOWAK and DRASZAWKA-BOLZAN (2001) also reported high phosphorus concentrations in perennial ryegrass, at 4.6–5.4 g P kg⁻¹. STĘPIEŃ and MERCIK (2002) demonstrated that among various animal by-products, meat-and-bone meal supported the highest increase in the phosphorus content of test plants. In the current experiment, a significant increase in phosphorus concentrations was noted in the first regrowth (2005) of perennial ryegrass fertilized with the highest meat-and-bone meal dose and

in the second regrowth (2006) of plants fertilized with lower meal doses. Relative to the control, the second perennial ryegrass regrowth fertilized with the lowest meal dose and the third regrowth of plants in all treatments contained significantly less phosphorus in the first year of the study.

The optimum potassium content of animal feed is $17.0 \text{ g K kg}^{-1} \text{ d.m.}$ Potassium deficiency is seldom observed in grasslands (FALKOWSKI et al. 2000). The average potassium content of perennial ryegrass was high, at 23.89 to $43.32 \text{ g K kg}^{-1} \text{ d.m.}$ Equally high potassium concentrations in perennial ryegrass were reported by WOŁOSZYK and KRZYWY (1999), and NOWAK and DRASZAWKA-BOLZAN (2001). In the first year of the current study, meat-and-bone meal applied in a dose of 0.25 and 0.5% caused a significant increase, and the highest meal dose contributed to a significant decrease in the potassium content of the first perennial ryegrass regrowth, compared with the control treatment. The highest meal dose significantly increased potassium concentrations in the second regrowth. In the second year, perennial ryegrass contained over 30% less potassium than in the first year. Grass fertilized with the highest and lowest dose of animal meal was most abundant in potassium.

The present results and the findings of other authors (WOŁOSZYK, KRZYWY 1999, FALKOWSKI et al. 2000, NOWAK, DRASZAWKA-BOLZAN 2001, NOGALSKA, CZAPLA 2009) indicate that potassium has an antagonistic effect on magnesium uptake by grasses (Table 3). Excess potassium accumulation in perennial ryegrass decreased the magnesium content of plants. Perennial ryegrass fertilized with the highest meat-and-bone meal dose contained large amounts of nitrogen, which could inhibit magnesium uptake (KOCHANOWSKA, NOWAK 1992). The magnesium content of perennial ryegrass varied subject to fertilization and regrowth, ranging from 1.69 to $4.11 \text{ g Mg kg}^{-1} \text{ d.m.}$ In both years of the experiment, perennial ryegrass had the highest magnesium content in treatments fertilized with 0.5% meal (18% increase relative to the control). In the second year, perennial ryegrass was less abundant in magnesium, by 30% on average, although all meal doses contributed to an increase in magnesium content, compared with the control treatment. Hay produced in Poland often contains insufficient quantities of magnesium. In animal nutrition, the threshold value is $2.0 \text{ g Mg kg}^{-1} \text{ d.m.}$ plants (FALKOWSKI et al. 2000).

The average calcium content of perennial ryegrass was in the range of 4.55 to $12.92 \text{ g Ca kg}^{-1} \text{ d.m.}$ The optimum value of $7.0 \text{ g Ca kg}^{-1} \text{ d.m.}$ (FALKOWSKI et al. 2000) was achieved in all treatments in the first year of the experiment, and in the treatment fertilized with the highest meat-and-bone meal dose in the second year. In the first year of the study, lower meal doses caused a significant (1.5-fold) increase in the calcium content of the first ryegrass regrowth. All meal doses exerted a similar effect on calcium concentrations in the third regrowth. In the second year of the experiment, perennial ryegrass contained

Table 3

The effect of meat-and-bone meal dosage on the concentrations of Mg, Ca and Na in perennial ryegrass biomass [g kg⁻¹ d.m.]

Year	Treat- ment	Mg				Ca				Na			
		regrowth											
		I	II	III	weig- hted mean	I	II	III	weig- hted mean	I	II	III	weig- hted mean
2005	NPK [%]	1.95	3.84	4.00	3.04	7.65	9.00	8.10	8.29	0.68	0.72	0.83	0.71
	0.25	2.73	2.92	3.68	2.93	11.35	10.75	10.70	11.03	0.93	1.26	1.24	1.10
	0.5	2.66	3.64	5.25	3.60	11.15	12.18	12.80	11.96	1.82	3.33	2.32	2.70
	1	1.41	2.79	3.51	2.94	7.4	12.30	15.00	12.92	1.17	2.67	3.88	3.04
	Mean	2.19	3.30	4.11	3.20	9.40	11.06	11.65	10.70	1.30	2.00	2.07	1.78
Weighted mean		–	–	–	3.13	–	–	–	11.05	–	–	–	1.89
NIR _{0.01}		0.23	0.65	0.92	–	1.48	r.n.	1.69	–	0.49	1.08	0.98	–
2006	NPK [%]	1.60	2.15	2.45	1.97	4.15	4.55	5.53	4.55	1.33	1.53	2.30	1.58
	0.25	1.65	2.75	1.93	2.13	5.13	3.85	5.47	5.02	1.38	1.08	1.28	1.24
	0.5	1.65	2.98	2.23	2.31	4.63	4.15	6.08	4.93	1.08	0.8	1.30	1.05
	1	1.85	2.58	2.05	2.17	7.03	8.20	6.75	7.27	1.88	3.05	2.92	2.72
	Mean	1.69	2.61	2.16	2.16	5.23	5.19	5.96	5.46	1.42	1.61	1.95	1.66
Weighted mean		–	–	–	2.14	–	–	–	5.44	–	–	–	1.65
NIR _{0.01}		r.n.	0.40	0.36	–	2.23	r.n.	r.n.	–	r.n.	r.n.	r.n.	–

2-fold less calcium, and only the highest meal dose caused a significant increase in the calcium content of the first regrowth, compared with the control treatment.

Both grass quality and intake by animals are affected by sodium content. Sodium-deficient diets have to be supplemented with minerals. The sodium content of perennial ryegrass fertilized with higher doses of meat-and-bone meal in the first year, and with the highest dose in the second year was close to the value of 1.5–2.5 g Na kg⁻¹ d.m. cited by FALKOWSKI et al. (2000). Sodium concentrations increased in successive regrowths in both years of the study. In the first year of the experiment, a meal dose of 0.5 and 1% contributed to a significant (approx. 4-fold) increase in the sodium content of perennial ryegrass, relative to the control. In the second year, plants fertilized with the highest dose of meat-and-bone meal accumulated the largest amounts of sodium, but the observed differences were statistically non-significant.

Conclusions

1. The results of the two-year experiment show that meat-and-bone meal applied pre-sowing in a single dose of 0.5% and 1% per pot caused an 11%

increase in the dry matter yield of perennial ryegrass, compared with the control treatment. The above doses satisfied the N and P demand of perennial ryegrass, while the lowest dose (0.25%) of meat-and-bone meal was insufficient to meet the nutrient requirements of plants in the second year of the study.

2. The highest dose of meat-and-bone meal contributed to a significant increase in the concentrations of nitrogen, potassium and sodium in perennial ryegrass, while the medium dose increased the levels of magnesium and calcium. The effect of increasing doses of meat-and-bone meal on the phosphorus content of the studied grass varied.

3. Meat-and-bone meal applied in a dose of 0.5% contributed to the most desirable mineral composition of perennial ryegrass, whereas the highest dose of meat-and-bone-meal resulted in excess accumulation of nitrogen and potassium in plants, thus inhibiting magnesium uptake.

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**THE EFFECT OF FUNGICIDE TREATMENT
ON THE PRODUCTIVITY AND HEALTH
OF BUCKWHEAT SEEDS
(*FAGOPYRUM ESCULENTUM* MOENCH)***

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Key words: buckwheat, gas exchange parameters, fungi, *Fusarium* spp., traditional method, multiplex PCR, protein fractions.

A b s t r a c t

The experiment investigated the effect of Funaben T fungicide treatment on the yield, gas exchange parameters and health status of buckwheat seeds cv. Kora. The results indicate that the applied seed dressing fungicide contributed to an increase in buckwheat seed yield. Gas exchange parameters were similar in both experimental variants. Buckwheat seeds were colonized mostly by fungi of the species *Alternaria alternata*, followed by fungi of the genus *Fusarium*. The presence of fusariotoxins was not noted in the analyzed buckwheat nutlets. Fungicide treatment had no effect on the content of the analyzed protein fractions.

**WPŁYW ZAPRAWY FUNGICYDOWEJ NA PRODUKTYWNOŚĆ I ZDROWOTNOŚĆ
ORZESZKÓW GRYKI (*FAGOPYRUM ESCULENTUM* MOENCH)**

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Słowa kluczowe: gryka, wskaźniki wymiany gazowej, grzyby, *Fusarium* spp., metoda tradycyjna, multiplex PCR, frakcje białek.

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Abstrakt

W doświadczeniu badano wpływ zaprawy fungicydowej Funaben T na plonowanie, wskaźniki wymiany gazowej i zdrowotność nasion gryki odmiany Kora. Wykazano, że zaprawa fungicydowa spowodowała wzrost plonu nasion gryki. Parametry wymiany gazowej w obu doświadczalnych wariantach kształtowały się na zbliżonym poziomie. Nasiona gryki w największym stopniu zasiedlone były przez gatunek *Alternaria alternata*, duży udział miały również grzyby z rodzaju *Fusarium*. Nie stwierdzono obecności fuzariotoksyn w analizowanych orzeszkach gryki. Nie wykazano również wpływu zaprawy fungicydowej na zawartość frakcji badanych białek.

Introduction

Buckwheat is a popular plant on the global health food market. It is a rich source of nutrients as well as biologically active compounds that are not classified as nutrients (KLEPACKA and FORMAL 2006). Owing to their chemical composition, buckwheat nutlets are suitable for use in the production of functional foods and food additives (TROSZYŃSKA et al. 2000). Buckwheat seeds contain gluten-free proteins with balanced amino acid levels, high quantities of crude fat comprising mostly unsaturated fatty acids, macronutrients, micronutrients, B vitamins and antioxidants (HOLASOVA et al. 2002, DIETRYCH-SZÓSTAK and SUCHECKI 2006). Buckwheat proteins contain no gliadins that cause food intolerance (celiac disease) (FORMAL and SORAL-ŚMIETANA 1985, DIETRYCH-SZÓSTAK and OLESZEK 1998).

As an abundant source of nutrients, buckwheat nutlets are used in the production of functional, medicinal and preventive foods (KWIATKOWSKI 2008). In view of their high dietary value, buckwheat plants have to be provided with optimum conditions for growth and development. NOWOROLNIK (1999), PODOLSKA and PECIO (1999) observed a correlation between buckwheat yield and various cultivation and habitat factors. Seed quality is also an important consideration. Seed treatment prior to sowing, including seed dressing, the use of plant growth regulators and laser radiation, has been shown to improve the germination and growth of buckwheat seedlings (CIUPAK et al. 2006).

Cereal plants as well as buckwheat are often infected with various species of pathogenic fungi which lower productivity and deteriorate yield quality. Fusariosis infections are among the most serious diseases affecting cereal plants. They are caused by fungi of the genus *Fusarium* which produce the following mycotoxins: trichothecene, enniatin, zearalenone and fumonisin. When present in food and feedstuffs, those mycotoxins pose a health risk for humans and animals (CHEŁKOWSKI 1985, YU 2000, EDWARDS 2004).

The following research problems were formulated in the present study: Is seed dressing treatment justified? What is the effect of seed dressing treatment on the productivity and health of buckwheat nutlets? The objective of this

study was to determine: the yield of buckwheat seeds and gas exchange parameters as affected by different seed dressing treatments, the rate of infection with fungi of the genus *Fusarium* and the toxin-producing capacity of *Fusarium* spp. in buckwheat nutlets, and the content of protein fractions in buckwheat seeds.

Materials and Methods

A large-area experiment was carried out at the Production and Experimental Station in Bałcyny (NE Poland) in 2005 and 2006. The experimental material consisted of buckwheat seeds cv. Kora. The investigated parameters were determined in two replications per each treatment with an area of 20 m². The seeds were divided into two groups:

- seeds treated with the fungicide Funaben T,
- untreated seeds.

The scope of the study was as follows:

1. Determination of the key biometric parameters of buckwheat plants and seed yield per hectare at 15% moisture content.
2. Determination of gas exchange parameters.

Gas exchange parameters were determined using a LI-COR 6400 portable gas analyzer. The studied indicators were determined at a fixed CO₂ concentration of 400 ppm and light intensity of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The photon source was a LED Light Source lamp emitting light with the main peak spectrum at 670 nm and the second peak at 465 nm. Measurements were carried out at the following growth stages: I – 4th–5th leaf blade stage, II – at the beginning of flowering (at the highest, fully developed leaf), III – at the seeds formation stage (at the highest, fully developed leaf). The noted values were registered, the measurements were carried out in five replications, and the presented results contain average values.

3. Determination of the health status of buckwheat seeds by traditional methods and molecular techniques.

The harvested buckwheat seeds were subjected by a mycological analysis using the artificial culture method and multiplex PCR. The phytopathological evaluation of seeds by the artificial culture method was carried out on 100 randomly seeds which were rinsed with water and surface disinfected with 70% ethanol and 1% sodium hypochlorate. The seeds were placed on Petri dishes with solidified PDA. The cultures were incubated for 7–10 days at a temperature of 20–24°C. Fragments of the emerged mycelia were transferred onto agar slants. The fungi colonizing the harvested seeds were identified to genus and species by traditional microscopic observation, based on the available references (ELLIS 1971, KWAŚNA et al. 1991).

Molecular analyses were carried out using the PCR and the multiplex PCR technique following the extraction of genomic DNA by the spin column method (KULIK et al. 2007). Species-specific primers described by the referenced sources (HUE et al. 1999, KULIK et al. 2004, KULIK 2008, PSZCZÓŁKOWSKA 2008) and PCR thermal profiles were used to identify *Fusarium* pathogens isolated from seeds. The toxin-producing capacity (ability to produce trichothecenes /NIV and DON chemotypes/ and enniatins) of *Fusarium* spp. species was analyzed using primers specific for genes involved in mycotoxin synthesis (EDWARDS et al. 2001, CHANDLER et al. 2003, KULIK et al. 2007) – Table 1. The PCR reaction mixture comprised FailSafe™ PCR 2X Premix E, 0.2U Fail Safe™ Enzyme Mix Only polymerase, a combination of primers, 5.75 µl deionized water and 5 µl matrix DNA. The prepared samples were subjected to temperature changes in the Eppendorf Mastercycler gradient thermocycler. The PCR assay was carried out in two replications. To visualize the product after the PCR, electrophoresis was carried out using 1.5% agarose gel with ethidium bromide. Electrophoresis was conducted in a 50 V electric field for 1.5 hours. PCR product size was evaluated by comparison with the Step Ladder 50 bp (Sigma-Aldrich, USA) molecular mass reference.

4. Determining the content of protein fractions in buckwheat nutlets

A 3 g seed sampled was ground in the IKA A10 (Labortechnik) analytical mill, and the resulting particles were passed through a sieve with 400 µm mesh size (particles smaller than 250 µm ether had a 90% share). The samples were degreased with petroleum benzine in a Soxhlet extractor (16 hours). The solvent was evaporated, 100 mg of the powdered seeds was placed in Eppendorf test tubes, and three protein fractions were extracted according to the method proposed by WIESER et al. (1998):

- albumins + globulins – 1 cm³ of the mixture (0.4 mol/L NaCl + 0.067 mol/L HKNaPO₄ with pH of 7.6) was extracted in two replications (*three replications for buckwheat);

- prolamin – 1 cm³ of the mixture (60% ethanol) was extracted in three replications;

- glutelins – 1 cm³ of the mixture (50% propanol-1 + 2 mol/L urea + 0.05 mol/L Tris HCl with pH of 7.5) was extracted with 1% DTE under nitrogen, in two replications.

The first two protein fractions were extracted at room temperature using the Eppendorf thermomixer (10-minute extraction). Glutelins were extracted in the thermomixer at 60°C. After each extraction, proteins were centrifuged at 11 000 x g. The collected fractions were freeze-dried, dissolved in 2 cm³ of the corresponding phase (1–3), purified using the Spartan-3NY 0.45 µm filter and transferred to glass vials. Protein fractions were identified using the Hewlett Packard Agilent 1050 HPLC with the following parameters: RP-18 Vydac 218TPP54 column, 5 µm, 250 x 4,6 mm, Zorbax 3000SB-C18 precolumn,

Table 1
Species-specific primers for the detection of *Fusarium* species and chemotypes in seeds buckwheat

Fungal species, chemotyp, gene	Primer	Size of PCR product (bp.)	References
1	2	3	4
<i>Fusarium</i> spp.	P58SL 5'-AGT ATT CTG GCG GGC ATG CCT GT-3' P28SL 5'-ACA AAT TAC AAC TCG GGC CCG AGA-3'	339	HUE F.X., HUERRE M., ROUFFAULT M.A., BIEVRE C. 1999. <i>Specific Detection of Fusarium Species in Blood and Tissues by PCR Technique</i> . Journal of Clinical Microbiology, 37(8): 2434–2438.
<i>Fusarium avenaceum</i>	FaR 5'-CAA GCA TTG TCG CCA CTC TC-3' FaF 5'-GTT TGG CTC TAC CGG GAC TG-3'	920	DOOHAN F.M., PARRY D.W., JENKINSON P., NICHOLSON P. 1998. <i>The use of species-specific PCR-based assays to analyse Fusarium ear blight of wheat</i> . Plant Pathology 47: 197–205.
<i>Fusarium culmorum</i> <i>Fusarium graminearum</i>	igscl1 5'-CGG CTC CCG GGT AGG CAA CTC-3' igscln2 5'-CAT TCC CTA GGC CCC TTA ACT GG-3' igsgr1 5'-TTC AGG GTA GGC TTT CAG TTA GGA-3' igsgr2 5'-GGA GAA GAG GGC TGC AGC GTT GG-3'	340 499	PSZCZÓŁKOWSKA A. 2008. <i>Diagnostyka patogenów grzybowych metodą PCR i tradycyjną oraz produktywność pszenicy ozimej (Triticum aestivum L.) w warunkach różnicowanej ochrony fungicydowej</i> . Rozprawy i monografie, 140, UWM Olsztyn.
<i>Fusarium poae</i> <i>Fusarium sporotrichioides</i>	poa1 5'-CTT GGT AGG GGG GAC AGA CAC GC-3' poa2 5'-CCA TTC CAC GCT CGA CAG ACC TG-3' fspits2k 5'-CTT GGT GTT GGG ATC TGT GTG CAA-3' P28SL 5'-ACA AAT TAC AAC TCG GGC CCG AGA-3'	203 288	KULIK T. 2008. <i>Development of duplex PCR assay for the simultaneous detection of Fusarium poae and F. sporotrichioides from wheat</i> . Journal of Plant Pathology, 90(3): 441–447.
<i>Fusarium</i> spp.	HATrif 5'-CAG ATG GAG AAC TGG ATG GT-3' HATyir 5'-GCA CAA GTG CCA CGT GAC-3'	260	EDWARDS S.G., PIRGOZLIEV S.R., HARE M.C., JENKINSON P. 2001. <i>Quantification of trichotecene-producing Fusarium species in harvested grain by competitive PCR to determine efficacies of fungicides against Fusarium head blight of winter wheat</i> . Applied and Environmental Microbiology, 67: 1575–1580.

cont. table 1

1	2	3	4
<i>Chemotyp-NIV</i>	Tri13/NIVF 5'-CCA AAT CCG AAA ACC GCA G-3' Tri13R 5'-TTG AAA GCT CCA ATG TCG TG-3'	312	CHANDLER E., SIMPSON D.R., THOMSET T M.A., NICHOLSON P. 2003. <i>Development of PCR assays to Tri7 and Tri13 trichothecene biosynthetic genes, and characterization of chemotypes of F. gramine-arum, F. culmorum and F. cerealis.</i> Physiological and Molecular Plant Pathology, 62: 355-367
<i>Chemotyp 15-acetyl DON</i>	Tri315F 5'-CTC GCT GAA GTT GGA CGT AA-3' Tri315R 5'-GTC TAT GCT CTC AAC GGA CAA C-3'	864	
<i>Chemotypes 3-acetyl DON</i>	Tri303F 5'-GAT GGC CGC AAG TGG A-3' Tri303R 5'-GCC GGA CTG CCC TAT TG-3'	586	
Gene esyn1 (<i>F. avenaceum</i> , <i>F. trincinctum</i> , <i>G. pulicaris</i>)	esya1 5'-GGT CTC GAT CCA TCC AAG TC-3' esya2 5'-GTG AAG AAG GCA GGC TCA AC 3'	401	KULIK T., PSZCZÓLKOWSKA A., FORDŃSKI G., OLSZEWSKI J. 2007. <i>PCR approach based on the esyn1 gene for the detection of potential enniatin-producing Fusarium species.</i> International Journal of Food Microbiology, 116: 319-324
Gene esyn1 (<i>F. poae</i> , <i>F. Sporotrichioides</i>)	esysp1 5'-GGC CTT GAG CCA TCC AGA TC-3' esysp2 5'-CTC GTT GGT AGC CTG CGA TCG-3'	273	
Gene esyn1 (<i>F. Proliferatum</i>)	esypro1 5'-GAT CAA CTC AGT CGC GCA GTA-3' esypro2 5'-TCC TCC TCA CGC TCC TCC A-3'	225	
Gene esyn1 (<i>Giberella pulicaris</i>)	esysam1 5'-TGA TTC TCA ACT CCG TCG TTC A-3' esysam2 5'-CAC AGC CTT CAT GTT CTT GGG-3'	332	

4.6 x 12.5 mm, column temperature – 45°C, mobile phase flow rate – 1 ml/min, injection volume – 20 µl. The separation was performed using a two-component gradient. Share of component A: 0 min 75%, 5 min 65%, 10 min 50%, 17 min 25%, 18 min 15%, 19 min 75% (the first component, A, was water with the addition of 0.1% TFA, the second component, B, was ACN with the addition of 0.1% TFA). A HP detector was applied at a wavelength of 210 nm. The results were analyzed using the Hewlett Packard HPLC 3D Chem Station application. Protein fraction analyses were carried out at the Department of Plant Raw Materials Processing and Chemistry, Faculty of Food Sciences at the University of Warmia and Mazury in Olsztyn.

5. Statistical analysis

The results were processed statistically in STATISTICA software, version 6 (StatSoft, Inc. 2003), using an analysis of variance. Differences between means were determined at a significance level of $p = 0.05$. The mean values of the investigated parameters were classified into uniform groups with the use of Fisher's test. The strength of linear relations between the studied parameters was tested using Pearson's correlation coefficient.

Results and Discussion

A higher buckwheat nutlet yield was noted in the treatment where seeds were dressed with the fungicide Funaben T (Table 2). The values of the investigated yield components were also higher in the above treatment, indicating that seed dressing improves yield, in particular in years marked by high agrophage infestation levels. According to ROŻEK and WNUK (1994), plant protection products have a minor phytotoxic effect on crops, and they significantly inhibit disease incidence and maximize seed yield. The results of this study point to significant correlations: negative correlation between plant length and the number of seeds per plant ($R = -0.30$), positive correlation was noted between TSW and the number of seeds per plant ($R = 0.31$), the number of seeds per plant and yield ($R = 0.45$), as well as between TSW and yield ($R = 0.40$) – Figure 1a–1d.

Selected biometric parameters of buckwheat

Table 2

Cultivar	Treatment	Plant height [cm]	Number of seeds per plant [no.]	Thousand seeds weight [g]	Seeds yield [t ha ⁻¹]
Kora	Seeds dressed	97.0 ^a	88.6 ^a	29.5 ^a	1.7 ^a
	Seeds undressed	83.2 ^a	57.0 ^b	28.9 ^a	1.1 ^b

Homogeneous groups *a*, *ab*, *b*; LSD $\alpha = 0.05$

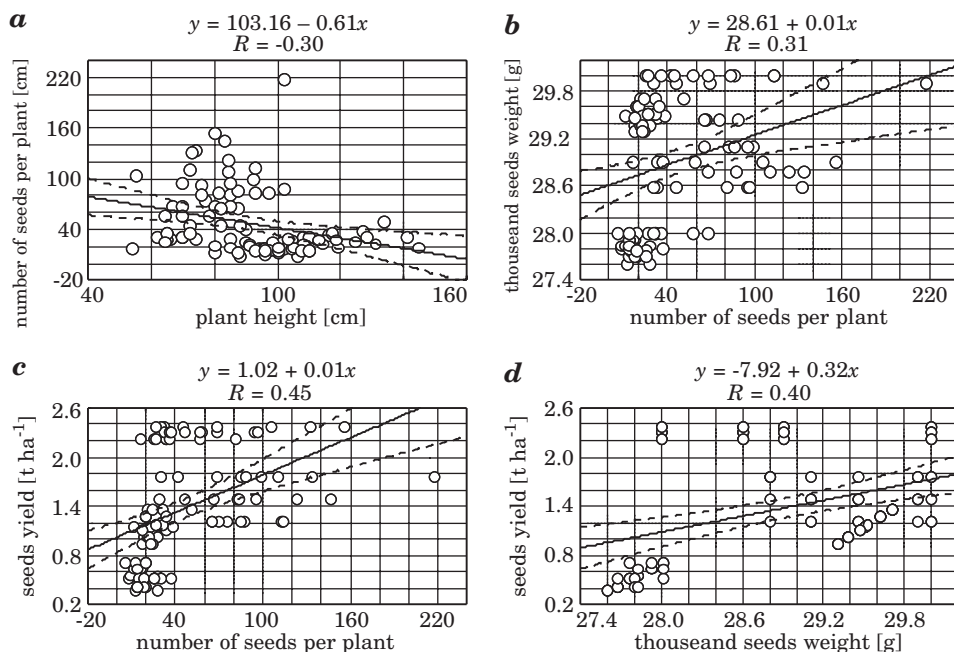


Fig. 1. Linear regression between: *a* – thousand seeds weight and number of seeds per cluster; *b* – number of seeds per cluster and plant height; *c* – seeds yield and thousand seeds weight; *d* – seeds yield and number of seeds per cluster

The noted results suggest that the applied fungicide did not affect the studied gas exchange parameters (photosynthesis rate, stomatal conductance, intercellular CO₂ concentrations and transpiration rate) – Table 3. Similar results were reported by OLSZEWSKI et al. (2007) in an experiment investigating the field pea where fungicide treatment did not alter the intensity of gas exchange.

Gas exchange parameters in buckwheat

Table 3

Fungicide treatment	Photosynthesis [$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$]			Transpiration [$\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$]			Intercellular CO ₂ concentration [$\mu\text{mol CO}_2 \text{ mol}^{-1}$]			Stomatal conductance [$\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$]		
	I	II	III	I	II	III	I	II	III	I	II	III
Seeds dressed	15.2 ^a	15.0 ^a	13.0 ^a	4.6 ^a	7.1 ^a	5.5 ^a	298 ^a	269 ^a	296 ^a	0.25 ^a	0.58 ^a	0.21 ^a
Seeds undressed	14.8 ^a	14.9 ^a	14.2 ^a	5.6 ^a	4.0 ^b	6.8 ^a	309 ^a	266 ^a	289 ^a	0.25 ^a	0.29 ^b	0.43 ^a

I – measurement of gas exchange parameters at the four- or five leaf stage; II – measurement of gas exchange parameters at beginning flowering; III – measurement of gas exchange parameters at the seeds formation stage)

Homogeneous groups *a*, *ab*, *b*; LSD $\alpha = 0.05$

Buckwheat seeds from both treatments were characterized by similar fungicide infection levels (Tables 4 and Table 5). In 2005, the predominant species with potentially pathogenic effects in both experimental variants was *Botrytis cinerea*, whereas *Alternaria alternata* was the main saprophyte species (Table 4). In 2006, *Fusarium* species had a 27% share of total fungal isolates. *Fusarium equiseti* accounted for 20% of total isolates (Table 5). As reported by WACHOWSKA and KWIATKOWSKI (2006), buckwheat nutlets were infected mostly with yeast-like fungi, and filamentous fungi were represented by *Cladosporium* spp., *Penicillium* spp. and *Aspergillus* spp. In this study, buckwheat nutlets were colonized mainly by *Alternaria alternata*.

Table 4

Number of fungal isolates in buckwheat seeds cv. Kora in 2005

Fungal species	Seeds dressed	Seeds undressed
<i>Alternaria alternata</i> Keissler Nees	40	31
<i>Ascochyta fagopyri</i> Bres.	–	4
<i>Botrytis cinerea</i> Pers, ex Fries	30	34
<i>Epicoccum purpurascens</i> Ehrenberg	2	1
<i>Penicillium</i> spp.	2	1
<i>Ramulkaria fagopyri</i> Abramov.	–	1
Non-spore-forming mycelia	3	–
Total	77	72

Table 5

Number of fungal isolates in buckwheat seeds cv. Kora in 2006

Fungal species	Seeds dressed	Seeds undressed
<i>Alternaria alternata</i> Keissler Nees	62	64
<i>Cladosporium cladosporioides</i>	1	–
<i>Epicoccum purpurascens</i> Ehrenberg	2	–
<i>Fusarium avenaceum</i>	2	2
<i>Fusarium equiseti</i>	22	17
<i>Fusarium poae</i>	–	1
<i>Fusarium solani</i>	3	6
<i>Mucor</i> spp.	3	6
<i>Penicillium</i> spp.	–	2
<i>Ramulkaria fagopyri</i> Abramov.	–	–
<i>Rhizopus</i> spp.	1	–
Non-spore-forming mycelia	–	–
Total	96	98

Toxic fungi of the genus *Fusarium* pose a growing problem in global crop production. *Fusarium* fungi decrease crop yield, causing seed infections that lead to the accumulation of various toxins. Increased metabolite concentrations in cereal plants and food products of plant origin have an adverse effect on the health of humans and animals (DESJARDINS 2006, KULIK and JESTOI 2009).

The presence of *Fusarium* spp. was determined by the PCR method in both treatments (Table 6). The length of the amplified DNA fragment was 339 bp, which corresponds with the findings of HUE et al. (1999). A multiplex PCR reaction performed with the involvement of primers specific for *Fusarium poae*, *F. culmorum*, *F. graminearum* and *F. sporotrichioides* revealed the presence of only *Fusarium poae* (Table 6). The amplified PCR product size was 203 bp, which is consistent with the findings of KULIK (2008). A negative result was reported in the multiplex PCR reaction using primers specific for genes involved in the biosynthesis of trichothecenes and enniatins. The obtained results imply that the studied buckwheat seeds constituted safe material for the production of food and feedstuffs.

Table 6
PCR analysis of the response of buckwheat seeds cv. Kora to fungi of the genus *Fusarium* and their toxin-producing potential

Fungal species, chemotyp, gene	Primer	Size of PCR product [bp]	Treatment			
			seeds dressed		seeds undressed	
			2005	2006	2005	2006
<i>Fusarium</i> spp.	P58SL/P28SL	339	+	+	–	+
<i>Fusarium avenaceum</i>	Far/Faf	920	–	–	–	–
<i>Fusarium culmorum</i>	igscul1/igsculn2	340	–	–	–	–
<i>Fusarium graminearum</i>	igsgra1/igsgra2	499	–	–	–	–
<i>Fusarium poae</i>	poa1/poa2	203	+	+	–	+
<i>Fusarium sporotrichioides</i>	fspits2k/P28SL	288	–	–	–	–
Gene tri5	HATri13R/HATri13F	260	–	–	–	–
Chemotyp NIV	Tri1NIVF/Tri13R	312	–	–	–	–
Chemotyp 15-acetyl DON	Tri315F/Tri315R	864	–	–	–	–
Chemotyp 3-acetyl DON	Tri303F/Tri303R	586	–	–	–	–
Gene esyn1 (<i>F. avenaceum</i> , <i>F. tricinctum</i> , <i>G. pulicaris</i>)	esya1/esya2	401	–	–	–	–
Gene esyn1 (<i>F. poae</i> , <i>F. sporotrichioides</i>)	esysp1/esysp2	273	–	–	–	–
Gene esyn1 (<i>F. proliferatum</i>)	esypro1/esypro2	225	–	–	–	–
Gene esyn1 (<i>G. pulicaris</i>)	esysam1/esysam2	332	–	–	–	–

Mycotoxin contamination of seeds and grain is preceded by plant infections already during the growing season. The quantity and type of mycotoxins accumulated in plant material is largely determined by the species composition

and the toxic potential of *Fusarium* fungi colonizing cereal grain. The diagnosis of pathogenic fungi's toxicity with the use of PCR methods supports forecasts of the level of seed infestation with various mycotoxins in a given area (DESJARDINS 2006).

As demonstrated by the results of this experiment, fungicide treatment did not have a significant effect on the content of the investigated protein fractions in buckwheat seeds (Figure 2). A decreasing trend was observed in respect of albumin + globulin fractions in untreated seeds, while a reverse trend was noted for prolamin and glutelin fractions. Similar results were reported in respect of fungicide-treated winter and spring wheat (unpublished study).

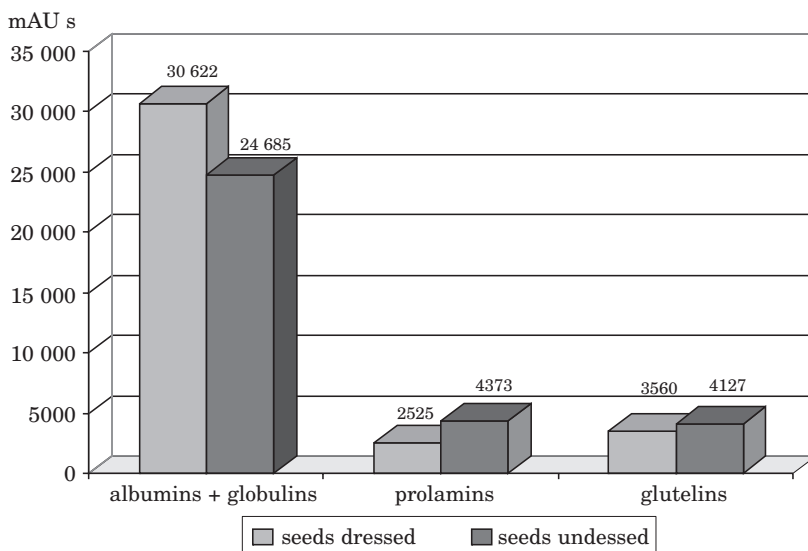


Fig. 2. Content of protein fractions in buckwheat seeds (calculated as mAU s)

Conclusions

1. Fungicide treatment increased buckwheat seed yield.
2. Gas exchange parameters were similar in both experimental variants.
3. The results of traditional analyses showed similar levels of fungal infections in both treatments. The presence of toxic fungi of the genus *Fusarium* was noted only in the second year of the study.
4. The results of PCR analyses indicate that the seeds of buckwheat cv. Kora were colonized by fungi of the genus *Fusarium* with *Fusarium poae* being the predominant species.

5. The presence of fusariotoxins was not determined in buckwheat seeds.
6. Fungicide treatment had no significant effect on the content of the analyzed protein fractions.

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THE COMPARISON OF HORSES MANAGEMENT CONDITIONS IN THE BOX STALL STABLE AND THE HORSE-BARN

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Key words: box stall stable, horse-barn, microclimate, welfare.

Abstract

The aim of this study was to compare the breeding environmental conditions in the box stall stable and the horse-barn. Assessment of the breeding environment was performed on the basis of the stable area and the cubic capacity as well as on microclimate measurements over the autumn and winter period. The results of the study indicate that both the box stall stable and the horse-barn provided a proper areal and cubic conditions, but the microclimate conditions were better in the stall box stable. In the horse-barn most of microclimate parameters (air temperature, relative humidity, air movement, cooling power and natural illumination conditions) exceeded the allowable level in larger degree than in the box stall stable. The dust pollution level in both stables, did not exceeded the allowable level and its size dependent on work conducted in the stables.

PORÓWNANIE WARUNKÓW UTRZYMANIA KONI W STAJNI BOKSOWEJ I BIEGALNI

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Słowa kluczowe: stajnia boksowa, biegałnia, mikroklimat, dobrostan.

Abstrakt

Celem pracy była ocena i porównanie warunków utrzymania koni w stajni boksowej i białalni. Ocenę wykonano na podstawie wskaźników powierzchniowo-kubaturowych oraz wyników pomiaru mikroklimatu w okresie jesienno-zimowym. W badaniach wykazano, że oba obiekty zapewniały prawidłowe wskaźniki powierzchniowo-kubaturowe, natomiast w stajni boksowej panowały korzystniejsze warunki mikroklimatyczne. W białalni większość parametrów mikroklimatycznych (temperatura powietrza, wilgotność względna, prędkość ruchu powietrza, ochładzanie i fotoklimat) w większym stopniu niż w stajni boksowej odbiegało od zalecanych norm zoohigienicznych. Poziom zapylenia powietrza w obu obiektach nie przekraczał wartości dopuszczalnych, a jego wielkość zależała od rodzaju wykonywanych prac w obiektach.

Introduction

In Poland, horses in mass breeding system are kept in three types of stables: tether stalls stables, box stall stables and horse-barns. Boxes and horse-barns are much more comfortable for horses than tether stalls. Boxes allow each animal to have its own space and personal management routine according to the wishes of individual owners (WARAN 2002). In the horse-barn horses are group housed, which gives them opportunity to both: free movement and social interactions between members of a team. It is cost effective and also relatively low maintenance. The main concern with the system relates to the risk of injury resulting from aggression between animals, and also the fact that some aspects of horses' health are more closely dependent upon that of other members of the group (WARAN 2002). But regardless of type of a stable, their main role is to provide favourable environmental conditions, which are one of the most important elements guaranteeing animals; proper health, condition, and productivity, that is – a very good welfare. Microclimate conditions play an important role, in assuring the good welfare (JEZIEFSKI, JAWORSKI 2006). The basic values of the quality of stable microclimate are: natural illumination conditions, thermal-humidity conditions and the level of air cleanliness. The illumination is the element, which influences the development, psyche and reproduction functions of animals (BETLEJEWSKA-KADELA 1990). Air temperature, relative humidity, air movement, cooling power are not less important, and they should be correlated in such a way to create the best as possible thermal-humidity conditions (PRUCHNIEWICZ 2003). The resultant of air temperature, humidity, air movement, solar radiation intensity and temperature of inside construction surfaces is the cooling rate, which is used to the complex evaluation of the climate factors impact on living organisms (KOŚLA 2001). The optimal microclimate of buildings, where animals are kept, is the basic condition to raise healthy herds of animals. However, the maintenance of microclimate parameters in stables is one

of main problems in horse husbandry. The conditions in which horses are kept are also important due to the Common Agricultural Policy (CAP) reforms in EU countries, which brings changes principles in receiving direct supplement by farm owners. Since 2013 the condition to obtain such supplement is going to be the fulfillment of cross-compliance stipulation involving the animal welfare obligation. Due to this fact, the owners of the stables are going to be interested in such breeding systems, that will be the easiest to provide the best welfare conditions to their horses.

The aim of this study was to compare the breeding environmental conditions in the box stall stable and horse-barn.

Material and Methods

The study was carried out over a period of four months in autumn and winter (November- February) in the box stall stable and the horse-barn. They were located in the north-eastern region of Poland. Both buildings differed in general area, surface area per one horse, cubic capacity and existence of windows in the building. Basic areal and cubic conditions indexes are shown in Table 1.

Table 1

Area-cubature indices of stable buildings

Specification	Box stall stable	Horse-barn
Number of housed horses	17	16
General area [m ²]	452.2	229.6
Box area surface/ area surface per one horse [m ²]	10.5	14.3
Cubature of the building [m ³]	994.8	1115.8
Cubic capacity per one horse [m ³]	58.5	69.7

Box stall stable was a building from the 18th century, completely renovated in 1996. The long axis run in the north-south direction. The length of the building was 32.3 m, width 14.0 m, height 2.2 m. Cubature of the building came to 994.8 m³, whereas cubic capacity 58.5 m³. The building had a total usable area of 452.2 m². Seventeen box stalls, for seventeen recreational horses, with a surface area of 10.5 m² (3.5 × 3.0 m) formed two rows separated by a corridor. In both gable walls were entrance gates divided into four sections that opened individually to the outside. In side walls there were ten windows measuring 1.75 × 0.73 m and five windows measuring 1.2 × 0.9 m. The loft of the stall box stable was the place, where the straw and hay was kept.

Horse-barn was a building converted from a shed. There were 16 weanling horses kept in the horse-barn stable. The long axis run in the north-south direction. The length of the building was 20.50 m, width 11.20 m, height 4.86 m. Cubature of the building came to 1115.8 m³, whereas cubic capacity to 69.7 m³. The building had a total usable area of 229.6 m² while surface area per one horse came to 14.3 m²/horse. The entrance gate was localized in the long wall of a building and it was divided into four sections that opened individually. There were no windows in the horse-barn stable, and the only source of daylight was the opened entrance gate. The loft was a place where hay and straw was kept.

In both stables there was a lack of planed ventilation system, and the air change was carried out by opening entrance gate (horse-barn) and entrance gate and windows (box stall stable).

Temperature and relative air humidity were measured regularly throughout the period of the study. The following momentary measurements were performed: air flow rate, cooling rate, dust pollution of air. Regular temperature and humidity measurements were carried out using LB-520 digital thermohygrometers (LAB-EL) which registered the above parameters at two-hour intervals. Thermohygrometers were placed outside and inside the box stall stable and the horse-barn at permanent monitoring points (6 points in the stall box stable and 4 points in the horse-barn stable). Thermohygrometer data were used to calculate average daily temperatures and relative humidity. Extreme values were recorded. Momentary measurements of microclimatic conditions (air flow and cooling rate) were performed at permanent monitoring once a week, three times a day at 6.00–7.00, 12.00–13.00 and 17.00–20.00 using Hill's katathermometer. Dust pollution was measured once a fortnight, three times a day- during the work conducted in the buildings in the morning and in the evening, and during the quiet time. Dust pollution was determined with the use of Zeiss conimeter. Samples were gathered from every box stall from the level of horses' heads. The classification of air dustiness in the investigated stable was determined on the basis of number of particles in 1 cm³ of air. The intensity of the dust pollution was defined as follows: medium – number of particulates to 100–200 particles/cm³, significant – number of particulates 200–300 particles /cm³, intense – number of particulates to 300–400, particles /cm³, very intense – number of particulates > 400 particles/cm³ (GRZEGORZAK et al. 1983).

The stables' natural illumination conditions were based on the W:F ratio (ratio of glazed window area to floor area), and the daylight coefficient (DC) was computed based on luxometric readouts. Daylight was measured using the L-51 luxometer (SONOPAN). All measurement and calculations were performed with methods commonly used in zoohygiene investigations (KOŚLA 2001).

The values of the investigated traits ($\bar{x} \pm \text{SD}$, min-max) were processed statistically using Statistica 8.0 PL software. The significance of differences between mean values in experimental groups was determined by a one factor analysis of variance in an orthogonal design and the new multiple range test.

Results and Discussion

From the data shown in Table 1, arises that both the surface area of the box and the surface area per one horse in the horse-barn fulfilled the recommendations. According to Ministry of Agriculture and Rural Development Regulation (Rozporządzenie... Dz.U. z 2010 r., nr 116, poz. 778) the minimal surface area of the box should not be less than 9 m². WARAN (2002) recommends different dimension of boxes according to the size of a horse. For ponies advised dimension is 9 m² whereas for large breeds not less than 13 m². The box stall area of investigated stable measured 10.5 m², thus it fulfilled the norm contained in the Regulation. However as it comes to loose system barns, including horse-barns the recommended surface per one adult horse should be 10 m² (WARAN 2002, Rozporządzenie... Dz.U. z 2010 r., nr 116, poz. 778). The area surface per one horse in the investigated horse-barn amount to 14.3 m², therefore it was in compliance with recommendations and the Regulation.

The important element having an influence on the stable's microclimate is the cubic capacity of a building, that is a volume of air per one horse. In both investigated buildings values of cubic capacity were higher than 24–45 m³ (KOŚLA 2001) and 30 m³ recommended by FIEDOROWICZ et al. (2004).

From the data presented in Table 2 it arises that the average temperature in the horse-barn for the whole period of study was significantly lower ($P \leq 0.05$) than in the box stall stable (appropriately 3.6 and 7.5°C). During the study period extreme temperature values were also lower in the horse-barn than in the box stall stable (appropriately -7.1 and -0.9°C). Other authors revealed in the winter period also variable temperatures which values oscillated as follows: -0.3 to 10.3°C in the box stall stable (SOWIŃSKA et al. 2010), 7.8–15.9°C in the box and 5.6–14.0°C in the standing stall (BOMBIK et al. 2009) and 3.4–5.7°C (ŁOJEK et al. 2005).

The level of relative humidity during the whole period of study was on similar level in both buildings (box stall stable 91.8%, horse-barn 94.0%). It is necessary to mention that in both stables oscillation range as well as the highest extreme values were approximate, which indicates the full atmospheric water vapor content. Other authors' results show also variable values of the relative humidity of air in stables. Generally, compared to results presented in Table 2 the values were lower and they occurred in extent as follows: 49.7–80%

Table 2

Microclimatic conditions in stable buildings (\pm SD, min-max)

Parameter	Stall box stable	Horse-barn
Air temperature [°C]		
\bar{x}	7.5 ^b	3.6 ^a
SD	4.80	6.03
range	-0.9–15.5	- 7.1–13.4
Relative air humidity [%]		
\bar{x}	91.8	94.0
SD	9.30	8.55
range	70.6–100.0	71.00–100.0
Air flow rate [m s ⁻¹]		
\bar{x}	0.23 ^a	0.37 ^b
SD	0.13	0.12
Range	0.04–0.50	0.11–0.70
Katathermometric estimation of cooling [mW cm ²]		
\bar{x}	43.4 ^A	53.9 ^B
SD	4.24	9.99
range	37.39–49.45	37.47–67.12

Explanation: A, B – $P \leq 0.01$; a,b – $P \leq 0.05$

(BUDZIŃSKA-WRZESIEŃ, WRZESIEŃ 2005), 52.3–99.0% (FIEDOROWICZ, ŁOCHOWSKI 2008), 67.0–86.0% (in the box) (HOUBEN 2008).

Average values of air flow rate for the whole study period was significantly lower ($P \leq 0.05$) in the box stall stable than in the horse-barn and it reached the following values: 0.23 and 0.37 m s⁻¹. While the oscillation range of this parameter for the whole period of study was to total: 0.04–0.50 and 0.11–0.76 m s⁻¹. Other authors' results imply the fluctuation of the parameter. Obtained results were: 0.02–1.89 m s⁻¹ (SOWIŃSKA et al. 2010), 0.2–0.6 m s⁻¹ (in the box) (HOUBEN 2008) and 0.15–0.35 m s⁻¹ (ŁOJEK et al. 2005).

As crucial determinants of the horse welfare, temperature, humidity conditions and air flow rate in stables have been investigated by numerous authors (BOMBIK et al. 2009, BUDZIŃSKA-WRZESIEŃ, WRZESIEŃ 2005, CURTIS et al. 1996, FIEDOROWICZ, ŁOCHOWSKI 2008, HOUBEN 2008, ŁOJEK et al. 2005, SOWIŃSKA et al. 2010). It is specified that the temperature inside of a stable should not be lower than 5°C (Rozporządzenie... Dz.U. z 2010 r., nr 116, poz. 778, RAJCHERT 2009), the relative humidity should not exceed the level of 80% (Rozporządzenie... Dz.U. z 2010 r., nr 116, poz. 778, FIEDOROWICZ, ŁOCHOWSKI 2008), and the air flow rate 0.3 m s⁻¹ (BEK-KACZKOWSKA 2005, PIETRZAK, TIETZE 1999, KOLBUSZEWSKI et al. 1995). On the contrary according to PRUCHNIEWICZ (2003) the highest temperature during winter should not be higher than 8°C.

To relate the above results to the own study results, it ought to be stated, that the average temperature in the horse-barn was lower than 5°C, while relative humidity exceeded allowable level of 80% with the air flow rate indicating occurrence of draughts. Whereas in the box stall stable, although

the equally high air humidity, the temperature was higher and the air flow rate was lower. It can be said that the thermal conditions in the box stall stable were more favourable than in the horse-barn. The results of cooling rate, which are the resultant of concurrence of temperature, humidity and air flow rate values seem to confirm the above statement. Average values of cooling rate for whole study period were higher ($P \leq 0.01$) in the horse-barn (53.9 mW cm^{-2}) than in the box stall stable (43.4 mW cm^{-2}). Referring the above data to the recommended values of cooling rate ($29\text{--}45 \text{ mW cm}^{-2}$) (FIEDOROWICZ, ŁOCHOWSKI 2008) it needs to be said that, the top level of that scope was exceeded in the horse-barn. The maximum values of cooling rate for whole period of study surpassed the top allowable level in both buildings, but to a greater degree in the horse-barn (67.1 mW cm^{-2}) than in the box stall stable (49.5 mW cm^{-2}).

Cooling rate is believed to be a good indicator of the animals' subjective perception of cold, heat and thermal comfort, the problem of cooling in horse stables during winter was investigated by other authors (SOWIŃSKA et al. 2010, BOMBIK et al. 2009, FIEDOROWICZ, ŁOCHOWSKI 2008). FIEDOROWICZ and ŁOCHOWSKI (2008) obtained too low values of cooling rate (11.73 mW cm^{-2} with the stable gate closed and $15.08\text{--}18.69 \text{ mW cm}^{-2}$ with the stable gate open), which may indicate the probability of overheating the horses' organisms, especially during the night with the gate closed. Proper average values of the cooling rate were gained by BOMBIK et al. (2009), and they amount to: in the box stall stable 36.4 mW cm^{-2} and 41.3 mW cm^{-2} in the tether stall stable. Approximate to the top allowable level cooling rate in two box stall stables were obtained by SOWIŃSKA et al. (2010), the average values during the study period shall amount to 47.15 and 44.82 mW cm^{-2} . However, the authors revealed, that the maximum values of this index in all investigated buildings exceeded significantly allowed values (appropriately: 98.9 mW cm^{-2} ; 76.8 mW cm^{-2}).

The required supply of daylight in stables guarantees the proper development and psychological and physical form. The recommended values of window-floor (W:F) ratio should not exceed 1:15 (FIEDOROWICZ 2007, JODKOWSKA 2007). In the own investigation (Table 3) the obtained results of W:F ratio amount to: 1:25 (box stall stable), while in the horse-barn the parameter was immeasurable because of the lack of windows in the building. The daylight illumination factor in the box stall stable (0.03%) and in the horse-barn (0.01%) did not correspond with the minimal recommended value for stables of 0.5% (JODKOWSKA 2007). The other authors investigations results concerning daylight illumination condition, also indicate that not all stables provide horses with adequate exposure to daylight. In the study of two stables BUDZIŃSKA-WRZESIEŃ and WRZESIEŃ (2005) reported W:F ratios of 1:32 and 1:25, even less satisfactory W:F ratio of 1:47 was noted by ŁOJEK et al. (2005). Comparing the

results of daylight illumination from own investigation to the recommended values from zoohygiene norms, it needs to be concluded that neither box stall stable, nor horse-barn failed to fulfill the requirements in this extent. The reasons for this fact was insufficient number of windows in the box stall stable and unsuitable cleanliness of glazed windows and walls. Instead in the horse-barn there were no windows, and the only source of daylight was the open gate.

Table 3

Illumination conditions in stables

Specification	Stall box stable		Horse-barn
	number	dimension	
Windows	10 5	1.75×0.73 1.20×0.90	none
Ratio of window area to floor area [W:F]	1:25		–
Daylight coefficient DC [%]	0.03		0.01

The level of air dustiness is presented in Table 4. The data noted in this study met standards of allowable 400 particle per cm^3 (GRZEGORZAK et al. 1983), but the more fulfilling conditions were noted in the horse-barn stable. It needs to be stated that the dustiness of air depend on both: work conducted in the stable and the housing system – in the box stall or horse-barn.

Table 4

The level of air dustiness (particles/ cm^3) with the consideration of work conducted during the day

Measurement number	Activity	Box stall stable	Horse-barn
1	morning work	238	205
	quiet time	150	122
	evening work	524	208
2	morning work	250	215
	quiet time	102	109
	evening work	241	208
3	morning work	551	127
	quiet time	170	202
	evening work	262	111
4	morning work	270	132
	quiet time	129	100
	evening work	162	160
5	morning work	230	123
	quiet time	113	101
	evening work	149	130
6	morning work	233	174
	quiet time	130	116
	evening work	110	123

Concluding the received results it is possible to state that box stall stable provided more beneficial values of factors characterizing the thermal comfort of the environment (temperature, relative humidity, cooling rate, air flow) than the horse-barn. However both buildings failed to provide the proper daylight illumination. On the contrary it needs to be taken under consideration that although the microclimatic conditions were worse in the horse-barn, horses kept in this housing system had the possibility of movement on larger area surface than horses kept in the box stall stable, as well they had freedom of social interactions.

Owing to a long life span and a wide range of uses, horses have special requirements which distinguish them from other domestic animals. Building new stables, and adapting the existing buildings for stables does not always correspond with the expertise knowledge related to the environmental needs of the horse. Stall box area surface, feed mangers and automatic waters properly and functionally placed are mostly the main concern for horse stable owners. While efficient ventilation providing favorable microclimate, and also the appropriate illumination conditions, having important impact on horses; quality of life are often underestimated by both designers and users of the stables.

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THE EFFECT OF GRAIN SPECIES AND FEED ENZYMES ON PRODUCTION RESULTS, SLAUGHTER VALUE AND MEAT QUALITY IN PIGS

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Key words: grains, feed enzymes, growth performance, slaughter value, meat quality, pigs.

Abstract

The objective of this study was to determine production results, slaughter value and meat quality in growing-finishing pigs fed complete diets containing various grain species, supplemented with exogenous feed enzymes. The feeding trial was performed on 36 crossbred (Polish Large White x Polish Landrace) x Duroc pigs divided into six experimental groups fed the following complete grower and finisher diets: diet *B* (control) based on barley, diet *W* based on wheat and diet *T* based on triticale. Diets *B*, *W* and *T* were supplemented with microbial β -glucanase and xylanase, pentosanase, hemicellulase and pectinase. Apart from the above grains and enzymes, the diets contained also a grower and finisher protein concentrate composed of soybean meal, rapeseed meal, faba bean, pea, and mineral, vitamin and amino acid supplements. Pigs were fattened from 30 kg to 105 kg body weight. Growth performance, the feed conversion ratio, the slaughter value of pigs, the chemical composition, lipid profile, cholesterol content, physicochemical and sensory properties of *m. longissimus dorsi lumborum* were determined.

Higher daily gains and better feed conversion were noted in pigs fed wheat and triticale, in comparison with those fed barley. The carcasses of pigs fed barley were characterized by a higher fat content and a lower lean content as well as worse chemical composition and higher water-holding capacity, compared with the carcasses of pigs fed triticale. Similar carcass quality traits were determined in the groups fed wheat. A higher improvement growth performance, slaughter value and pork quality was observed following enzyme supplementation of a diet containing triticale, in comparison with wheat- and barley-based diets.

WPLYW GATUNKU ZBOŻA I ENZYMÓW PASZOWYCH NA EFEKTY TUCZU, WARTOŚĆ RZEŻNĄ I JAKOŚĆ MIĘSA ŚWIŃ

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S ł o w a k l u c z o w e: zboża, enzymy paszowe, efekty tuczu, wartość rzeźna, jakość mięsa, tuczniki.

Abstrakt

Celem badań było określenie efektów tuczu, wartości rzeźnej i jakości mięsa tuczników żywionych mieszankami pełnoporcjowymi zróżnicowanymi gatunkiem zboża i uzupełnionymi enzymami paszowymi. Badanie żywieniowe przeprowadzono na 36 tucznikach mieszańców ras (wielka biała polska x polska biała zwisłoucha) x Duroc, podzielonych na 6 grup doświadczalnych żywionych mieszankami pełnoporcjowymi grower i finisz: mieszanka B (kontrolna) z udziałem jęczmienia, W – z pszenicą i T – z pszenżytem. Mieszanki paszowe B, W i T były uzupełnione mikrobiologiczną β -glukanazą i ksyłanazą, pentoanazą, hemicelulazą i pektinazą. Oceniane zestawy paszowe oprócz wyszczególnionych zbóż i enzymów paszowych zawierały koncentrat białkowy grower i finisz obejmujący poekstrakcyjną śrutę sojową, rzepakową, nasiona bobiku, grochu oraz dodatki mineralno-witaminowo-aminokwasowe. Tucz przeprowadzono na zwierzętach o masie ciała od 30 do 105 kg. Oceniano przyrosty dobowe, wykorzystanie paszy, wartość rzeźną tusz tuczników, skład chemiczny, wskaźniki profilu lipidowego, poziom cholesterolu, właściwości fizykochemiczne i sensoryczne mięśnia *longissimus dorsi lumborum*.

W wyniku przeprowadzonych badań stwierdzono lepsze przyrosty dobowe i wykorzystanie paszy u tuczników otrzymujących w dawce pszenicę i pszenżyto niż u zwierząt żywionych jęczmieniem. Tusze pochodzące od tuczników karmionych mieszanką zawierającą jęczmień charakteryzowały się większym otluszczeniem, mniejszą mięsnością oraz gorszym składem chemicznym i większą wodochłonnością w porównaniu z mięsem świń żywionych pszenżytem. Były one porównywalne do mięsa tuczników otrzymujących w dawce pszenicę. Dodatek enzymów paszowych do analizowanych zestawów paszowych poprawił w większym stopniu efekty tuczu, wartość rzeźną i jakość mięsa świń żywionych mieszanką z udziałem pszenżyta w porównaniu z mieszanką zawierającą pszenicę i jęczmień.

Introduction

In pig nutrition, cereal grains are the main source of energy accumulated in carbohydrates which account for 60 to 70% grain dry matter. Ground barley, wheat and triticale are used as components for both traditional farm-made feeds and commercial diets. The above cereals contain considerable amounts of non-starch polysaccharides (NSP), including β -glucans and arabinoxylans (BHATTY et al. 1991), whose adverse effects on nutrient digestibility and production results have been described by BEDFORD et al. (1992) and PARTRIDGE (2001). The nutritive value of cereal-based diets for pigs could be increased by adding exogenous feed enzymes (LYNCH et al. 2007, O'SHEA et al. 2010).

Nutritional regime is a key environmental factor affecting production results and meat quality. Previous research has demonstrated that the tissue composition, quantity and quality of meat can be modified by changing the levels of protein and metabolizable energy in the ration fed to pigs (WIESEMÜLLER 1996, WOOD et al. 2003). The type of feed and feed additives may affect production results and slaughter quality as well as the chemical composition, physicochemical and nutritional properties of pork (GRELA 2004, MIGDAŁ et. al. 2004).

The objective of this study was to determine growth performance, slaughter value and meat quality in growing-finishing pigs fed complete diets containing various grains, supplemented with feed enzymes (β -glucanase, xylanase, pentosanase, hemicellulase and pectinase).

Materials and Methods

The feeding trial was performed on 36 crossbred (Polish Large White x Polish Landrace) x Duroc pigs divided into six experimental groups, as follows:

Item	Grower and finisher diets					
Grain species	barley (<i>B</i>)		wheat (<i>W</i>)		triticale (<i>T</i>)	
Feed enzymes	–	+	–	+	–	+

–/+ – absence/presence of feed enzymes: β -glucanase, xylanase, pentosanase, hemicellulase and pectinase;

The content of enzyme supplements in grower and finisher diets: Ronozyme VP – 0.05%, Ronozyme WX – 0.04%.

Experimental groups received complete grower and finisher diets based on grains supplemented or not supplemented with enzymatic preparations: Ronozyme VP containing microbial β -glucanase (50 FBG g⁻¹), pentosanase, hemicellulase and pectinase, and Ronozyme WX containing microbial xylanase (1000 FXU g⁻¹). The grain (B, W, T) content of grower and finisher diets ranged from 69 to 71% and from 76 to 78%, respectively. Apart from the above grains and enzymes, the diets contained also a grower and finisher protein concentrate composed of soybean meal, rapeseed meal, faba bean, pea and mineral, vitamin and amino acid supplements. The concentrate content of experimental diets was determined by grain species, and it ranged from 29 to 31% in grower diets and from 22 to 24% in finisher diets. The experimental grower and finisher diets contained 165/145 kg⁻¹ crude protein and 12.7–13.0/12.7–13.1 MJ ME kg⁻¹, respectively. Pigs had free access to feed and water.

Daily gains and feed conversion were determined for the entire fattening period (from 30 to 105 kg body weight). Carcass quality parameters were assessed on all 36 animals. Carcass dressing percentage, carcass conformation and fatness (according to the EUROP classification system) were determined immediately after slaughter, using a CGM 100 ultrasound grading probe. Measurements were performed at the level of the last thoracic vertebra and 7 cm from the dorsal midline. The weight of loin and ham was determined on carcasses chilled for 24 hours at 2–4°C, and samples of *m. longissimus dorsi lumborum* were collected to analyze the chemical composition of meat, including the content of dry matter, crude ash, total protein and intramuscular fat, by standard methods (*Official Methods...* 1990). Lipids, extracted as described by FOLCH et al. (1957), were assayed for fatty acid concentrations using a PV 4600 gas chromatograph equipped with a flame-ionization detector (FID). Total cholesterol levels were also determined in lipid samples of *m. longissimus dorsi lumborum*, by the method proposed by ARNETH and AL-AHMAD (1995). The values of pH₄₅ and pH₂₄ were measured using a Radiometer pH-meter with a PHC 4406 electrode, the water-holding capacity of pork was estimated by the Grau and Hamm method (OECKEL VAN et al. 1995) and meat color was assessed in the CIELAB (L*, a*, b*) system, using a Minolta CR-200 chroma meter. The sensory attributes of pork were evaluated on a five-point scale, in accordance with the Polish Standard (*Analiza sensoryczna...* PN-ISO 4121:98 (1998)).

The obtained results were processed by a two-factorial analysis of variance (ANOVA) and Duncan's multiple range test, using STATISTICA software (2009).

Results and Discussion

Data in Table 1 show that higher daily gains (from 3.8% to 4.3%) and better feed conversion (from 5.2% to 6.2%) were observed in pigs fed diets W (wheat) and T (triticale), compared with diet B (barley). Diet supplementation with enzymatic formulas containing microbial β -glucanase and xylanase, hemicellulase, pentosanase and pectinase increased the growth rate of pigs (from 2.2% to 2.5%) and decreased the feed conversion ratio (from 1.6% to 3.8%). Feed enzymes exerted a more profound effect in the groups fed diet T (triticale). The production results noted in our study were very good and comparable with those reported by other authors for diets based on barley, wheat (GARRY et al. 2007) and triticale (FLIS et al. 2003).

Table 1
Production results and slaughter quality traits

Specification	Groups*						SEM	Grains (G)			Enzymes (E)		Interaction (G x E)			
	B			W				T			B	W		T	-	+
	-	+		-	+			-	+							
Grain species																
Feed enzymes																
Daily gain from 30–105 kg BW																
[g]	808	828	839	841	843	882	10.44	818	840	863	830	850	0.39			
[%]	100	–	103.8		104.3			100	102.7	105.5	100	102.4				
	100	102.5	100	102.2	100	104.6										
Feed conversion ratio from 30–105 kg BW																
[kg/kg]	3.10	3.05	2.94	2.92	2.91	2.80	0.03	3.08	2.93	2.86	2.98	2.92	0.42			
[%]	100	–	94.8	–	93.8			100	95.1	92.8	–	–				
	100	98.4	100	99.3	100	96.2					100	97.9				
Dressing percentage [%]	78.31	78.44	79.11	77.45	79.36	78.60	0.61	78.37	78.28	78.98	78.92	78.16	0.09			
Back fat thickness [mm]	17.20	17.13	17.50	17.26	16.32	14.25	0.59	17.16	17.38	15.30	17.00	16.21	0.42			
Meat content of carcass [%]	50.21	50.63	50.61	50.78	51.15	52.34	0.75	50.42	50.69	51.74	50.65	51.25	0.27			
Weight of ham [kg]	10.95	10.89	11.03	11.40	11.78	11.91	0.17	10.92	11.21	11.85	11.25	11.40	0.56			
Weight of loin [kg]	2.64 ^B	2.69 ^B	2.72 ^B	2.67 ^B	2.79 ^B	3.06 ^A	0.04	2.67 ^B	2.70 ^B	2.93 ^A	2.72 ^B	2.81 ^a	0.00			

* B – barley (control group); W – wheat; T – triticale;

-/+ – absence/presence of feed enzymes: β -glucanase, xylanase, pentosanase, hemicellulase and pectinase

a, A... Within rows means bearing different superscripts differ significantly at: small letters – $P \leq 0.05$; capitals – $P \leq 0.01$

SEM – standard error of mean

The values of carcass dressing percentage were high (from 78.31 to 79.36%) and similar in all experimental groups (Table 1). The carcasses of pigs fed triticale and a grower and finisher protein concentrate (diet *T*) were characterized by an insignificantly lower fat content and a higher lean content, compared with the carcasses of pigs fed wheat (diet *W*) and barley (diet *B*). Highly significant differences in loin weight were found between pigs fed diet *T* and those fed diets *W* and *B*. No differences between groups were reported with respect to ham weight. The addition of microbial β -glucanase, xylanase, pentosanase, hemicellulase and pectinase to experimental diets containing various grains slightly improved the analyzed carcass quality parameters (except for dressing percentage). The slaughter value indicators noted in our study were typical of pork and comparable with the findings of other authors (SOBOTKA 2000, ROTH-MAIER et al. 2004, GARRY et al. 2007).

Pork quality was evaluated based on the chemical composition and nutritional, physicochemical and sensory properties of *m. longissimus dorsi lumborum* (Table 2 and Table 3). Meat from pigs fed a barley-based diet containing a grower and finisher concentrate had an insignificantly lower total protein content and a higher intramuscular fat content, in comparison with meat from pigs fed wheat- and triticale-based diets. The intramuscular fat levels noted in the present experiment were within the 2.32–2.15% range, considered optimal for pork (ŁYCZYŃSKI et al. 2003). Diet supplementation with feed enzymes resulted in a further decrease (from 5.7 to 11.7%) in the intramuscular fat content of *m. longissimus dorsi lumborum*, from 2.32 to 2.19% in the group fed diet *B*, from 2.29% to 2.16% in the group fed diet *W* and from 2.15% to 1.90% in the group fed diet *T*. The investigated experimental factors had no significant effect on the nutritional properties of pork. The fatty acid profile and total cholesterol concentrations were typical of this animal species.

The values of pH_{45} were similar in meat samples of all experimental groups, ranging from 6.08 to 6.12, whereas pH_{24} varied within a wide range of 5.45 to 5.56 depending on grain species. The lowest pH_{24} value was reported in meat from pigs fed triticale (diet *T*), and the highest – in meat from pigs fed wheat (diet *W*). The applied feed enzymes had no significant effect on meat acidity. The examined pork samples were characterized by normal quality, with pH_{45} and pH_{24} in the 5.6–6.8 and 5.2–6.4 range, respectively (MELLER et al. 1977).

The water-holding capacity of meat is considerably affected by acidity. Feeding diets based on three different grain species to growing-finishing pigs had a statistically insignificant effect on the water-holding capacity of pork, which reached the highest level of 8.61 cm² in the groups fed barley (diet *B*), followed by 8.41 cm² and 8.43 cm² in the groups fed wheat and triticale (diets *W* and *T* respectively). An increase in water-holding capacity was observed

Table 2
Chemical composition and lipid profile of *longissimus dorsi lumbarum* muscle

Specification	Groups*						SEM	Grains (G)			Enzymes (E)		Interaction (G x E)
	B		W		T			B	W	T	-	+	
Grain species	-	+	-	+	-	+							
Feed enzymes													
Dry matter [%]	25.36	25.41	25.99	25.73	25.82	25.71	0.22	25.38	25.86	25.76	25.72	25.61	0.65
Crude ash [%]	1.11	1.13	1.15	1.14	1.18	1.17	0.01	1.12	1.15	1.17	1.14	1.15	0.12
Crude protein [%]	21.14	21.24	21.96	22.11	21.67	21.82	0.08	21.19	22.04	21.74	21.59	21.72	0.85
Intramuscular fat [%]	2.32	2.19	2.29	2.16	2.15	1.90	0.09	2.26	2.23	2.03	2.25	2.08	0.24
SFA [%]	55.29	54.98	54.05	55.11	55.01	56.32	2.05	55.13	54.58	55.67	54.78	55.57	0.44
UFA [%]	44.71	45.02	45.95	44.89	44.99	46.69	1.77	44.86	45.42	45.84	45.22	45.53	0.55
MUFA [%]	37.15	36.99	37.97	36.18	37.22	39.04	1.69	37.07	37.07	38.13	37.44	37.40	0.33
PUFA [%]	7.56	8.03	7.98	8.71	7.77	7.65	0.97	7.79	8.34	7.71	7.77	8.13	0.26
Total cholesterol [mg/100 g]	77.32	68.62	72.72	74.41	74.40	83.51	4.89	72.97	73.56	78.96	74.81	75.51	0.12

* Explained at the bottom of Table 1
SFA – saturated fatty acids; UFA – unsaturated fatty acids (MUFA+PUFA); MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids;
SEM – standard error of mean

Table 3
Physico-chemical and sensory properties of *longissimus dorsi lumborum* muscle

Specification	Groups*						SEM	Grains (G)			Enzymes (E)		Interaction (G x E)
	B		W		T			B	W	T	-	+	
	-	+	-	+	-	+							
Grain species													
Feed enzymes													P<
Water holding capacity [cm ²]	8.61	8.82	8.41	8.67	8.43	9.35	0.25	8.72	8.54	8.89	8.48	8.95	0.53
Colour meat:													
L*	59.68 ^b	58.38 ^b	62.13 ^a	59.66 ^b	60.48	59.41 ^b	1.95	60.03	60.89	59.94	60.76 ^a	59.15 ^b	0.33
a*	7.85 ^{ab}	7.69 ^{ab}	7.27 ^b	8.07 ^a	8.01 ^a	7.96 ^{ab}	1.19	7.77	7.67	7.99	7.71	7.91	0.40
b*	14.97	14.56	15.37	14.76	15.65	15.36	0.72	14.76	15.06	15.50	15.32	14.89	0.53
pH ₄₅	6.12	6.09	6.10	6.19	6.11	6.08	0.03	6.11	6.14	6.10	6.11	6.12	0.35
pH ₂₄	5.54 ^A	5.55 ^A	5.56 ^A	5.53 ^A	5.45 ^B	5.46 ^B	0.01	5.55 ^A	5.54 ^A	5.45 ^B	5.52	5.51	0.25
Aroma – intensity	5.0	5.0	5.0	5.0	5.0	5.0	–	5.0	5.0	5.0	5.0	5.0	–
Aroma – desirability	5.0	5.0	5.0	5.0	5.0	5.0	–	5.0	5.0	5.0	5.0	5.0	–
Tenderness	4.48	4.56	4.58	4.50	4.91	4.42	0.11	4.52	4.54	4.67	4.66	4.49	0.27
Juiciness	4.26	4.32	4.16	4.41	4.17	4.25	0.06	4.29	4.28	4.21	4.19	4.33	0.73
Taste – intensity	4.65	4.59	4.83	4.75	4.71	4.42	0.06	4.62	4.79	4.56	4.73	4.59	0.74
Taste – desirability	4.65	4.55	4.83	4.67	4.72	4.67	0.05	4.60	4.75	4.70	4.73	4.63	0.87

* Explained at the bottom of Table 1

aA... Within rows means bearing different superscripts differ significantly at: small letters – $P \leq 0.05$; capitals – $P \leq 0.01$

SEM – standard error of mean

in meat from pigs fed enzyme-supplemented diets, and it was highest in the group receiving a triticale-based diet.

Another important determinant of meat quality is color whose parameters are affected by the traits analyzed above. The mean values of color parameters in the CIELAB system, i.e. $L^* = 59.68\text{--}62.13$, $a^* = 7.27\text{--}8.07$, $b^* = 14.56\text{--}15.65$, indicate that the analyzed pork samples had a typical, normal color, with a low contribution of redness and yellowness.

Neither grain species nor feed enzymes exerted a significant influence on the sensory attributes of pork, which was characterized by a highly desirable aroma, desirable tenderness, juiciness and palatability.

Conclusions

It may be concluded that diets containing wheat or triticale and a grower and finisher protein concentrate composed of soybean meal, rapeseed meal, and legume seeds fed to pigs, contribute to better fattening results than barley-based diets. Grain species had no effect on slaughter value and carcass quality parameters. Diet supplementation with β -glucanase and xylanase supports an improvement in production results, slaughter value and meat quality, primarily in pigs fed triticale-based diets.

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INFLUENCE OF TRANSPORT ON CADMIUM, LEAD AND ARSENIC CONTENT IN SOIL

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Key words: heavy metals, soil, motorization, traffic.

A b s t r a c t

The purpose of the study was showed the transport effect on cadmium, lead and arsenic content in soil. The research was carried out in Morąg city in the places with high traffic intensity near garden plots, agricultural areas, wasteland and birchen woodland and at different distance from the route, and near Skierniewice lake situated with a long way to roads. There were analyzed pH, granulometric composition and humus, cadmium, lead and arsenic content in soil. It was showed the lead pollution of soil near the busy roads. Moreover, there was more cadmium content, but in the acceptable level.

WPLYW KOMUNIKACJI NA ZAWARTOŚĆ W GLEBIE KADMU, OŁOWIU I ARSENU

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Słowa kluczowe: metale ciężkie, gleba, motoryzacja, natężenie ruchu.

A b s t r a c t

Celem przeprowadzonych badań było określenie wpływu ruchu komunikacyjnego na zawartość kadmu, ołowiu i arsenu w glebie. Przeanalizowano punkty o dużym natężeniu ruchu samochodowego w Morągu. Uwzględniono tereny ogródków działkowych, gruntów użytkowanych rolniczo, nieużytku i młódziny brzozy znajdującej się w różnej odległości od tras komunikacyjnych oraz położoną w dalszej odległości od dróg okolicę jeziora Skierniewice. Program badawczy obejmował analizę gleb z uwzględnieniem pH, składu granulometrycznego oraz zawartości próchnicy, kadmu ołowiu i arsenu. Stwierdzono, że w pobliżu ruchliwych tras komunikacyjnych gleba była zanieczyszczona ołowiem. W glebie było również więcej kadmu, ale w ilościach nieprzekraczających dopuszczalnych norm.

Introduction

The estimate of the different factors and processes which influence on soil quality is one of the leading and still topical problem. The physical, chemical or biological soil properties changes for the worse can decide not only about fertility decreased but even about total production excluding. The factors responsible for the degradation of the soil environment include an excessive cumulation of heave metals. They occur as natural component in nature, but belong to particularly dangerous elements, which create a potential risk to the biological environment and decide about human health (CURZYDŁO 1995, ZIMDAHL, SKOGERBOE 1997, GORLACH, GAMBUS 2000, BIELIŃSKA 2005, AELION et al. 2009, DOMSKA, WARECHOWSKA 2009). The elements with a very high risk degree counted among others cadmium, lead, copper and zinc, and those of a medium risk degree – arsenic. Mainly, in this problem, the attention is to heavy metals content in the surface area of soil (BIERNACKA, MAŁUSZYŃSKI 2007, NIEDŹWIECKI et al. 2007).

The soil pollution of heavy metals is showing on town areas with the high urbanization degree, near the industrial factories and the communication roads (CURZYDŁO 1995, GAŁKA, SZERSZEŃ 1996, INDEKA, KARACZUN 1999, WIATER 1999, STEINER et al. 2007, CHRISTOFORIDIS, STAMATIS 2009). In this case, the most changes of soil are as effect of human activity, while the natural factors (matrix, climate, landform features) have secondary importance (OLEŚKÓW 2007).

The purpose of our investigation was determination of cadmium, lead and arsenic content in soil area adjacent to the busy route of the Morąg town.

Experimental Procedures

The investigation was carried out in 2007 in the Morąg town near the communication roads with intensity the road traffic (Figure 1). The study sites were on the area of town allotments (sites 1 and 2), cultivated field (sites 3–5), waste land (site 6), birchen woodland (sites 7 and 8) and near Skiertąg Lake (site 9).

Soil was sampled from the surface soil layer about 2 m by the diagonally method and, near the communication road at the distance of 20 and 250 m (sites 1 and 2) and 20, 250 and 500 m (sites 3–5 and 6–8). In mean soil samples (formed by mixing 10 individual samples), the granulometric composition was determined by areometric Bouyoucos method modified by Cassagrande and Prószyński, pH – electrometrically in 1 mol dm⁻³ KCl, the humus content – according to Tiurin, and the contents of cadmium, lead and arsenic

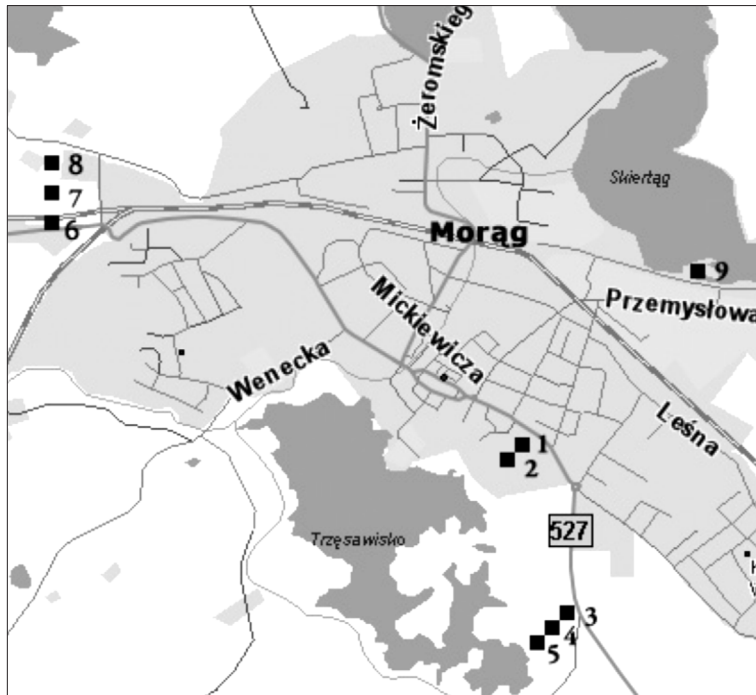


Fig. 1. The research stands: 1, 2 – garden plots, 3, 4, 5 – agricultural area, 6 – wasteland, 7, 8 – birchen woodland, 9 – near Skiertąg Lake

– with the atomic absorption spectrometry technology after a sample mineralization using nitric and hydrochloric acid.

The significance of variations has been calculated using the Tuckey's test, at the level of $p = 0.05$.

Discussion of Results

It has been found out that the study soil samples were not too much different with acidity, which was from pH of 6.5 to 7.0 (Table 1). The soil with a good agricultural fertility was only in an allotment with light loam granulometric composition and with the largest humus content (sites 1 and 2) and soil of cultivated field with strong loamy sand granulometric composition and with lower humus content (sites 3–5). The rest of soils were worse, with granulometric composition of light loamy sand and with small humus content.

Table 1

Some physical and chemical soil properties

Research stands	Granulometric composition	Acidity [1 mol dm ⁻³ KCl]	Humus [%]
1	light loam	7.0	0.5
2	light loam	7.0	0.5
3	strong loamy sand	7.0	0.3
4	strong loamy sand	6.5	0.3
5	strong loamy sand	7.0	0.3
6	light loamy sand	6.5	0.2
7	light loamy sand	6.5	0.1
8	light loamy sand	7.0	0.1
9	light loamy sand	6.5	0.2

1, 2 – garden plots, 3, 4, 5 – agricultural area, 6 – wasteland, 7, 8 – birchen woodland, 9 – near Skiertąg Lake

The physical and chemical properties of the study soils, as well as acidity, granulometric composition and humus content, like to BIENIEK (2005) studies, was not favourable for excessive cumulation of heavy metals. However, some authors (BIELIŃSKA 2005) was not showed relation between organic carbon content and granulometric composition with heavy metals content but others (MEDYŃSKA, KABAŁA 2007) think that humus show high abilities of heavy metals absorption, which makes difficult to wash them out of the soil. A high mobility of heavy metals is in the condition of acid acidity and, the soil graining typical of sands and sandy loam indicates a possibility of an occurrence of water permeability and easy migration of pollutions into the soil profile (GORLACH, GAMBUŚ 1992, GONG, DONAHOE 1997).

The cadmium content in the Polish soils amounts from 0.01 to 24.75 mg kg⁻¹ s.m., average 0.22 mg kg⁻¹, while the standard norm in the surface soils is from 1 to 3 mg kg⁻¹ s.m. (TERELAK et al. 1995).

In the area of study soils the content of cadmium was in natural norm (0°) from 0.09 to 0.81 mg kg⁻¹ s.m. (Table 2) of lower standard norms of soil quality (regulation of the Minister of Environment 2002), in amount not higher than acceptability limited content (CZARNOWSKA, BORUCKI 1996). The higher cadmium content was directly near the road to the Olsztyn in soil of the arable areas (site 3). A half lower cadmium content in soil was in a long way from the road (sites 4 and 5), in soil of an allotments (sites 1 and 2) and in soil of wasteland near the approach route to the E7 country road (site 6) depend to a small cadmium content (0.09–0.13 mg kg⁻¹) in soil near this route, but at a longer distance in the birchen woodland and in place with the most distance

from the communication roads (near Skiertąg Lake). The obtained results were not showed the clear influence of the communication activity on cadmium content in soil. However, OLEŚKÓW (2007) in the study on an allotments area of the Wrocław has been showed high cadmium content in degree from I to V, not only near communication roads, but also near big industrial plants. By some authors (MEINHARDT 1995), the most source of soil cadmium pollution is the dusts emission from a mill of non-iron metals and dusts from a smelter wastes carried out by the wind. The cadmium content in soil higher than natural values can be also connected with properties of the geological base, applying of the sewage deposits, the higher phosphorus fertilization or as effect the town dumps with industrial and energetic wastes, the remains of paints and lacquers (DOMSKA, WARECHOWSKA 2009).

Table 2

Cadmium, lead and arsenic content in soil (mg kg^{-1} s.m.)

Research stands	Cd	Pb	As
1	0.42	25.0	1.51
2	0.40	15.0	1.44
3	0.81	45.0	1.31
4	0.40	20.0	1.48
5	0.42	10.0	1.60
6	0.39	30.0	1.61
7	0.09	40.0	1.59
8	0.13	25.0	1.51
9	0.12	20.0	1.54
NIR; $\text{LSD}_p = 0.05$	0.03	0.08	0.07

* see Table 1

The lead content in the Polish soils amounts from 0.1 to 992.5 mg kg^{-1} s.m. and generally it depend on the mineral and granulometric composition and on the origin of base rocks. The lead availability also depends on soil acidity and in lower degree on humus and sorption properties of soil (TERELAK et al. 1995).

The lead content in the surface soil level of the study area was higher than in GAŁKA and SZERSZEŃ study (1996) of Oleśnica area and it was from 10.06 to 45.05 mg kg^{-1} s.m. (Table 2). In the most cases there was not higher content than limit norm of 40 mg kg^{-1} (TERELAK et al. 1995). The lead content, highest than limit norm, was in soil directly near the busy communication road to Olsztyn (site 3). Similarly, the others authors (GAŁKA, SZERSZEŃ 1996, INDEKA, KARACZUN 1999) have been showed, that the most lead content is near the communication route and it is smaller together from the roadway distance.

The own investigations carried in Morąg were showed the higher lead content, a little lower than the limit norm and near limit norm (30.02 and 40.00 mg kg^{-1}) was near the route to the E7 country road at the distance of 20 and 250 m (sites 6 and 7). By CZARNOWSKA and BORUCKI (1996) in the soils with low pollution, the lead content is from 20 to 50 mg kg^{-1} , so it can found, that in the own study experiments, only in two cases (sites 2 and 5), the soils was not polluted with lead. OLEŚKÓW (2007) has been showed the communication influence on the heavy metals pollution of soils in the study of the Wrocław vicinity and, there was average 70% of lead pollution from I to III scale degree. In the investigation of LASKOWSKI and TOŁOCZKO (1995), near the agglomerations of urbanized and industrial area was showed that lead concentration more depend on kind of the base rock, the granulometric composition or the organic substance content than on the location of the sites research in the land. However, the authors was showed that even a low content of heavy metals can be dangerous with the high soil acidity because it connected with the high participation of the available forms in total content of heavy metals.

The arsenic is element very popular in the environment. It is used in various industry branches and in agriculture as a component of pesticides, moreover is in small amounts in the all food agents (PLAK 2007, DOMSKA, WARECHOWSKA 2009). The anthropogenic sources of arsenic can be from pesticides with arsenic content and also from agents used for wood conservation or paints and lacquers production, but the most dangerous is non-ferrous metallurgy, particularly copper metallurgy and solid and liquid fuel burning (PLAK 2007). In the soil arsenic is absorbed by organic substances, ferric oxides, aluminum hydroxides and manganese compounds, and its content is highly variable and ranges from 0.1 to 95 mg kg^{-1} . The arsenic content is higher in clayey soils and soils rich in organic components, ferric, aluminum and phosphorus compounds, and in the region of the metallurgic and chemical industry, and in large urban agglomeration its concentration in soil can reach the values of as much as 2500 mg kg^{-1} (KABATA-PENDIAS, PENDIAS 1999 by PLAK 2007). The dissolvability of lead in soil and also fhyto-assimilability of its can be depended on the competitive ions content, for instance phosphate ions or iron salts (GULZ et al. 2005).

The arsenic content in soil of the Morąg select area was very low from 1.31 to 1.61 mg kg^{-1} (Table 2). These values were in the range of the acceptable standard norm not more than 20 mg kg^{-1} s.m. arsenic content (PLAK 2007). In the MEINHARDT (1995) study which has been conducted on the Wrocław province and in the Wrocław town was polluted of heavy metals, and also arsenic pollution of soils with the similar granulometric composition (light loamy sand), but with larger humus content (from 2.1 to 4.9%), directly near industrial factors, communication routes and sewage deposits (the Czechnica

Heat and Power Station and slag heap of the Siechnice Mill). Next, SZERSZEŃ et al. (1996) was showed a big influence of wastes on arsenic content in soil the Legnica province, when near of these there was until 29.8 mg kg⁻¹ s.m. of its.

Conclusion

1. There was showed that soil located in the direct distance of the busy communication routes (to Olsztyn and E7 country road) in the Morąg town was polluted with lead.

2. In the soil near the study communication routes was also more cadmium content, but in this case, it was not higher than acceptable of standard norms and law regulations.

3. There was not observed of the communication routes influence on arsenic content in soil of the study area.

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**REGULARITIES AND ANOMALIES
IN THE STRUCTURE OF GONADS IN COREGONID
FISHES***

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Key words: coregonid fishes, ovaries, testes, anomalies in the structure of gonads.

Abstract

The anatomical structure of gonads in coregonids is characteristic for teleost fishes with follicular, synchronous group tape-like ovaries and acinose type, lobulated testes. In some alpine lakes in Switzerland and in water bodies in northern Poland, certain disorders and changes in the macroscopic structure of whitefish *Coregonus* sp. gonads have been observed in the pre-spawning period. In males, the most frequently observed alteration was the division of testes into a larger cranial lobe and a smaller caudal one connected by the spermatic duct, although this has been described as a normality for the species. However, some evident anomalies in the morphological structure of ovaries and testes have been noticed, such as adherence or adhesion of lobes to the peritoneal wall and lateral muscles, asymmetry in the size of lobes, their atrophy or deep narrowing of both gonads. There have also been noted cases of hermaphroditism among both fish fry and adult coregonids. The reasons why such anomalies appear remain unclear, although it has been documented that the formation and deformities of fish gonads can be strongly affected by environmental factors, chemical substances which produce crypto-endocrine effect, pathogens and parasites. The aforementioned changes may have also been an effect of interspecies hybridization, both natural and artificial, or a result of irresponsible actions undertaken by person involve in fishery practice.

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PRAWIDŁOWOŚCI I ANOMALIE W STRUKTURZE GONAD RYB SIEJOWATYCH

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Słowa kluczowe: ryby siejowate, jajniki, jądra, anomalia struktury gonad.

Abstrakt

Ryby siejowate cechują się posiadaniem charakterystycznych dla ryb kostnoszkieletowych anatomicznych planów budowy gonad, tj. pęcherzykowych jajników grupowo synchronicznych typu taśmowatego oraz acinozowych jąder typu zrazikowego. W kilku alpejskich jeziorach Szwajcarii oraz zbiornikach północnej Polski zanotowano zaburzenia i zmiany makroskopowej struktury gonad siei *Coregonus* sp. w okresie przedtarłowym. U samców najczęściej obserwowano separację jąder na większy płat kranialny i mniejszy kaudalny połączone nasieniowodem, co jednak uznano za prawidłowość dla tego gatunku. Obserwowano ponadto wyraźne anomalie struktury morfologicznej jajników i jąder, tj.: przyleganie bądź zrośnięcie płatów ze ścianą otrzewnej i mięśniami bocznymi, asymetrię wielkości płatów, ich atrofię czy wreszcie głębokie przewężenia obu gonad. Notowano również przypadki obecności osobników hermafrodytycznych wśród narybku, jak i u dorosłych siejowatych. Przyczyny tego typu nieprawidłowości pozostają niejasne, aczkolwiek udokumentowano, że na kształtowanie i deformację gonad u ryb mogą istotnie wpływać czynniki środowiskowe, substancje chemiczne o działaniu kryptoendokrynowym, patogeny i pasożyty. Opisywane zmiany mogły być też wynikiem hybrydyzacji międzygatunkowej, tak naturalnej, jak i sztucznej będącej efektem nieodpowiedzialnych działań człowieka w warunkach hodowlanych.

The macro- and microscopic structure of gonads in teleost fishes

Gonads of teleost fishes, like those of all other vertebrates, appears as paired organs situated in the dorsal part of the body cavity near the mesentery. Although they always develop as paired organs, some adult individuals of certain species have single gonads (JOBLING 1995). Outermost, gonads are enveloped in a thin layer of fibrous connective tissue. In a transverse cross-section, they are typically oval or triangular structures. The left and the right gonad are usually clearly separated. However, sometimes they can be partly (e.g. in perch *Perca fluviatilis* L.) or completely (e.g. in guppy *Peocilia reticulata* Peters) joined (fusion) (BILLARD 1986). In some species, such as smelt *Osmerus eperlanus* (L.), testes and ovaries can be arranged in the body cavity asymmetrically, with distinct disproportion in the size of both parts

of the gonads. Consequently, the lengths of oviducts or spermatic ducts running from either of the two gonads are likewise different (KOWALSKI et al. 2006). In sexually mature salmonid or coregonid fishes, oviducts undergo involution and during ovulation mature oocytes fall into the body cavity, from which they are released straight into water through a short, funnel-like duct and the genital aperture located just posterior to the anus (TAKASHIMA and HIBIYA 1995).

Regarding the histological structure of fish gonads, they are composed of two types of cells, i.e. germ cells from which mature gametes (eggs or spermatozoa) are formed, and somatic cells, which support, nourish and regulate the activity and development of germ cells. Apart from cells, gonads also contain acellular components, such as basement membranes (NAGAHAMA 1983). Ovaries of teleost fishes, in contrast to other vertebrates, are predominantly follicular structures, which contain a distinct cavity (ovocell), into which oocytes are released during ovulation. Ovarian lamellae containing developing oocytes typically stretch alongside this ovarian cavity (HOAR 1969). Functionally, the basic component of an ovary are ovarian follicles, whose arrangement is similar in all teleost fishes. Centrally located oocytes are enveloped in a cytoplasmatic membrane and yolk layer, known also as *zona radiata* or the chorion. This layer is surrounded by a follicular theca, innermost composed of one layer of granular cells and outermost – of a layer of secretion cells producing steroids, which are accompanied by fibroblasts, collagen fibers and blood vessels (TAKASHIMA and HIBIYA 1995).

In teleost fish males, two types of microscopic structure of testes are distinguished: tubular and lobular (BILLARD 1986). In tubular testes, characteristic for such fish as percids, canaliculi (tubes) create a regular systems of structures, in which spermatozoa are formed in the peripheral part of a testis. During the final stage of spermatogenesis, they are gradually directed to the central part of a gonad (GRIER et al. 1980). In turn, lobular testes, characteristic for cyprinids, salmonids and coregonids, are composed of numerous and irregular in shape ampules (lobules), separated by a thin layer of connective tissue (Figure 1 a, c). In this case, spermatozoa are formed in ampules and during spawning they move to a large canal in the central part of a testis. Histological sections of testicular ampules during the pre-spawning period in European whitefish (Figure 1b) and vendace (Figure 1d) reveal a high degree of similarity. The central part of the structure of gonad lobules is full-filled with mature spermatozoa, while their walls are rather thin at that time, with numerous and distinctly marked Sertoli cells. The primary function of Sertoli cells is to control the development and physiological functions of germ cells. Sertoli cells are a source of hormonal substances secreted to the lumen of seminal ampules. They also act as phagocytes consuming apoptotic or damaged spermatids occurring during spermatogenesis (SCHULZ et al. 2010).

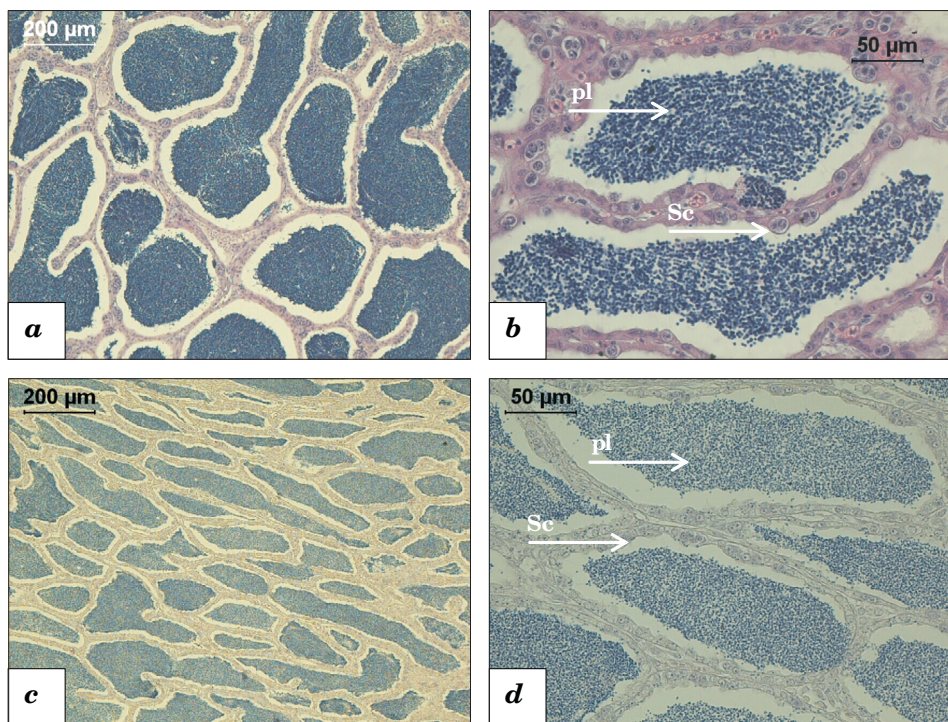


Fig. 1. Histological cross-section of testis: *a*, *b* – whitefish; *c*, *d* – vendace. Descriptions: pl – spermatozoa, Sc – Sertoli cells.

Formation of the anatomical and histological structure of gonads in coregonid fishes

According to TAKASHIMA et al. (1980), sex differentiation in gonochoristic fish follows a similar course as in reptiles. The two phases are distinguishable during the development of the reproduction system: determination, when the main role is played by sex chromosomes, and differentiation of gonads, when steroid hormones are released to regulate the proper development of male or female gonads. Under laboratory conditions, an analysis of the formation of anatomical structure of gonads and their cytological differentiation in European whitefish has been described by DLUGOSZ and DEMSKA-ZAKES (1992). The experiment realized based on material obtained during artificial reproduction of spawners captured in Isag Lake (the Mazurian Lake District).

Sex differentiation in European whitefish, similarly to other Salmoniformes (HURK VAN DEN and SLOF 1981, SACOBIE and TILLMANN 2005) ongoing through the two stages, and the anatomical differentiation of gonads was preceded by cytological differentiation. At the same time, female gonads

were always larger than male ones and were characterized by much higher intensity of karyokinetic divisions of their cells. The primordial germ cells (PGC) in gonadal anlagae of European whitefish larvae were observed from 47th day of post-embryonic development. In turn, ovaries with distinct protoplasmatic oocytes were visible in histological sections from 108th day after hatching, when the fish reached the total length from 4.2 to 4.8 cm and weighed between 780 and 1100 mg (Figure 2a). Sex differentiation towards becoming male fish was much slower as no typical testes with spermatogonial cells were observed until the termination of the experimental rearing, i.e. until 166th day after hatching (Figure 2b) (DLUGOSZ and DEMSKA-ZAKES 1992).

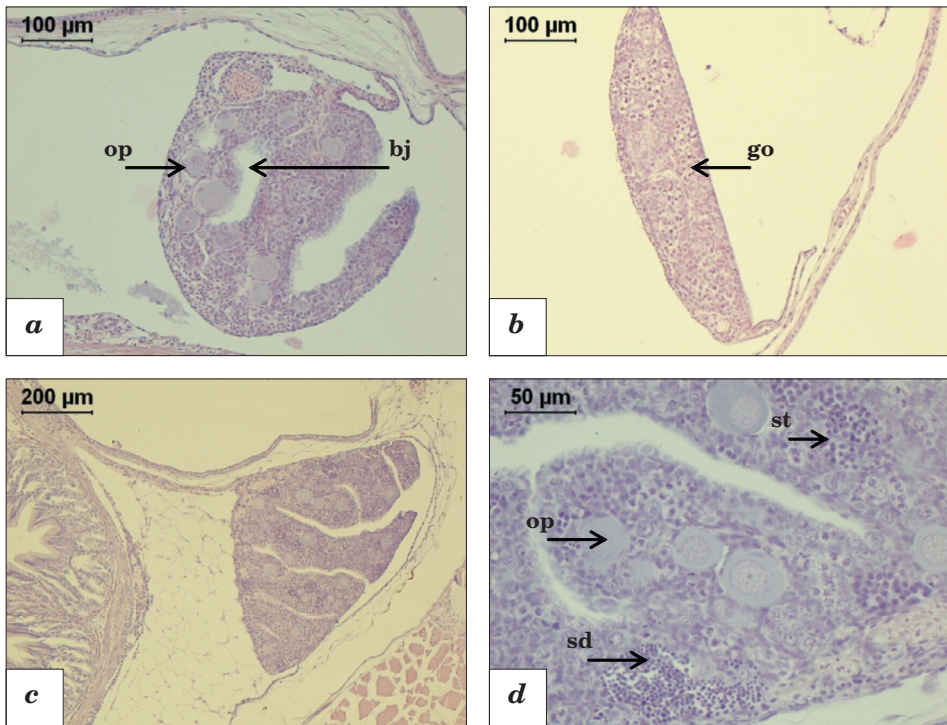


Fig. 2. Histological cross-section of coregonids gonads: *a* – an ovary whitefish on 133th day of post-embryonic development; *b* – a testis of whitefish on 166th day after hatching; *c*, *d* – a gonad of a hermaphroditic individual (a hybrid of whitefish and peled), containing female and male germ cells. Descriptions: bj – ovarian lamellae, go – gonocytes, op – previtellogenic oocytes, sd – spermatids, st – spermatocytes

A very similar course of sex differentiation has been observed in peled *Coregonus peled* Gmel., in which gonadal anlagae transform directly into male or female gonads, which means that they are differentiated gonochoristic

species. In an experiment concerning analysis of potential influence of varied rearing temperature (10, 17 and 21°C) on formation of gonads in this species, first symptoms of anatomical differentiation of gonads were noticed on 81st day of post-embryonic development in all experimental groups. At the same time, presence of first cells of the female sex line, i.e. oogonia, was confirmed in fish reared in water of the temperature close to optimal and sublethal. It was also found out that a rise in the temperature, by making the growth rate of fish more dynamic, affected directly the time and rate of sex differentiation of peled (KRÓL et al. 2003).

Anomalies in the morphological structure of gonads

Irregularities in the development of the reproduction system may occur as early as the stage of gonad formation. Anomalies during the anatomical sex differentiation have been found, for example, in hybrids of European whitefish and peled reared in cage culture (DEMSKA-ZAKES and MAMCARZ 1996). These alterations most often included occurrence of both male and female gonads, i.e. testes and ovaries, in the same individual. There were also cases when male germ cells were found next to previtellogenic oocytes in typical ovaries (Figure 2 c, d).

Our analysis of the quality of semen and the work undertaken towards improvement of its cryopreservation based on anadromous whitefish spawners from Łebsko Lake (CIERESZKO et al. 2008) demonstrated the presence of two separate, morphologically clearly distinct testicular lobes in males of this species. These consisted of a larger (cranial) and smaller (caudal) part, the latter located close to the anus (Figure 3a). Additionally, through the application of computer-assisted sperm analysis (CASA), we compared parameters of semen obtained from two fragments of testes in fish captured in two water bodies, located far from each other, that is from Łebsko Lake on the Baltic Sea coast and Gaładuś Lake, lying near the Polish-Lithuanian border. The histological sections of both fragments of testes was likewise examined. No significant differences in the histological structure or quality of semen sampled from the cranial and caudal parts of testes were found (HLIWA et al. 2008, 2010).

Similar results obtained from macroscopic observations of gonads and anomalies in their morphological structure in three ecotypes of *Coregonus* sp. during the pre-spawning period were reported by BERNET et al. (2004). In oligotrophic alpine Lake Thun (Switzerland), characteristic “separation of lobes in gonads”, repeating in many examined individuals, was observed and regarded as a normal event in whitefish. The macroscopic and microscopic analysis of gonads demonstrated that 35%, i.e. 281 out of 808 examined fish,

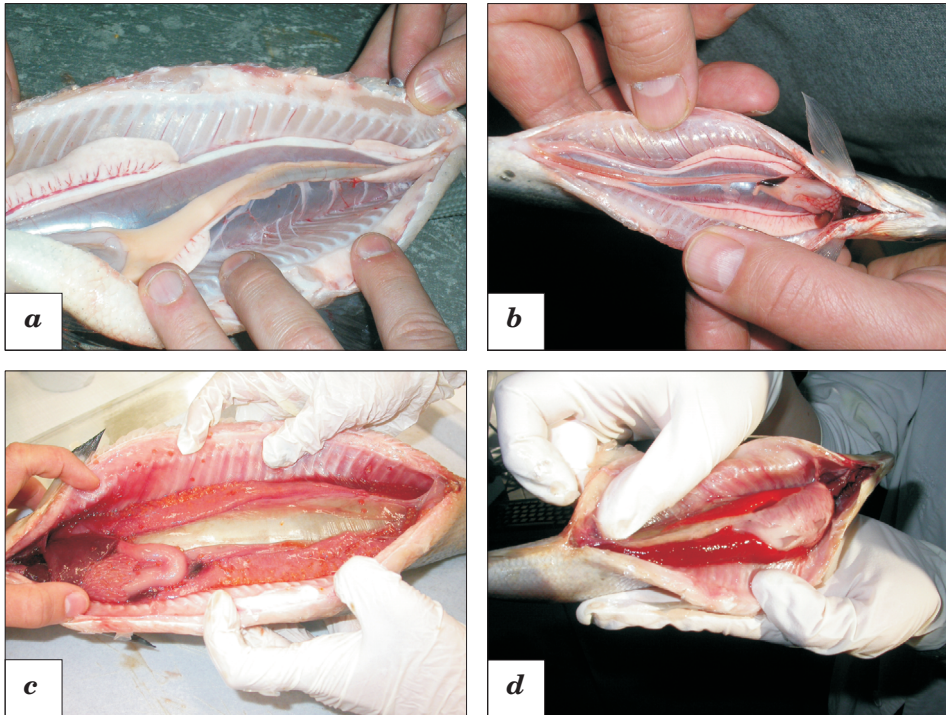


Fig. 3. The gonads of coregonids: *a* – a macroscopic image of whitefish testes during the pre-spawning period; visible is the division of gonads into the cranial (larger) and caudal (smaller) lobe; *b* – a macroscopic image of vendace testes; *c* – a macroscopic image of whitefish ovaries during the post-spawning period, visible are single oocytes remaining in the body cavity of a female; *d* – a macroscopic image of vendace ovaries during the pre-spawning period

experienced a variety of pathological changes. In general, anomalies appeared more frequently in males (40% of captured individuals) than in females (26%), and predominantly in the age groups 3+ to 5+. The morphological changes in gonads included adherence or adhesion of lobes to the peritoneal wall and lateral muscles, distinct asymmetry in size of both gonads, atrophy, segregation of lobes into subunits or their deep narrowing. Moreover, the histological analysis revealed a small percentage (1.1%) of hermaphroditic individuals, in which testicular and ovarian tissues were present, lying sequentially next to one another in gonads, or such individuals, in which one strip consisting of ovarian tissue and another one being testicular tissue were found, or finally a mosaic type of gonads, in which oocytes were set in gonads which morphologically resembled a typical testis or else spermatozoa were present in ovaries.

The morphological asymmetry of female gonads during the pre-spawning period was observed by HEESE (1990) in whitefish *Coregonus lavaretus*

(L. 1758) from the Gulf of Pomerania and the Szczecin Lagoon. In this case, the left lobe of a gonad in females was typically 15-20% shorter than the right one. However, in females of whitefish captured in the coastal area of the Łeba River or in females of the vendace *Coregonus albula* (L.), related to whitefish, and caught in Gaładuś and Hańcza Lakes in the Suwałki Lake District, no such anatomical changes in ovaries and testes were found (Figure 3 b, c, d) (HLIWA et al., unpublished).

Causes of macro- and microscopic changes in the structure of coregonids gonads

Until now, changes in the morphological structure of gonads have frequently been reported for salmonid fishes (FITZSIMONS and CAIRNS 2000, KINNISON et al. 2000, DZIEWULSKA and DOMAGAŁA 2009) and for coregonids, phylogenetically related to the former family (URBACH et al. 2008, BITTNER et al., 2009, HLIWA et al. 2010). The causes of this type of observed anomalies remain unclear, although it has been documented that the formation and deformities of fish gonads as well as the sex structure of fish natural populations can be affected by a variety of environmental factors, mainly the temperature (TESSEMA et al., 2006, BAROILLER et al. 2009), chemical substances which produce crypto-endocrine or toxic effects (JOBILING et al. 1998, NOAKSSON et al. 2001) or even parasites (WICKLUND and BYLUND 1994).

One of the most frequent genetically based cause, which may considerably determine the development and the final macroscopic structure as well as functions performed by fish gonads is interspecies hybridization (CHEVASSUS 1983). Similar dates and places of spawning as well as the spawning behavior itself favour natural and uncontrollable hybridization of coregonid fishes. Such hybridization, however, is frequently a result of thoughtless and irresponsible actions of people in aquaculture. Back in the 1980s, in eutrophic Legińskie Lake, 15.2% of hybrids of indigenous whitefish *Coregonus lavaretus* L. and introduced peled *Coregonus peled* Gmel. revealed aplasia or atrophy of one of the gonads (DEMSKA-ZAKES and MAMCARZ 1996). Moreover, hermaphroditic specimens possessing one female and one male gonad were found, and the histological assays confirmed that in gonads of fish aged 3+ there were both previtellogenic and vitellogenic (during the vacuolization stage) oocytes as well as male spermatogonial cells. In turn, BOGDANOVA (2002) described such changes in *C. peled* × *C. nasus* hybrids as degeneration of maturing oocytes or/and disrupted differentiation of ovaries.

All things considered, it cannot be excluded that a specific division into two asymmetric lobes, observed in whitefish specimens from Polish (HLIWA et al.

2010) or Swiss lakes (BERNET et al. 2004, URBACH et al. 2008) is a side-effect of long-lasting hatchery practice, including such techniques as using a limited number of spawners for artificial reproduction or selecting spawners according to phenotypes, thus diminishing genetic diversity of breeding populations (WEDEKIND 2002). Negative effects can also appear due to maintaining spawners in water recirculatory systems or using inadequate artificial feeds, which leads to certain 'domestication' of this fish species. The latter hypothesis is noteworthy because vendace living in the wild, which is not subjected to breeding practice, has not been observed to undergo segregation of testes or other atypical changes in the macroscopic image of gonads.

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THE EFFECT OF APPLICATION OF NEW SPAWNING AGENTS IN ARTIFICIAL REPRODUCTION OF WILD COMMON TENCH, *TINCA TINCA* (L.)

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Key words: Ovopel, Ovaprim, hCG, ovulation rate, induced spawning.

Abstract

The aim of this study was compare the results obtained after testing new spawning agents (Ovaprim and PG-600) in artificial reproduction of common tench in comparison to Ovopel, the spawning agent, which worked very well in this species. The tench spawners were collected from open waters. After catching, the selected fish were transported to the hatchery, where the fish were treated with the spawning agents: Ovopel at 1 pellet kg⁻¹ Ovaprim at 0.5 ml kg⁻¹ or PG-600 at 600 IU kg⁻¹. The fish from the control group received saline (0.9% NaCl). The best parameters recorded during the artificial reproduction, such as the percentage of ovulated females and embryo survival at the eyed-egg-stage were obtained in the group treated with GnRH analogues. In general, the obtained results indicate that all of the tested spawning agents might be used on successful tench artificial reproduction. However, after application of Ovopel better results were recorded.

WPŁYW ZASTOSOWANIA NOWYCH ŚRODKÓW HORMONALNYCH NA EFEKTYWNOŚĆ SZTUCZNEGO ROZRODU LINA *TINCA TINCA* (L.)

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Słowa kluczowe: Ovopel, Ovaprim, hCG, owulacja, rozród kontrolowany.

Abstract

Celem pracy było porównanie wyników kontrolowanego rozrodu lina pozyskanego z wód otwartych po zastosowaniu nowych środków hormonalnych (Ovaprim i PG-600) z wynikami uzyskanymi po użyciu Ovopelu, który był stosowany z powodzeniem u tego gatunku wcześniej.

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Krótko po odłowieniu wyselekcjonowane tarlaki przetransportowano do wylęgarni, gdzie zostały poddane stymulacji hormonalnej. Ryby otrzymały Ovopel (1 granula kg^{-1}), Ovaprim (0,5 ml kg^{-1}) lub PG-600 (600 IU kg^{-1}). Ryby z grupy kontrolnej otrzymały placebo (0.9% NaCl). Najlepsze wyniki kontrolowanego rozrodu, wyrażone jako odsetek owulujących samic, oraz najwyższą przeżywalność embrionów uzyskano w grupach, którym podano analogi GnRH. Otrzymane wyniki wskazują na wysoką skuteczność wszystkich testowanych preparatów w indukcji owulacji u lina. Najlepszy wynik jednak odnotowano po zastosowaniu Ovopelu.

Introduction

Common tench *Tinca tinca* (L.) is one of the most perspective cyprinid fish species for freshwater aquaculture (WANG et al. 2006). For many years this species had been important for both lake exploitation (MAMCARZ and SKRZYPCZAK 2006, SKRZYPCZAK and MAMCARZ 2006) and pond culture (CELADA et al. 2009). For those reasons many papers aimed at different aspects of tench reproductive cycle (BRETON et al. 1980a, 1980b, EPLER et al. 1981, HOROSZEWICZ et al. 1981), artificial reproduction (e.g. LINHART et al. 2006, MAMCARZ et al. 2006, KOURIL et al. 2008), physiology of reproduction and rearing larvae and juveniles under controlled conditions (i.e. CELADA et al. 2009). Most of them were carried out on cultured stocks (i.e. GELA et al. 2006, RODINA et al. 2007). These data were contrary to those concerning the different aspects of wild fishes reproduction (KUCHARCZYK et al. 2007). Controlled reproduction of wild spawners is much more difficult and much more irregular than the cultured ones (KREJSZEFF et al. 2009, 2010a). Thus, improvement of our knowledge regarding reproduction of wild stocks is needed. Especially, when monitoring and preventive methods are undertaken to avoid gene pool shallowing what could be linked with introduction to culture stock external gene pool (HARADA et al. 1998).

Many different hormonal combinations have been tested to induce artificial spawning of wild cyprinids, including common tench. Most commonly used were: carp pituitary homogenate (CPH), human chorionic gonadotrophin (hCG) and different forms of GnRH analogues frequently with dopamine antagonist (i.e. Ovopel) (e.g. BARTH et al. 1997, CAILLE et al. 2006, KUCHARCZYK et al. 2007, KUJAWA et al. 2009). However, the results were very often inconsistent (KUCHARCZYK et al. 1996, 1998). Thus, the new spawning agents were introduced for wild species controlled reproduction (TARGOŃSKA et al. 2010) due to decrease production costs (HAKUĆ-BŁAŻOWSKA et al. 2009, 2010) and improve gametes quality (ŹARSKI et al. 2009).

Widely applied method in artificial reproduction of wild cyprinids is recognition of oocytes maturity stage prior to hormonal injection (KUCHARCZYK et al. 1997a,b,c, 2005, 2008, KREJSZEFF et al. 2008, 2009). Due to the fact that

tench is batch spawner it is very difficult to predict effectiveness of wild stock reproduction regarding latency time and quantity of obtained eggs (KUCHARCZYK et al. 2001, KUJAWA et al. 2010). The same difficulties for other batch spawning species were reported earlier (KREJSZEFF et al. 2010a). Thus, other aspects of wild stock propagation were undertaken. It regards, among others, time of hormonal stimulation after fish collection from natural reservoirs during spawning season (KUCHARCZYK et al. 2007) and comparable investigation regarding wild and cultured stocks reproduction effectiveness (KUJAWA et al. 2010). So far, there is no published data regarding different hormonal preparations effectiveness. The aim of the study was to compare the effectiveness of three commercial preparations (Ovopel, Ovaprim and PG-600) in controlled reproduction of tench collected from natural waters (lakes) during spawning season. The tested preparations were not used for induction of spawning in common tench so far.

Material and Methods

Broodstock collection

Tench spawners were collected during two subsequent spawning seasons from wild stock in mid-June from Sasek Wielki Lake, north Poland. Females, for further treatments, were strictly selected according to the following criteria: the belly had to be fully distended and bulging, soft and resilient to touch. For experimental procedures all captured males were used. The size of spawners ranged from 0.6 to 1.2 kg. Shortly after catching, the fish were moved to the hatchery. Water temperature in the lake during fish catching was about 17°C. The selected males and females were kept in separate 1000 dm³ tanks in the hatchery with controlled temperature and photoperiod (16 hrs light and 8 hrs dark) (KUJAWA et al. 1999). Water temperature in the hatchery unit was gradually increased from 18 to 20°C. The maximum load of spawners was 25 kg m⁻³. The level of dissolved oxygen was minimum 6 ppm.

Experiment design

In each year the same experimental procedure was done. The females were randomly divided into four groups ($n = 20$ for each treatment in each year): three experimental and control one. After two days of acclimation to 20°C, the females were treated with respective single hormonal injection. Ovaprim (0.5 ml kg⁻¹), Ovopel (1 pellet kg⁻¹) and PG-600 (600 IU kg⁻¹) were used for

stimulation while the control group received saline (0.9% NaCl). Ovaprim (Syndel, Canada) is a preparation containing salmon GnRH analogue [D-Arg⁶, Pro⁹Net-sGnRH] (at 20 µg) and 10 mg dopamine antagonist (domperidone) in 1 ml solution (Peter et al. 1993). One Ovopel (Unic-trade, Hungary) pellet (average weight about 25 mg) contains mammalian GnRH analogue [D-Ala⁶, Pro⁹Net-mGnRH] (at 18–20 µg) and 8–10 mg dopamine antagonist (metoclopramide) (HORVATH et al. 1997). PG-600 (Intervet, Poland) is a preparation containing human and horse chorionic gonadotropin. Males ($n = 10$ for each treatment in each year) were randomly divided into four groups (as well as females) and received the same preparations as females but in a half of doses. All spawning agents (except Ovaprim, which is produced ready to use) were prepared with 0.9% NaCl: gonadotrophins was dissolved and Ovopel pellets were pulverized in a mortar and then dissolved. Injections were done intraperitoneally at the base of the ventral fin. When ovulation occurred collection of gametes and controlled incubation was applied. It allowed determining the reproduction effectiveness in each group. Before any manipulations fish were anaesthetized with 2-phenoxyethanol ($0.5 \text{ cm}^3 \text{ dm}^{-3}$).

Gametes management

Ripe gamete donors were anaesthetized in the solution of 2-phenoxyethanol ($0.5 \text{ cm}^3 \text{ dm}^{-3}$). Milt samples were collected with plastic syringes and kept at 4°C. Next, spermatozoa motility in each experimental group was estimated subjectively under the light microscope (magnification 500x) according to methodology described by GLOGOWSKI et al. (1999). Females were checked every hour between hours 12 and 16 after injection. Eggs were stripped into a plastic vessels. Weight of obtained eggs was noted. Two egg samples (250–300 eggs each) from each female were placed on Petri dishes. Next, eggs were mixed with 0.05 ml of pooled freshly obtained milt sample and gametes were activated with hatchery water. Eggs samples were incubated at 24°C in closed recirculation system as described KREJSZEFF et al. (2010b). During eyed-egg-stage, the survival of embryos was recorded.

Statistical analysis

The statistical analysis of the data was carried out using the STATISTICA for Windows ver. 8.0 PL software package (StatSoft) and MS Excel. All of the values expressed as percentages were arcsine transformed prior to statistical analysis. Statistical differences between groups were analyzed by the analysis of variance (ANOVA) and Tukey's *post hoc* test ($\alpha = 0.05$).

Results

Hormonal stimulation had a positive influence on achievement of spawning readiness and ovulation by the females. The largest percentage of ovulating females was recorded in the group stimulated with Ovopel (98%). That result differed significantly from those obtained in other groups stimulated with Ovaprim (80%) and PG-600 (65%) respectively between which no differences were recorded ($P > 0.05$). In case of the control group ovulation was detected in just 10% of the females (Table 1). The highest weight of obtained eggs were recorded in groups treated with preparations containing GnRHa and the lowest in the control group. During incubation of eggs obtained from the control group females the lowest percentage of live embryos at eyed-egg stage as compared to the other groups where hormonal stimulation was applied was recorded. No statistically significant differences regarding embryos survival were recorded among the experimental groups ($P > 0.05$) (Table 1). No differences in the latency time were found either and for all the groups it was 16 ± 1 hours.

Table 1
Results of controlled reproduction of wild common tench, *Tinca tinca* (L.), stock with application of different hormonal preparations

Group	Ovulation [%]		Spermiation [%]		Spermatozoa motility [%]		Embryos survival [%]		Weight of eggs per kg of females body weight	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Control	10 ^c	0	55 ^b	7	53 ^b	9	65.3 ^a	3.5	2.51 ^c	2.23
Ovaprim	80 ^b	14	85 ^a	7	81 ^a	6	88.9 ^b	2.9	13.94 ^a	2.31
PG-600	65 ^b	7	70 ^{ab}	0	75 ^a	11	85.6 ^b	6.2	8.93 ^b	1.95
Ovopel	98 ^a	3.5	90 ^a	0	80 ^a	9	86.4 ^b	4.2	14.11 ^a	2.42

Results were obtained during two subsequent reproductive seasons. Data in columns marked with different superscript were statistically different

Hormonal stimulation was observed to be efficient in induction of spermiation in males (Table 1). A better result in spermiation rate was observed after application of preparations contained GnRHa ($P < 0.05$). However, no statistical differences were noted as concerned spermatozoa motility among experimental groups. The worst results were recorded in control group (Table 1).

Discussion

Controlled reproduction of freshwater fish, including cyprinids, depends of hormonal stimulation (KUCHARCZYK et al. 1996, 1998, 2008, SZCZERBOWSKI et al. 2009, YARON et al. 2009), where the dose and type of hormonal preparation applied influence the effectiveness of reproduction directly (BRZUSKA 2006, KREJSZEFF et al. 2008). This concerns the percentage of ovulations and the survival of embryos resulting from the quality of the gametes conditioned by hormonal stimulation (CEJKO et al., 2010, ŻARSKI et al. 2009, 2010). This depends on both the mechanism of functioning of the individual hormonal preparations and their specificity for the fish organism (BRZUSKA 2006, PODHOREC and KOURIL 2009, YARON et al. 2009). In case of this study the best results obtained after application of Ovopel indicate high effectiveness of mammalian gonadoliberrine analogue (mGnRHa) in ovulation stimulation in tench. The studies conducted so far indicate that this analogue is much less specific in cyprinids as compared to the salmon analogue contained, among others, in Ovaprim, which created the necessity of the increased mGnRHa dosage administration in one injection (PODHOREC and KOURIL 2009, ŻARSKI et al. 2009, TARGOŃSKA et al. 2010).

Application of pure gonadotropins in induction of cyprinid fish reproduction caused ovulation only in batch spawners, including common tench. However, this kind of hormonal preparations effectiveness were usually worst than after stimulation with GnRH analogues (KUCHARCZYK et al. 1997a,b,c, 2005, 2007). In the present study results after application of PG-600 in females were comparable with those obtained in Ovaprim group. Such high effectiveness of PG-600 could be linked with different gonadotropins contained in this preparation (mixed human and horse gonadotropin). However, it should be studied much more closely to find which form of gonadotropins would be more effective for induction of ovulation in cyprinids.

It was previously reported that different hormonal preparations affects gametes quality significantly. The positive or negative effect of application of different hormonal preparations usually intensified when ovulation and spermiation were induced with more or less effective preparations (ŻARSKI et al. 2009, TARGOŃSKA et al. 2010). In the present study all the hormonal preparations tested offered positive influence on the quality of the gametes expressed as the percentage of live eyed-egg embryos. Only eggs obtained from the females of the control group were characterized by a significantly lower quality. These results are contrary with those obtained for other cyprinids. In ide, *Leuciscus idus* (L.), and dace, *Leuciscus leuciscus* (L.) application of Ovaprim improved gametes quality as compared to Ovopel (ŻARSKI et al. 2009). Positive effect of Ovaprim was also reported for asp, *Aspius aspius* (L.) (TARGOŃSKA et al.

2010). In those studies Ovaprim improved probably quality of eggs as well as semen quality. However, very good embryos survival in common tench is commonly observed (KUCHARCZYK et al. 2007, KUJAWA et al. 2009). Thus, positive effect of various hormonal preparations and high survival rate of tench embryos regardless of hormonal preparation used indicates that effective induction of ovulation in common tench is quite easy to achieve with various hormonal preparations. However, on the base of obtained results it should be strongly recommended to use preparations which contains GnRH analogues.

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FATTY ACID COMPOSITION AS A COEFFICIENT OF WAYS OF USAGE OILS FROM SEEDS OF DIFFERENT VARIETIES OF RAPESEED*

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Key words: rapeseed, varieties, fatty acids, omega-6 to omega-3 ratio, oils oxidizability.

A b s t r a c t

The aim of researches was to indicate effective ways of usage oils obtained from seeds of nine national varieties of rapeseed: four pollinated and two hybrid of winter rape as well as three pollinated of spring rape. Fatty acid composition was determined by gas chromatography and it was the base to determine omega-6 to omega-3 ratio as well as calculate oxidizability index.

The researches showed that shares of individual fatty acids in oil samples from analyzed rapeseed varieties were statistically different (significance at level $p \leq 0.05$). The most diversified in terms of fatty acids share, especially oleic and linolenic acids, were oils from rapeseeds of spring varieties. The most distinctive oil sample in terms of fatty acids content was oil from rapeseeds of spring variety Bios, which characterized by the lowest share of oleic acid and the highest linolic and linolenic acids shares. Moreover, this oil sample had very good omega-6 and omega-3 ratio and hence it can be conceded as the most healthy. What is more, by dint of the highest oxidizability index this oil should be recommended to consumption without heating. However, the most relevant to biodiesel production oils, because of low oxidizability index, were oils from rapeseeds of varieties: Monolit, Pomorzanin and Huzar.

SKŁAD KWAŚÓW TŁUSZCZOWYCH JAKO WSKAŹNIK KIERUNKU WYKORZYSTANIA OLEJU Z NASION RÓŻNYCH ODMIAN RZEPAKU

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Słowa kluczowe: rzepak, odmiany, kwasy tłuszczowe, proporcja kwasów omega-6 do omega-3, utlenialność olejów.

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Abstrakt

Skład kwasów tłuszczowych oznaczono w celu wskazania optymalnego kierunku wykorzystania olejów otrzymanych z nasion dziewięciu krajowych odmian rzepaku: czterech ozimych populacyjnych, dwóch ozimych mieszańcowych oraz trzech jarych populacyjnych. Skład kwasów tłuszczowych oznaczono metodą chromatografii gazowej, a na ich podstawie wyznaczono proporcje kwasów omega-6 do omega-3 oraz obliczono wskaźniki utlenialności olejów.

Stwierdzono, że udział poszczególnych kwasów tłuszczowych w olejach z nasion badanych odmian rzepaku różnił się statystycznie na poziomie istotności 0,05. Najbardziej zróżnicowane pod względem udziału kwasów tłuszczowych, zwłaszcza oleinowego i linolenowego, były oleje z nasion odmian jarych. Olejem najbardziej odróżniającym się pod względem składu kwasów tłuszczowych był olej z nasion odmiany jarej Bios zawierający najniższy udział kwasu oleinowego, a najwyższy – linolowego i linolenowego. Olej ten, cechujący się równocześnie bardzo dobrą proporcją kwasów omega-6 i omega-3, można uznać za najzdrowszy olej jadalny, który z uwagi na najwyższy współczynnik utlenialności, powinien być rekomendowany do spożywania na zimno. Za najprzynajmniej do produkcji biodiesla, z uwagi na niskie wartości współczynników utlenialności, uznano oleje z nasion odmian Monolit, Pomorzanin oraz Huzar.

Introduction

Cultivation of double-improved variety of rapeseed (with low content of erucic acid and glucosinolates) provided raw material to production edible oil of good quality and caused high interest of its cultivation. Therefore, there is high quantity of national and foreign varieties of double-improved rape. Currently, the National Register of varieties in Poland includes 66 winter varieties and 19 spring varieties of rape, both pollinated and hybrid ones (*Lista odmian roślin...* 2008). These varieties differ in terms of fat, protein and glucosinolates content (*Lista odmian roślin...* 2008). The most important qualitative feature for rapeseed producers is the productivity rate, while for technologists – the content of fat in seeds. Possibilities of using rapeseed oil are determined by fatty acid composition. Oils that are aim to direct intake should have proper omega-6 and omega-3 ratio – from 1:1 to 5:1 (ACHREMOWICZ et al. 2005, JELIŃSKA 2005, KOLANOWSKI 2007, SIMOPOULOS 2008, KRZYMAŃSKI 2009). In organism acids of these both families are metabolized to individuals with longer carbon chain and with higher amount of double bonds which are precursors of eicosanes that are named tissue hormones. During metabolizing omega-6 and omega-3 the same tissue enzymes take part, desaturases and elongases, therefore an excess of one that acids makes slower or prevents changes of other one (JELIŃSKA 2005). The only commonly available oil that fits this ratio is rapeseed oil. Usage for frying requires oil which contains lower polyunsaturated fatty acid share and higher unsaturated and monounsaturated fatty acid shares, such as oil that is thermooxidative resistant. In case of usage rapeseed oil to biodiesel production, high share of polyunsaturated fatty acids is not desirable because in fuel they will be oxidized in high

pace and hence it can cause drying of oils, plugging fuel system and creation precipitates on injectors (TYS et al. 2006).

Currently, simultaneous interest of rapeseed as a raw material to production edible oil and biodiesel caused that the aim of research was to determine the share of fatty acids in cold-pressed oils from selected national varieties of rapeseed, in terms of indicating optimal possibilities of their use.

Material and methods

Material

Oil samples cold-pressed from nine varieties of rape: four winter pollinated (Bogart, Bojan, Bosman and Monolit), two winter hybrid (Kaszub and Pomorzanin) and three spring pollinated (Bios, Feliks and Huzar) were the experimental material originating from the harvest of 2008. The seeds were obtained from the Plant Breeding Station in Strzelce.

Methods

All the seeds were cold-pressed using a screw oil expeller featuring a cylindrical perforated strainer basket Komet laboratory CA 59 G, with temperatures $\leq 40^{\circ}\text{C}$. Mechanical impurities were removed from the pressed oil by centrifugation in a centrifuge type MLW T 24D (10 000 rpm, 10 min).

Fatty acid composition was evaluated by standard gas chromatography method (*Analiza estrów...* PN-EN-ISO-5508:96). Methyl esters were prepared according to the method described by ZADERNOWSKI and SOSULSKI (1978). The analysis of methyl esters were carried out applying GC 8000 series FISIONS Instrument Gas Chromatograph equipped with a flame-ionization detector using a column type DB-225 (30 m x 0,25 mm) packed with chromosorb GP and helium as a carrier gas. Fatty acids were identified according to retention time determined for fatty acid standards.

According to share of individual fatty acids omega-6 to omega-3 ratio and oxidizability index of oils (U) were calculated using formula given by COSGROVE et al. (1987):

$$U = \frac{(0.02 \cdot C_{18:1} + 1 \cdot C_{18:2} + 2 \cdot C_{18:3})}{100}$$

where:

$C_{18:1}$ – share of oleic acid,

$C_{18:2}$ – share of linolic acid,

$C_{18:3}$ – share of linolenic acid.

Statistical analysis

Obtained results of researches were statistically analyzed using the Statistica 9.0 PL (StatSoft Poland) program. In order to indicate significance of differences between oil samples unvaried analysis of variance (ANOVA) with Tukey's test of $p \leq 0.05$ significance level was used.

Results and discussion

Results of carried out research revealed significant diversity of fatty acids content in oils samples obtained from seed of analyzed varieties of rapeseed (Table 1).

Unsaturated fatty acids ($C_{16:0}$, $C_{18:0}$ and $C_{20:0}$) accounted for 6.13 to 7.55% and oils obtained from seeds of winter pollinated varieties turned out to be more diversified in this case than oils from seeds of spring varieties. In all researched oil samples palmitic acid was dominated among unsaturated fatty acids, accounted for > 62% sum of all unsaturated acids. The lowest share of this acid, 4.20%, indicated in oil pressed from seeds of spring pollinated variety Feliks and the highest one, 4.99%, in oil obtained from seeds of winter pollinated varieties Bosman (Table 1). Another unsaturated fatty acid, stearic, accounted for – from 1,15% sum of fatty acids in oil from seeds of winter pollinated varieties Bogart to 2.14% in oil from seeds of Monolit variety that belongs to the same group of varieties. Share of arachidic acid in oils obtained from seeds of all varieties did not exceeded 0.7% (Table1).

Long-chain saturated fatty acids have been perceived recently as hiperlipidemic and/or hipercholesterolemic but works that are currently carried out do not confirm that. YU et al. (1995) in a review based on working out results of research performed in 18 source material, indicated that stearic acid has neutral lipid- and cholesterolemic and effects. BONANOME et al. (1988) having analyzed metabolic results of a highstearic diet found out that in the same time stearic acid decreases cholesterol content and increases oleic acid content in triglycerides of serum. Authors of that research made a suggestion that increase oleic acid content is an effect of the stearic acid conversion and that stearic acid is equally effective in decreasing level of cholesterol as oleic acid.

HUNTER et al. (2010), in conclusion of clinical research connected with dietary predisposition cardiovascular disease claimed that in food required solid fat trans fatty acids should be substituted on stearic acid that decreases level of cholesterol LDL and influences neutrally on level of HDL. Opinions connected with palmitic acid influence on level of cholesterol are divided. DENKE et al. (1992) confirmed that palmitic acid increased concentration of general cholesterol and LDL in higher level than lauric acid. SUNDRAM et al. (1994) revealed that in comparison to lauric acid with myristic acid – palmitic acid decreased concentration of cholesterol for young normocholesterolemic men. CLANDININ et al. (2000) revealed that palmitic acid did not influence on lipoprotein profile of serum in case of recommended intake of linolic acid.

The highest share, 61.09–66.53%, sum of all fatty acids in researched oils were monounsaturated acids: oleic, eicosanoic and palmitoleic. In that group of fatty acids oleic acid was dominated and accounted for from 59.16% sum of fatty acids in oil from seeds of Bios variety to 65.34% in oil from seeds Feliks variety (Table 1).

The mentioned varieties, with marginal different share of this acid, belong to the same group of spring pollinated varieties. Oils obtained from seeds of winter varieties were shown to be characterized by more aligned level of oleic acid. Total share of other monounsaturated acids, palmitoleic and eicosanoic, in all oils ranged from 1.19 to 1.94%. Fatty acids share does not vary from results obtained in other publications mentioned above (ROTKIEWICZ et al. 1995, TAŃSKA et al. 2009).

All contained in research oils monounsaturated fatty acids – oleic, eicosanoic and palmitoleic, decrease risk of appearance of cardiovascular disease decreasing level of triglycerides and total cholesterol (KRIS-ETHERTON et al. 1999), especially LDL fraction (GILL et al. 2003) that slows down pace of oxidation LDL to atherogenic and increases level of HDL fraction (ASHTON et al. 2001). The result of these activities is a lower accumulation of vascular cholesterol and higher of hepatic one (MATTHAN et al. 2009).

Polyunsaturated fatty acids, linolic and linolenic, constituted 26–32% sum of all fatty acids in research oils. The most diversified in this case turned out to be oils from seeds of spring varieties where extreme difference of this fatty acids share accounted for above 5 percentage individuals. Share of linolic acid in oils of seeds of majority varieties ranged from 19–20% (Table 1). Significantly lower share this fatty acid was in pressed oils from seed of two pollinated varieties – winter one Monolit (17.92%) and sprig one Feliks (18.06%). However, in oil from seed of spring variety Bios share of linolic acid was the highest (20.87%) and significantly higher than in oils from seeds of other varieties. Share of α -linolenic acid turned out to be more diversified than share

of linolic acid because it oscillated in range from 7.77–11.34%. Oils from seeds of spring varieties, Huzar and Bios were extremely different in this case.

Polyunsaturated fatty acids belonging to NNKT have the biggest influence on a formation of the nutrition value and oils stability. Recently, it has been said that the higher sum of polyunsaturated fatty acids the higher nutrition value of oils. Currently, there it is claimed that not only share of NNKT is important but also omega-6 to omega-3 ratio (ACHREMOWICZ *et al.* 2005, JELIŃSKA 2005, KOLANOWSKI 2007). The proper omega-6 and omega-3 ratio in consuming oils should range from 1:1 to 5:1 (ACHREMOWICZ *et al.* 2005, JELIŃSKA 2005, KOLANOWSKI 2007, SIMOPOULOS 2008, KRZYMAŃSKI 2009).

In all analyzed oils ratio omega-6 and omega-3 acids ranged from 1.8:1 – 2.5:1 (Table 2), that is why it was proper. However, taking into account that in a contemporary “westward” diet omega-6 and omega-3 ratio is 20-30:1 (JELIŃSKA 2005 cited by SIMOPOULOS 1999), the oils that should be said to be dietary better are oils pressed from seeds of spring variety Bios, winter Pomorzanin which accounted for the lowest omega-6 to omega-3 ratio – 1.84:1 (Table 2). The most diversified in terms of this feature turned out to be oils pressed from seeds spring varieties where proportions of NNKT were extremely different.

Table 2

Ratio of omega-6 acid to omega-3 acid and oxidizability of oils

Form	Variety	Omega-6/omega-3	Oxidizability
Winter	Bogart	1.97 : 1	0.40
	Bojan	2.10 : 1	0.40
	Bosman	2.25 : 1	0.39
	Kaszub	2.25 : 1	0.39
	Monolit	2.12 : 1	0.36
	Pomorzanin	1.84 : 1	0.36
Spring	Bios	1.84 : 1	0.45
	Feliks	2.02 : 1	0.37
	Huzar	2.49 : 1	0.36

Desirable content and proportion of polyunsaturated fatty acids in oil for consumers is not positive for biodiesel because of decreased stability of this product. The literature data indicate that linolic acid oxidizes 10–40-fold quicker than oleic and that linolenic even from 2 to 4-fold quicker than linolic acid (FREGA *et al.* 1999, DROZDOWSKI 2002).

Oxidizability index was the highest for the oil from seeds of spring variety Bios (0.45) and that indicates on its lower suitability as a raw material to the biodiesel production. Varieties that gives oil with the lowest index of oxidizability, 0.36–0.37, can be indicated as the best to obtain biodiesel, such as: one

hybrid varieties (Pomorzani) and three pollinated varieties: winter one Monolit and two spring Huzar and Feliks. BOJANOWSKA (2006) carried out research connected with fatty acids content of five varieties of rapeseed originating from the harvest of 2003 and 2004. She indicated that the most suitable to biodiesel production was oil from seeds of Bosman variety originating from the harvest of 2004 which contained ca. 90% oleic acid, ca. 5% linolic acid and < 2% linolenic acid. This fatty acids composition is seemed to be surprisingly different from typical one for oil pressed from seeds of double-improved variety of rapeseed what indicates on higholeic variety.

In the work above fatty acid content was considered in terms of variety. It is known that feature of variety can be modified by variable agro-climatic conditions, such as: temperature (HASSAN et al. 2005) and rainfalls (ASLAM et al. 2009). System of these conditions, variable in different years of harvest, can largely modify fatty acids content what was mentioned in TAŃSKA'S (2005) and BOJANOWSKA'S (2006) research. In named publications indicating on diversification of mono- and polyunsaturated acids in oil from rapeseeds descends from different areas and years of harvest. Probably it has connection with individual development, growing and maturation cycles of seeds for varieties. In this case even plants growing in one area of harvest can afflict different temperature and moisture conditions in this same growing periods. Taking into account synthesis of reserve fat in seeds that runs from saturated fatty acids to unsaturated ones (JELIŃSKA 2005) it can be claimed that in situation of the optimal agro-climatic conditions during development and grooving, seeds will contain more acids with higher unsaturated degree than seeds of plants that afflict environmental stresses in this same growing period.

Conclusion

1. The most diversified in terms of share of individual fatty acids were oils from rapeseeds of spring varieties. Oils from seeds of Bios and Feliks, belonged to the same group of varieties, were characterized by extremely different shares of oleic, linolic and linoleic. Differences in shares of these acids were respectively, 6.18, 2.81 and 3.57 percentage units.

2. The most distinctive oil in terms of fatty acid composition was oil from seeds of spring variety Bios, characterized by the lowest share of oleic acid (59.16%) and the highest share of linolic (20.87%) and linolenic (11.34%) acids.

3. Among oils pressed from seeds of nine varieties the healthiest one turned out to be oil obtained from seeds of Bios variety, characterized by the

highest and simultaneously very suitable omega-6 and omega-3 proportion. By dint of the highest oxidizability index this oil should be recommended to cold-consuming.

4. The best oils in terms of biodiesel production turned out to be oils from seeds of Monolit, Pomorzanin and Huzar varieties, characterized by the lowest values of oxidizability index.

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