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THE EFFECT OF MULTI-COMPONENT FERTILIZERS ON SPRING BARLEY YIELD, THE CONTENT AND UPTAKE OF MACRONUTRIENTS*

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Key words: spring barley, yield, macronutrients, multi-component fertilizers.

A b s t r a c t

The aim of this study was to determine the effect of mixed multi-component fertilizers on spring wheat yield, the content and uptake of macronutrients. A three-year field experiment (2005–2007) was carried out in a randomized block design at the Research and Experimental Station in Tomaszkowo, at the University of Warmia and Mazury in Olsztyn. The experiment comprised three fertilization treatments in four replications: control treatment (simple fertilizers), Amofosmag 3 and Amofosmag 4. The tested crop was spring barley cv. Ortheqa.

Amofosmag 4 had the most beneficial influence on the yield of spring barley grain. The effect of Amofosmag 3 was similar to that of simple fertilizers. In most cases, simple and multi-component fertilizers exerted a comparable effect on the mineral composition of the tested crop. More pronounced differences were observed in this respect between successive years of the study. The highest total uptake of nitrogen and potassium by spring barley was noted in plots fertilized with Amofosmag 3, while the highest total uptake of phosphorus, calcium and magnesium was observed in treatments with Amofosmag 4. This indicates that the nutrients contained in mixed fertilizers are more readily available to plants, compared with simple fertilizers.

WPLYW NAWOZÓW WIELOSKŁADNIKOWYCH NA PLON, ZAWARTOŚĆ I POBRANIE MAKROELEMENTÓW PRZEZ JĘCZMIEŃ JARY

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S ł o w a k l u c z o w e: jęczmień jary, plon, makroelementy, nawozy wieloskładnikowe.

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Abstract

Celem pracy była ocena wpływu zastosowanych nawozów wieloskładnikowych mieszanych na plon, zawartość i pobranie makroelementów przez jęczmień jary. Trzyletnie doświadczenie polowe (2005–2007) przeprowadzono w Zakładzie Dydaktyczno-Doświadczalnym w Tomaszku należącym do Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Doświadczenie, założone metodą losowanych bloków, obejmowało trzy obiekty nawozowe w czterech powtórzeniach: obiekt kontrolny (nawozy jednoskładnikowe), Amofosmag 3 i Amofosmag 4. Rośliną testowaną był jęczmień jary odmiany *Orthegea*.

Z przeprowadzonych badań wynika, że najkorzystniej na plon ziarna jęczmienia jarego wpłynął Amofosmag 4. Amofosmag 3 działał natomiast na poziomie nawozów jednoskładnikowych. Nawozy jednoskładnikowe i wieloskładnikowe wykazywały, na ogół, działanie równorzędne na skład mineralny testowanej rośliny. Większe zróżnicowanie wystąpiło między poszczególnymi latami badań. Największe łączne pobranie N i K przez jęczmień jary stwierdzono po zastosowaniu Amofosmagu 3, a P, Ca i Mg w obiektach z Amofosmagiem 4. Świadczy to o lepszej przyswajalności składników pokarmowych z badanych nawozów wieloskładnikowych niż z nawozów jednoskładnikowych.

Introduction

Due to their balanced and complete composition, mixed fertilizers can meet the specific nutrient requirements of each plant species and can be adjusted properly to soil fertility, which is an important consideration in developing fertilization programs. Multi-component fertilizers, which provide three primary macronutrients, N, P and K, and secondary nutrients, Mg, Ca, S and Na, in varying proportions, are applied to correct magnesium deficiency in the soil and to reduce sulfur emissions, thus preventing nutrient deficiency in plants (FILIPEK 2001, FILIPEK-MAZUR, GONDEK 2005). A clear advantage of multi-component fertilizers over simple fertilizers is that the former supply a combination of nutrients at a time (GLABISZ et al. 1992). Compound fertilizers provide crops with essential nutrients in adequate amounts and proportions, and they help prevent or reduce nutrient leaching (CZUBA 1998, ZAWARTKA, SWIERAWSKA 2004a). The aim of this study was to determine the effect of mixed multi-component fertilizers, Amofosmag 3 and Amofosmag 4, on spring barley yield, the content and uptake of macronutrients.

Materials and Methods

A three-year field experiment (2005–2007) was carried out in a randomized block design at the Research and Experimental Station in Tomaszko, at the University of Warmia and Mazury in Olsztyn. The experiment, which comprised three fertilization treatments in four replications: control treatment (simple fertilizers), Amofosmag 3 and Amofosmag 4, was established on proper

brown soil developed from sandy loam, of quality class III b and very good rye complex. The physicochemical properties of soil in each year of the study are presented in Table 1. The tested crop was spring barley (*Hordeum vulgare L.*) cv. Orthega. The preceding plants were winter triticale in the first year, and winter rapeseed in the following two years. Plot surface area was 10 m².

Table 1
Selected physicochemical properties of soil used in the experiment [mg kg⁻¹]

Year	pH w 1 M KCl	Available forms		
		P	K	Mg
2005	6.15	56.7	112.0	31.0
2006	7.20	112.9	145.3	25.0
2007	5.60	116.0	224.0	87.0

Based on the average levels of available phosphorus in the soil, 300 kg ha⁻¹ Amofosmag 3 (NPKMg 3:14:20:2 + 22% CaO + 9% SO₃; 9 kg N, 18 kg P, 50 kg K on pure ingredient basis) and Amofosmag 4 (NPKMg 4:15:15:2 + 24% CaO + 9% SO₃; 12 kg N, 20 P, 37 kg K on pure ingredient basis) were applied pre-sowing. The nitrogen rate of 80 kg per ha was supplemented with two doses of ammonium nitrate applied by top-dressing in all treatments, including control. In the control treatment, the following fertilizers were applied pre-sowing: 12 kg N in the form of urea, 45 kg P₂O₅ (20 kg P) in the form of triple superphosphate and 45 kg K₂O (37 kg K) : ha⁻¹ in the form of potash salt.

Samples of spring barley were collected at the stage of full maturity. The grain and straw harvested in each plot was dried and weighed individually. Wet mineralized samples were assayed for the content of: total nitrogen – by the hypochlorite method, phosphorus – by the vanadium-molybdenum method, calcium and potassium – by atomic emission spectrometry (AES), and magnesium – by atomic absorption spectrometry (AAS). The results of chemical analyses were verified statistically by a two-factorial analysis of variance for a randomized block design. The experimental factors were as follows: *a* – fertilization, *b* – duration of the experiment. The least significant difference was assumed at *p* = 0.05.

Results and Discussion

The distribution of air temperatures in 2005 differed insignificantly from the long-term average (Table 2). Precipitation total in April was substantially lower than the long-term average, which could have contributed to uneven

emergence, whereas July was too wet. In 2006, mean monthly temperatures were similar to the long-term average. The highest temperature was noted in July. Precipitation levels differed considerably from the average values in July and August. Precipitation total in July in August was over 2.5-fold lower and nearly 2.5-fold higher, respectively, than the long period average, which made harvest difficult. In 2007, air temperatures during the growing season were slightly above the long-term average. July was wet, with a difference of 99.9 mm between mean monthly rainfall and the long period average. Weather conditions could have affected the yield of spring barley.

Table 2
Weather conditions in 2005–2007 – data provided by the Meteorological Station in Tomaszkowo

Month	Mean daily temperature [°C]				Precipitation total [mm]			
	2005	2006	2007	1970–2000	2005	2006	2007	1970–2000
April	8.2	7.3	7.5	6.9	22.0	25.6	24.7	36.1
May	11.6	12.5	13.8	12.7	68.2	89.2	93.5	51.9
June	14.2	16.0	17.7	15.9	35.4	79.2	88.1	79.3
July	19.7	20.9	17.7	17.7	83.9	29.3	173.7	73.8
August	16.9	17.2	18.3	17.2	39.6	165.0	68.0	67.1
September	18.1	14.8	12.6	12.5	17.9	51.0	57.9	59.0

In the first year of the study (2005), the average yield of spring barley grain reached 4.96 t ha⁻¹, and it was significantly lower than in the subsequent years (Table 3). The highest grain yield was attained in the treatment with Amofosmag 4 (5.41 t ha⁻¹) – it was by 10% and 18% higher than in the control treatment (simple fertilizers) and in the Amofosmag 3 treatment, respectively. In an experiment with winter wheat conducted by SZTUDER (2007), multi-component fertilizers had a more desirable yield-forming effect than simple fertilizers. Different results were reported by STEPIEŃ and MERCIK (2001). In the present study, barley straw yield corresponded to grain yield. In 2006, the yield of spring barley grain reached 6.58 t ha⁻¹, and it was considerably higher (by 32% on average) than the value noted in 2005. This could have resulted from more favorable temperatures. A comparison of fertilization treatments shows that Amofosmag 3 contributed to an increase in the yield of spring barley grain and straw. The yield of barley grain obtained in 2007 was similar to that noted in 2006 (6.43 t ha⁻¹ on average). In 2007, Amofosmag 4 had the most beneficial influence on barley grain yield, which was found to increase by around 5%, compared with the control treatment. Barley straw yield was affected by the applied fertilizers to a lower degree.

Table 3
Spring barley yield after the application of Amofosmag 4 and Amofosmag 3 [t ha⁻¹]

Treatment	Grain				Straw			
	2005	2006	2007	mean for <i>a</i>	2005	2006	2007	mean for <i>a</i>
NPK	4.92	6.46	6.28	5.89	3.74	5.84	4.87	4.82
Amofosmag 4	5.41	6.50	6.59	6.17	4.17	5.75	5.40	5.11
Amofosmag 3	4.57	6.78	6.43	5.93	360	6.29	5.60	5.16
Mean for <i>b</i>	4.97	6.58	6.43		3.84		5.29	
LSD _{<i>p</i>=0.05} for <i>a</i>	n.s.				n.s.			
<i>b</i>	0.41				0.49			
<i>ab</i>	n.s.				n.s.			

Legend: *a* – fertilization, *b* – duration of the experiment, *ab* – interaction, n.s. – non-significant difference

The means of three years show that Amofosmag 4 caused an approximately 5% increase in grain yield, in comparison with the control treatment. The effect of Amofosmag 3 was similar to that of simple fertilizers. An increase in the yield of spring barley grain following the application of mixed fertilizers was also reported by ZAWARTKA and SKWIERAWSKA (2004b), and by MAZUR et al. (2001).

The results of chemical analyses of spring barley grain and straw, presented in Table 4, suggest that the concentrations of the analyzed macronutrients varied insignificantly between fertilization treatments, and in most cases they remained within normal limits (CZUBA 1996). In all years of the study, the straw of spring barley fertilized with Amofosmag 3 had a significantly higher potassium content, compared with the other treatments. More pronounced differences were observed in this respect between successive years of the study. In the first year of the study, the grain of spring barley contained significantly less nitrogen and more magnesium, compared with the values noted in the two consecutive years. In the second year of the experiment, barley grain contained larger amounts of phosphorus, potassium and calcium than in the first and third year, and larger quantities of nitrogen than in the first year. The above differences were statistically significant. The highest nitrogen content (23.2 g kg⁻¹ d.m. on average) of barley grain was observed in 2007, and it was significantly higher than in 2005 and 2006 (by 67% and 45%, respectively). The findings of numerous authors (KRZYWY et al. 2000, FILIPEK 2001, KRZYWY et al. 2001, MAZUR et al. 2001) suggest that multi-component fertilizers have an insignificant effect on the macronutrient content of the tested plants.

Table 4
Macronutrient content of spring barley after the application of Amofosmag 4 and Amofosmag 3
[g kg⁻¹ d.m.]

Macro-nutrient	Treatment	Grain				Straw			
		2005	2006	2007	mean for a	2005	2006	2007	mean for a
Nitrogen	NPK	12.6	15.5	22.9	17.0	6.9	3.5	5.0	5.1
	Amofosmag 4	13.8	15.8	23.1	17.6	7.0	4.6	5.2	5.6
	Amofosmag 3	15.5	16.9	23.5	18.6	7.4	4.6	5.2	5.7
Mean of b		14.0	16.1	23.2	–	7.1	4.2	5.1	–
LSD _{p=0.05} for a		n.s.				n.s.			
b		1.38				0.66			
ab		n.s.				n.s.			
Phosphorus	NPK	2.8	4.1	2.4	3.1	0.38	1.11	0.80	0.76
	Amofosmag 4	3.0	4.2	2.3	3.2	0.39	1.20	0.77	0.79
	Amofosmag 3	2.9	4.0	2.2	3.0	0.61	1.10	0.83	0.85
Mean of b		2.9	4.1	2.3	–	0.46	1.14	0.80	–
LSD _{p=0.05} for a		n.s.				n.s.			
b		0.18				0.17			
ab		n.s.				n.s.			
Potassium	NPK	3.9	4.6	3.8	4.1	11.5	13.8	10.9	12.1
	Amofosmag 4	4.6	4.5	3.6	4.2	14.0	15.1	9.2	12.8
	Amofosmag 3	4.3	4.6	3.5	4.1	14.8	15.7	11.5	14.0
Mean of b		4.3	4.6	3.6	–	13.4	14.9	10.5	–
LSD _{p=0.05} for a		n.s.				1.12			
b		0.19				1.10			
ab		0.33				n.s.			
Calcium	NPK	0.47	1.00	0.49	0.65	4.7	5.0	2.7	4.1
	Amofosmag 4	0.45	1.11	0.47	0.68	4.7	5.6	2.7	4.3
	Amofosmag 3	0.42	1.02	0.48	0.64	4.2	5.5	2.4	4.0
Mean of b		0.45	1.04	0.48	–	4.5	5.4	2.6	–
LSD _{p=0.05} for a		n.s.				n.s.			
b		0.19				0.52			
ab		n.s.				n.s.			
Magnesium	NPK	1.10	0.86	0.81	0.92	0.39	0.65	0.45	0.50
	Amofosmag 4	1.20	0.86	0.80	0.95	0.40	0.66	0.43	0.50
	Amofosmag 3	1.11	0.83	0.83	0.92	0.43	0.62	0.44	0.50
Mean of b		1.14	0.85	0.81	–	0.41	0.64	0.44	–
LSD _{p=0.05} for a		n.s.				n.s.			
b		0.03				0.06			
ab		n.s.				n.s.			

Explantations as in Table 3

Macronutrient uptake [kg per ha] was estimated based on the yield and macronutrient content of spring barley grain and straw (Table 5). The highest nitrogen uptake by barley plants (183.6 kg N ha⁻¹) was noted in the third year of the experiment, following the application of Amofosmag 4. Nitrogen uptake

Table 5

Nutrient uptake by spring barley grain and straw [kg ha^{-1}]

Treatment	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
2005					
NPK	87.79	15.19	62.19	19.88	6.76
Amofosmag 4	103.84	17.85	83.26	22.02	8.15
Amofosmag 3	97.47	15.44	72.93	17.03	6.57
2006					
NPK	120.57	32.90	110.30	35.66	9.34
Amofosmag 4	129.45	34.20	116.07	39.35	9.28
Amofosmag 3	143.51	34.03	129.93	41.37	9.51
2007					
NPK	168.16	18.96	76.94	16.21	7.21
Amofosmag 4	183.63	19.30	73.40	17.67	7.59
Amofosmag 3	180.22	18.79	86.90	16.52	7.60

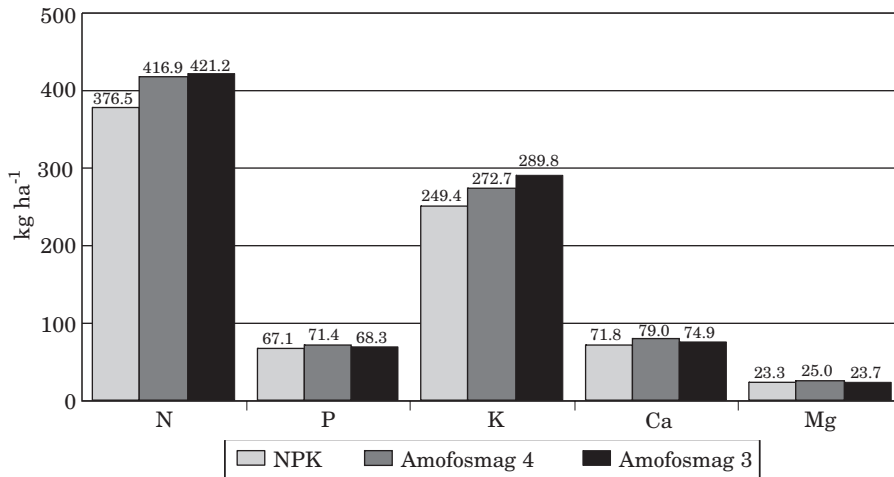


Fig. 1. Total macronutrient uptake by spring barley over a three-year experimental period

was affected by a high nitrogen content of barley grain. Nitrogen uptake in the third year of the study was 1.8-fold and 1.4-fold higher than in the first and second year, respectively. Phosphorus uptake levels were comparable in all treatments, and they were found to increase in plots fertilized with Amofosmag 4. Phosphorus uptake varied between years – it was highest in 2006 ($33.7 \text{ kg P ha}^{-1}$ on average), when barley grain and straw were abundant in phosphorus, and nearly two-fold lower in the first and third year of the experiment. A similar, albeit less pronounced, relation was observed with

regard to the uptake of potassium, calcium and magnesium, which was highest after the application of Amofosmag 3. The highest total (mean values of three years) uptake of nitrogen and potassium by spring barley was noted in plots fertilized with Amofosmag 3, while the highest total uptake of phosphorus, calcium and magnesium was observed in treatments with Amofosmag 4. This indicates that the nutrients contained in mixed fertilizers are more readily available to plants, in comparison with simple fertilizers (Figure 1). STĘPIEŃ and MERCIK (2001), ŚWIERCZEWSKA and SZTUDER (2006), and SZTUDER (2007) demonstrated that multi-component fertilizers, compared with simple fertilizers, contributed to higher nutrient uptake by various plants.

Conclusions

1. Amofosmag 4 had the most beneficial influence on spring barley yield – the application of this fertilizer enabled to increase grain yield by 5% on average, in comparison with the control treatment. The effect of Amofosmag 3 was similar to that of simple fertilizers.

2. The concentrations of the analyzed macronutrients in spring barley grain and straw varied insignificantly between fertilization treatments. Simple and multi-component fertilizers exerted a comparable effect on the chemical composition of the tested crop. Significant differences were observed in this respect between successive years of the study.

3. The highest total uptake of nitrogen and potassium by spring barley was noted in plots fertilized with Amofosmag 3, while the highest total uptake of phosphorus, calcium and magnesium was observed in treatments with Amofosmag 4. This indicates that the nutrients contained in mixed fertilizers are more readily available to plants, compared with simple fertilizers.

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VARIABILITY OF THE CARCASS WEIGHT OF THE RED DEER (*CERVUS ELAPHUS* L.) IN POLAND

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Key words: red deer, variability of carcass weight, population quality.

Abstract

The objective of this study was to determine the carcass weight of red deer (*Cervus elaphus* L.) stags, hinds and calves in Poland in successive months of the hunting season. The results of the study show that the average carcass weight of stags, hinds and calves in Poland is 115.05 kg, 75.38 kg and 42.48 kg, respectively. Stags characterized by the heaviest carcasses inhabit the provinces of Podkarpacie, Podlasie and Lublin, and those with the lightest carcasses can be found in the Lower Silesian and Pomeranian province. The highest carcass weight of hinds was noted in the Provinces of Podkarpacie and Lublin, while lowest – in the Lower Silesian and Pomeranian Province. In the group of calves, the highest and the lowest values of carcass weight were observed in the Provinces of Łódź and Lublin, and in the Lubuskie and West Pomeranian province, respectively. The average carcass weight of red deer varies significantly subject to the month of the hunting season. The heaviest stags are hunter-harvested in September, and the heaviest hinds and calves in December and February, respectively.

ZMIENNOŚĆ MASY TUSZY JELENIA SZLACHETNEGO (*CERVUS ELAPHUS* L.) W POLSCE

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Słowa kluczowe: jeleń szlachetny, zmienność masy tuszy, jakość populacji.

Abstract

Celem pracy była charakterystyka masy tuszy byków, łań oraz cieląt jelenia szlachetnego (*Cervus elaphus* L.) na terenie Polski oraz opisanie zmienności masy tuszy w poszczególnych miesiącach sezonu polowań.

Stwierdzono, że średnia masa tuszy byków, łań i cieląt jelenia szlachetnego na terytorium Polski wynosi odpowiednio: 115,05 kg, 75,38 kg i 42,48 kg. Samce jelenia charakteryzujące się najcięższą tuszą bytują w województwach: podkarpackim, podlaskim i lubelskiego, a o tuszach najlżejszych w dolnośląskim i pomorskim. Najcięższe samice występują w województwie podkarpackim i lubelskim, a najlżejsze w dolnośląskim i pomorskim. U cieląt wartości te stwierdzono odpowiednio w województwach: łódzkim i lubelskim oraz w lubuskim i zachodniopomorskim. Stwierdzono istotne zróżnicowanie średniej masy tuszy w poszczególnych miesiącach sezonu polowań. Najcięższe byki pozyskuje się we wrześniu, natomiast łanie i cielęta odpowiednio w grudniu i lutym.

Introduction

The Polish red deer population comprises various regional forms and ecotypes which differ with respect to body size, measurements and weight, antler shape, and color (TOMEK 2002), whose representatives inhabit the following regions: Carpathians, Warmia and Mazury, Wielkopolska and Lublin (STELIŃSKI 1957). The carcasses of red deer dwelling in the Carpathians and in Warmia and Mazury are characterized by the best quality (DZIĘCIOŁOWSKI 1969, KUBACKI and JAMROZY 1999, BREWCZYŃSKI 2002). The above differences result, among others, from climatic differences, the type of soil and vegetation, population density and genetic factors (BOBEK et al. 1984, BORKOWSKI 1989). The carcass weight of animals, considered an indicator of individual quality, changes in response to the above factors.

Quality is often referred to in ecological studies, but its measurement varies greatly across studies. It can be regarded as a variable that continuously changes throughout life, such as: annual body condition, annual reproductive success or body weight, etc (CLUTTON-BROCK et al. 1982, MOYES et al. 2006, PETTORELLI et al. 2001).

The objective of this study was to determine the carcass weight of red deer (*Cervus elaphus* L.) stags, hinds and calves in Poland in successive months of the hunting season.

Material and Methods

Research area

The study was conducted in 13 Polish provinces: Lower Silesian, Kujawy and Pomeranian, Lublin, Lubuskie, Łódź, Masovian, Podkarpacie, Podlasie,

Pomeranian, Świętokrzyskie, Warmia and Mazury, Wielkopolska and West Pomeranian, covering a total area of 275,750 km² (88.41% of Poland's territory).

Experimental materials

The experimental materials comprised 22 402 red deer carcasses, including 9 372 carcasses of males (stags), 10 098 carcasses of females (hinds) and 2 932 carcasses of individuals aged up to 1 year (calves). Hunting reports and the records of the venison meat purchase center, "LAS OLSZTYN" Ltd., were analyzed in the study. All animals were hunter-harvested.

Data were collected over eight successive hunting seasons, from 2000/2001 to 2007/2008, and comprised the following information: carcass weight, region and time of harvest of each animal, sex and age of each animal. Table 1 presents the number of analyzed carcasses subject to the sex and age classes and the hunting season.

Table 1

Number of analyzed carcasses [indiv.]

Hunting season	Stags	Hinds	Calves	Total
2000/2001	1669	2003	568	4240
2001/2002	1530	1774	587	3891
2002/2003	1254	1286	334	2874
2003/2004	1228	1489	364	3081
2004/2005	631	758	233	1602
2005/2006	978	1088	239	2295
2006/2007	1032	242	295	1569
2007/2008	1050	1458	312	2820
Total	9 372	10 098	2 932	22 402

All animals were shot during the red deer open season: stags – from 21 August to the end of February, hinds – from 1 October to 15 January, calves – from 1 October to the end of February (Rozporządzenie Ministra Środowiska... Dz.U. z 2005).

The carcasses were weighed using an electronic scale, accurate to 0.1 kg, after they had been transported to the storehouse of the "LAS OLSZTYN" company from local venison meat collection points. Prior to weighing, all carcasses were chilled for at least 24 hours.

Statistical analysis

The results were processed statistically using STATISTICA PL software. The statistical analysis of the investigated carcass traits involved the calculation of arithmetic means and standard deviations, and the estimation of significance of differences between mean values. The obtained results were verified by an analysis of variance for one-factor non-orthogonal design.

Results

The collected data and performed calculations show that the average carcass weight of red deer stags, hinds and calves in Poland is 115.05 kg, 75.38 kg and 42.48 kg, respectively.

Characteristics of red deer carcass weight in successive hunting seasons

As demonstrated by the data in Table 2, the lowest average carcass weight of stags (111.27 kg) was noted in the hunting season 2000/2001, while highest (120.63 kg) – in 2007/2008. The difference between this value and those observed in the remaining hunting seasons was highly significant. The highest average carcass weight of hinds (78.2 kg) was reported in 2006/2007, and it differed highly significantly from the average carcass weights noted in the hunting seasons 2001/2002, 2002/2003, 2005/2006 and 2007/2008. The lowest average carcass weight of hinds was 74.13 kg (2001/2002). The average carcass weight of calves reached the highest level in 2005/2006 (43.55 kg), and it was highly significantly higher than the mean values noted in 2001/2002 (40.96 kg) and 2002/2003 (41.71 kg).

The above data show that there were no clear relationships between the highest and the lowest average carcass weights in the sex and age classes of red deer during the eight analyzed hunting seasons. However, the pattern of changes in the average carcass weight of hinds and calves was similar during the first six hunting seasons. A decrease in the average carcass weight of females and young animals was noted at the beginning of the investigated period, followed by an increase which was observed until the hunting season 2004/2005. In subsequent seasons the trends in carcass weight changes in the groups of hinds and calves were different. Changes in the average carcass weight of stags, in comparison with hinds and calves, followed a different pattern in the majority of cases.

Tabela 2

Carcass weight stags, hinds and calves in successive hunting seasons [kg]

Statistical measures	Hunting seasons								Significance of differences
	2000/ /2001	2001/ /2002	2002/ /2003	2003/ /2004	2004/ /2005	2005/ /2006	2006/ /2007	2007/ /2008	
	1	2	3	4	5	6	7	8	
STAGS									
<i>n</i>	1669	1530	1254	1228	631	978	1032	1050	1, 2, 3, 4, 5, 6, 7 < 8**
\bar{x}	111.27	112.13	115.30	117.60	112.37	115.44	115.70	120.63	1, 2, 5 < 3, 4, 6, 7*
<i>S</i>	26.11	26.61	27.27	28.09	26.14	27.52	27.32	28.21	3 < 4*
HINDS									
<i>n</i>	2003	1774	1286	1489	758	1088	242	1458	7 > 2, 3, 6, 8**
\bar{x}	76.3	74.13	75.78	76.22	76.54	75.27	78.21	75.45	1, 4, 5 > 2, 3, 6, 8*
<i>S</i>	13.4	13.46	13.67	14.28	14.63	14.49	14.71	14.07	
CALVES									
<i>n</i>	568	587	334	364	213	229	295	312	1 > 2**, 6 > 2, 3**
\bar{x}	42.26	40.96	41.71	42.96	43.25	43.55	43.07	43.26	6 > 1, 4*, 4, 5, 7, 8 > 2**
<i>S</i>	7.53	7.88	7.57	8.00	7.90	7.64	7.17	7.64	4, 5, 7, 8 > 3*

* - $P \leq 0.05$ ** - $P \leq 0.01$

Table 3 illustrates the average carcass weight of red deer in each month of the hunting season. The average carcass weight of stags varied widely throughout the open season, reaching the highest values in September and August (over 128 kg and 118 kg, respectively). The difference in the average carcass weight of stags shot in September and in subsequent months of the hunting season (October to February) was highly significant. The hinds harvested in November and December were characterized by the heaviest carcasses (76.92 kg and 77.25 kg, respectively). The above values were significantly higher than those noted in October and January (around 74.3 kg in both months). The average carcass weight of calves also varied subject to the month of the hunting season. The highest average carcass weight of calves was reported in February and November (43.88 kg and 43.22 kg, respectively), while the animals shot in January and October were marked by the lowest average carcass weight (41.48 kg and 41.61 kg, respectively). The difference between the mean values noted in February and November and in the remaining months was highly significant.

Tabela 3
Carcass weight stags, hinds and calves in months of the hunting season [kg]

Statistical measures	Month of harvest							Significance of differences
	August 1	September 2	October 3	November 4	December 5	January 6	February 7	
	STAGS							
<i>n</i>	236	3499	2285	993	728	809	822	2 > 3, 4, 5, 6, 7**
\bar{x}	118.21	128.42	112.03	103.46	102.76	101.64	101.15	1, 3 > 4, 5, 6, 7*
<i>S</i>	31.33	26.73	25.28	22.88	22.11	21.76	20.12	2 > 1*
	HINDS							
<i>n</i>	-	-	2870	2547	2449	2232	-	3, 6 < 4, 5**
\bar{x}	-	-	74.30	76.92	77.25	74.28	-	
<i>S</i>	-	-	12.95	13.87	14.32	14.52	-	
	CALVES							
<i>n</i>	-	-	973	625	433	561	310	3, 6 < 4, 5, 7**
\bar{x}	-	-	41.61	43.22	43.01	41.48	43.88	
<i>S</i>	-	-	7.84	7.55	7.77	7.67	7.26	

* - $P \leq 0.05$ ** - $P \leq 0.01$

Tabela 4
Carcass weight stags, hinds and calves in particular analyzed provinces [kg]

Statistical measures	Province (see the legend below)													Significance of differences
	1	2	3	4	5	6	7	8	9	10	11	12	13	
	STAGS													
<i>n</i>	84	1256	753	109	161	174	1495	502	790	51	2575	171	1249	3, 7, 8, 11>1, 9**; 7>5, 6*
\bar{x}	92.11	107.23	121.19	105.08	115.66	117.86	122.80	121.73	101.72	107.59	120.83	106.86	105.43	3, 7, 8, 11>2, 4, 10, 12, 13**
<i>S</i>	19.50	24.20	26.27	21.86	24.00	25.37	28.17	26.98	23.48	22.32	27.79	23.08	23.96	2, 4, 9, 10, 12, 13>1*
	HINDS													
<i>n</i>	102	1489	440	115	138	117	1586	592	780	41	3132	153	1413	3, 6, 7>1, 4, 9, 13**
\bar{x}	63.97	72.74	81.22	68.91	78.07	80.97	81.93	79.74	69.36	78.83	77.32	71.83	69.19	3, 6, 7>2, 12*
<i>S</i>	11.83	11.38	12.70	10.79	9.87	12.77	15.05	13.84	10.27	12.03	14.40	10.71	11.61	5, 8, 10, 11>1, 4, 9, 13
	CALVES													
<i>n</i>	39	434	80	26	32	16	164	124	389	32	1088	43	465	3, 5, 6, 7, 8, 10>1, 2, 4, 9, 11, 12, 13**
\bar{x}	41.97	42.49	46.76	40.92	46.88	46.69	46.20	45.98	41.02	46.00	42.14	42.23	40.60	2, 11, 12>13**
<i>S</i>	8.31	7.21	5.67	7.71	6.26	6.74	6.66	6.31	6.84	5.66	8.28	6.78	7.52	2, 11, 12>4*

* - $P \leq 0.05$

** - $P \leq 0.01$

Provinces: Lower Silesian (1), Kujawy and Pomeranian (2), Lublin (3), Lubuskie (4), Łódź (5), Masovian (6), Podkarpacie (7), Podlasie (8), Pomeranian (9), Świętokrzyskie (10), Warmia and Mazury (11), Wielkopolska (12), West Pomerania (13)

Table 4 presents the average carcass weight of red deer stags, hinds and calves in 13 analyzed provinces. Stags characterized by the heaviest carcasses were shot in the Provinces of Podkarpacie (over 122 kg), Podlasie and Lublin (over 121 kg), and Warmia and Mazury (over 120 kg). Stags with the lightest carcasses were harvested in the Lower Silesian (92 kg) and Pomeranian Province (101 kg). The differences between the highest and the lowest mean values were highly significant. The highest carcass weight of hinds was noted in the Provinces of Podkarpacie (81.93 kg) and Lublin (81.22 kg), while lowest – in the Lower Silesian Province (63.97 kg), similarly as in the group of stags. The average carcass weight of calves also varied subject to the region of harvest. The data in Table 4 show that in the group of calves, the highest values of carcass weight were observed in the Provinces of Łódź (46.88 kg), Lublin (46.76 kg) and Masovia (46.69 kg), whereas lowest – in the West Pomeranian (40.60 kg) and Lubuskie Province (40.92 kg).

Discussion

The effect of environmental variation on traits such as body (carcass) weight were widely demonstrated in red deer (ALBON et al. 1983, CLUTTON-BROCK et al. 1987, COULSON et al. 2003) but although studies could show that heterogeneity exist among individuals in their response to such environmental effects, the majority of this work focuses on differences in the strength of others effects among stage, age and sex classes. For example, KRUK et al. 1999 showed that male, but not female, lifetime reproductive success in population of red deer was associated with birth mass. Similarly, Loison et al. (2004) found that maternal quality accounts for more variance in male offspring body mass than female body mass in red deer.

It should be noted that the analyzed data were collected for animals harvested in accordance with the culling strategy. As a result, the average values of carcass weight presented in the study could be slightly lower from the mean value determined for the entire red deer population in Poland. The body weight of red deer stags, which is a key indicator of their quality, is most often presented as carcass weight following evisceration, decapitation and partial bleeding. Therefore, the actual body weight of those animals may be by approximately 25% higher (DZIEGIELEWSKI 1970). For practical purposes, it seems important to determine the average carcass weight of red deer harvested in various regions of the country and in different months of the hunting season, as this information may be used by venison sellers and distributors while planning sale and purchase volume.

According to DROZD et al. (2000), the average carcass weight of stags in the macroregion of Central and eastern Poland in 1972–1996 reached 128.6 kg.

As demonstrated by BREWCZYŃSKI (2002), the average carcass weight of red deer stags (133.9 kg) harvested in the area managed by the State Forest Enterprise branch in Krosno (Carpathians) was also higher than the values noted in the present study. Insignificantly lower average carcass weight of stags, at 108.0 kg, was reported by ŻURKOWSKI et al. (2000) who conducted a study in the Pisz Forest in 1997–1998. CZYŻYK et al. (2007) analyzed the red deer population of Masurian Forests in 1990–2001 where the average carcass weight of stags reached 112.1 kg, and it was comparable to that noted in our study. Yet the mean values determined for individual years by the cited authors were lower than our results, at up to 110 kg. JAMROZY (1995) found, based on a study covering ten hunting seasons, that the carcass weight of red deer from the Carpathians ranged from 112 kg in the west to 148 kg in the eastern part of the region. According to DZIĘGIELEWSKI (1970), Carpathian red deer are larger than the animals dwelling in Wielkopolska, which is consistent with our findings.

The results of a study carried out by TOMEK (2002) in the region of Krynica indicate that the average carcass weight of red deer hinds older than 3 years is 90.4 kg. This value is higher than that noted in the present study. In a study by JANISZEWSKI and SZCZEPAŃSKI (2004) conducted in the Forest Division of Wipsowo, Province of Warmia and Mazury, the average carcass weight of hinds reached 76.6 kg, which is comparable with the value observed in our study in this province (77.32 kg). According to DZIĘCIOŁOWSKI (1970), the average carcass weight of hinds in the Polish Lowlands is 71.9 kg. In an earlier study by MYSTKOWSKA (1966), the average carcass weight of hinds dwelling in the lowlands in 1960–1962 reached 70.9 kg. ŻURKOWSKI et al. (2000) investigated the red deer population from the Pisz Forest in 1996/1997 and 1997/1998, and found that the average carcass weight of hinds oscillated around 70.1 kg, and it was lower than in our study. DZIĘCIOŁOWSKI (1969) demonstrated that the average carcass weight of 96 red deer hinds harvested in three hunting grounds in Poland reached 104.4 kg.

BOBEK et al. (1992) found that the carcass weight of calves in the red deer population of southern Poland ranged from 42.5 kg to 50 kg. This was validated by the findings of DZIĘCIOŁOWSKI (1969a) who carried out a study in the former provinces of Olsztyn and Lublin in 1964–1968, and reported that the average carcass weight of male and female calves was 44.8 kg and 43.90 kg, respectively. The data are comparable with our results. In the red deer population of the Krynica Forests, described by TOMEK (2002), the carcass weight of calves reached 55.80 kg, and it was higher than in the present study.

An analysis of average carcass weight values in the sex and age classes of red deer revealed that they were higher in the eastern parts of their range in Poland, compared with the west. This confirms Bergmann's rule stating that

the average body size of a given species tends to be larger at higher latitudes (BOBEK et al. 1984). The body size of warm-blooded animal species becomes larger in cold climates, and their surface area to volume ratio decreases.

In the present study, the heaviest red deer stags were harvested in September, and then a decrease in their carcass weight was observed. The noted differences were due to the rutting season which took place in September and at the beginning of October. Before the rut, stags are known to deposit fat reserves as an additional source of energy (BOBEK et al. 1984). KRUPKA et al. (1986) reported that the carcass weight of red deer stags in Central and eastern Poland decreases by 8.27% during the rut, and by 22.73% over the entire open season. In a study by DROZD et al. (2000), the decrease in the carcass weight of stags reached 25%. As demonstrated by BOBEK et al. (1984), the winter weight loss in large herbivorous mammals may be as high as 35%.

In the group of red deer hinds, the highest average carcass weight was noted in November and December, and it was significantly higher than the carcass weight of females harvested in October and January. In a study by JANISZEWSKI and SZCZEPAŃSKI (2004), the carcass weight of hinds in the fall and winter was at a similar level. According to the cited authors, the hunting season had an insignificant effect on the carcass weight of hinds, which was validated by the present study. TOMEK (2002) analyzed red deer hinds from the Krynica Forests and found that their carcass weights were highest in November and December, and that the noted values were similar. The above observations correspond to our findings, since the carcass weights of hinds in our study were at a similar level, and they were not affected by the hunting season. According to BOBEK et al. (1992), the average carcass weight of red deer hinds in the Bieszczady Mountains and in the Beskids is higher, reaching 82.5 kg in January and 89.7 kg in December.

Calves shot in February and November were characterized by the highest carcass weight. In a study by JANISZEWSKI and SZCZEPAŃSKI (2004), the average carcass weight of calves harvested in one of the forest divisions in the Province of Warmia and Mazury was 43.5 kg. According to the above authors, the month of harvest has no significant effect on the carcass weight of red deer aged up to one year, which is consistent with our findings.

Conclusions

The results of the study, which investigated red deer populations in Poland, support the following conclusions:

1. The average carcass weight of red deer stags, hinds and calves in Poland is 115.05 kg, 75.38 kg and 42.48 kg, respectively.

2. Stags characterized by the heaviest carcasses inhabit the Provinces of Podkarpacie (Subcarpathian), Podlasie and Lublin, and those with the lightest carcasses can be found in the Lower Silesian and Pomeranian Province.

3. The highest carcass weight of hinds was noted in the Provinces of Podkarpacie and Lublin, while lowest – in the Lower Silesian and Pomeranian Province. In the group of calves, the highest and the lowest values of carcass weight were observed in the Provinces of Łódź and Lublin, and in the Lubuskie and West Pomeranian Province, respectively.

4. The average carcass weight of red deer differs significantly subject to the month of the hunting season. The heaviest stags are hunter-harvested in September, and the heaviest hinds and calves in December and February, respectively.

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**EFFECT OF ORGANIC POLYELECTROLYTES
ON SEDIMENTATION PROPERTIES
OF POST-COAGULATION SLUDGE**

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Key words: flocculation, sedimentation, organic polymers.

Abstract

A study was carried out into sedimentation of sludge obtained in the process of coagulation-flocculation of pulp and paper wastewater with the use of PAC and organic polymers. An attempt was made to evaluate the effect of various organic polyelectrolytes on phase separation effectiveness. It was found that the application of cationic polymers at a dose of 1 mg dm^{-3} and 1.5 mg dm^{-3} (Z 63 and Z 92, respectively) in combination with PAC had a positive effect on the reduction in the sludge volume when compared with the sludge volume obtained in the process of coagulation with PAC without a flocculant. It was also noted that following the application of the optimum dose of either cationic or anionic polymers the sedimentation period was considerably reduced from 60 min (sample without flocculants) to even 30–35 min.

**WPLYW POLIELEKTROLITÓW ORGANICZNYCH NA WŁAŚCIWOŚCI
SEDIAMENTACYJNE OSADU POKOAGULACYJNEGO**

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Słowa kluczowe: flokulacja, sedymentacja, polimery organiczne.

Abstrakt

Przeprowadzono badania sedymentacji osadu ściekowego otrzymanego w wyniku koagulacji-flokulacji ścieków celulozowo-papierniczych za pomocą PAC i polimerów organicznych. Podjęto próbę oceny wpływu polielektrolitów organicznych o różnym charakterze jonowym na skuteczność separacji faz. Badania wykazały, że zastosowanie polimerów kationowych w dawce 1 mg dm^{-3} Z 63 i $1,5 \text{ mg dm}^{-3}$ Z 92 w kombinacji z PAC szczególnie korzystnie wpływa na obniżenie objętości osadu ściekowego w porównaniu z osadem otrzymanym w wyniku koagulacji za pomocą PAC bez flokulanta. Stwierdzono również, że po zastosowaniu optymalnej dawki polimerów zarówno kationowych, jak i anionowych następuje znaczne skrócenie czasu sedymentacji z 60 min (w próbie bez flokulanta) nawet do 30–35 min.

Introduction

Chemical engineering deals, among others, with the removal of dissolved substances and particles of various sizes from water and wastewater. Depending on their size, particles can be removed mechanically (particles $> 1 \text{ mm}$) or through sedimentation (particles $> 100 \text{ }\mu\text{m}$). In the case of particles smaller than $1 \text{ }\mu\text{m}$ this problem can be solved by the application of processes resulting in the aggregation of solid substances dispersed in water or wastewater, with a significant effect on sedimentation and filtration efficiency. Chemical coagulation and flocculation of particles are considered common effective aggregation techniques. The process of flocculation is widely applied in various branches of industry, including wastewater treatment. Increasing volumes of sludge constitute a significant problem for sludge management and possible methods of utilisation (BARAN, TURSKI 1999). Synthetic organic polymers find application in liquid-solid phase separation on an industrial scale. Combinations of inorganic coagulant and synthetic organic polymers as flocculating agents are used in order to improve the process efficiency. The main task of a flocculant is to improve stabilisation of flocs and separate them in the form of post-coagulation sludge. Considerably large molecular weights of flocculants enhance the formation of large flocs with better structure and resistance to damage. Active sites to be found along the polymer chain may bind to several particles at the same time and initiate their aggregation. The flocculation process induced by the bridge formation within the macromolecular polymers is the most effective way to produce large flocs. Both the size and the density of the resulting aggregates have a significant effect on the effectiveness of phase separation during, among others, sedimentation or filtration. Polyelectrolyte type and the adsorbed polymer chain conformation are the key factors in the flocculation mechanism (GREGORY 2009). The optimum flocculation occurs when the polyelectrolyte chains extend in the form of ribbons, adsorb themselves onto the particles; surfaces and form polymer bridges.

The macromolecular chain extends as the degree of hydrolysis of the anionic polymer increases (SASTRY 1999). The macromolecular conformation also depends on the polymer charge density. Electrostatic interactions between the particular polymer segments, especially between those with high charge density, unfold the macroion ribbon. According to BOLTO and GREGORY (2007), hydrodynamic conditions (stirring, diffusion) can also essentially affect the polymer adsorption degree, especially those of higher molecular weights. Organic polymers should be added in the optimum dose. If the dose is too low, the bridging is insufficient and, in consequence, the flocculation is inefficient. The application of organic polyelectrolytes offers many benefits such as high efficiency at low doses, reduction in the required dose of inorganic coagulant and considerably higher cost-effectiveness of treatment (approx. 25–30% lower costs) (NOZAIC 2001). Organic polymers are also less affected by pH changes.

The effectiveness of the entire technological process is determined by the effectiveness of flocculation and phase separation in a system. The aim of the presented study was to determine the effect of the ionic character of organic polymers on the phase separation in the process of coagulation/flocculation with PAC (poly-aluminium chlorides).

Material and Methods

PAC (poly-aluminium chloride), made up of 47–52 mg Al dm⁻³ and c.a. 97 g Ca²⁺ dm⁻³, was used as a coagulating agent. The process of coagulation/flocculation was performed in the presence of various high molecular weight organic polymers, both cationic (Z 63 and Z 92) and anionic (M 1011 and P 2540).

Chemical coagulation of wastewater was carried out following a standard jar-test procedure:

- fast stirring (400 rpm) – 1 min,
- slow stirring (30 rpm) – 15 min,

Following the fast stirring period, measurements of the volume of the sediment sludge were performed every 5–10 min for 1 h. Such a period was sufficient to complete the sedimentation process.

Results and Discussion

The effectiveness of sedimentation is determined, among others, by particle type, their size, degree of hydration and the time of their sedimentation.

Sedimentation curves $I_S = f(t)$ were plotted in order to determine the sedimentation parameters. The sedimentation index (I_S) defines gains in sludge volume over time ($V t^{-1}$ [$\text{cm}^3 \text{min}^{-1}$]). Figures 1a–d present the sedimentation curves of sludge obtained with the application of inorganic coagulant (PAC) and organic polymers of various ionic character in 1 h. The flocculants were added in doses ranging from 0.5–1.5 mg dm^{-3} . Power curve and R^2 ($R^2 > 0.96$) equations are given under each curve.

Figure 1a presents the effect of P 2540 polymer on the sludge sedimentation process. The resulting flocs fell at varied rates and formed sludge layers of various thickness. P 2540 polymer doses from 0.5–1.5 mg dm^{-3} reduced $I_S = 0.258\text{--}0.3 \text{ cm}^3 \text{min}^{-1}$ (after 60 min) when compared with $I_S = 0.4 \text{ cm}^3 \text{min}^{-1}$ obtained with the use of PAC (without flocculants). When comparing the sedimentation curves of the sludge produced with organic flocculants (Figures 1a–d), it was found that the I_S values obtained (after 60 min) were considerably lower for Z 63 ($I_S = 0.18\text{--}0.26 \text{ cm}^3 \text{min}^{-1}$) and Z 92 ($I_S = 0.25\text{--}0.29 \text{ cm}^3 \text{min}^{-1}$) than for P 2540 and M 1011 ($I_S = 0.26\text{--}0.3 \text{ cm}^3 \text{min}^{-1}$). The smallest gain in the sludge volume over times of $I_S = 0.18 \text{ cm}^3 \text{min}^{-1}$ and $I_S = 0.25 \text{ cm}^3 \text{min}^{-1}$ were observed for a 1 mg dm^{-3} dose of Z 63 a 1.5 mg dm^{-3} dose of Z 92, respectively. Differences in the I_S values and in the course of sedimentation curves obtained in the sedimentation with inorganic coagulant and organic polymers may indicate a change in the particle aggregation degree. The obtained I_S values also indicate the great effect of cationic flocculants on sludge sedimentation and a diversified aggregation mechanism. However, during visual observations of the flocculation process, an aiding capacity of flocculants in the formation of flocs of greater sizes was reported, especially for P 2540 and M 1011. This can be caused by the greater hydration degree of these flocs. The colloidal particles may have been agglomerated directly by the flocculant particles. It could be concluded that the slowly falling flocs were more hydrated than those falling faster.

Interactions of organic polymers with inorganic coagulants produce more stable flocs than those obtained with coagulants used alone (GREGORY and LI 2004). The application of organic polymers produces larger flocs up to a certain limit. When this limit is reached, the flocs disintegrate into several smaller parts. Sedimentation curves reflect the differences in the activity of flocculants with various ionic characters. The results show that both the ionic character and the applied dose of a polymer significantly affect the resulting sludge volume.

Figure 2 characteristics of sludge sedimentation with 15 mg dm^{-3} PAC and anionic flocculants.

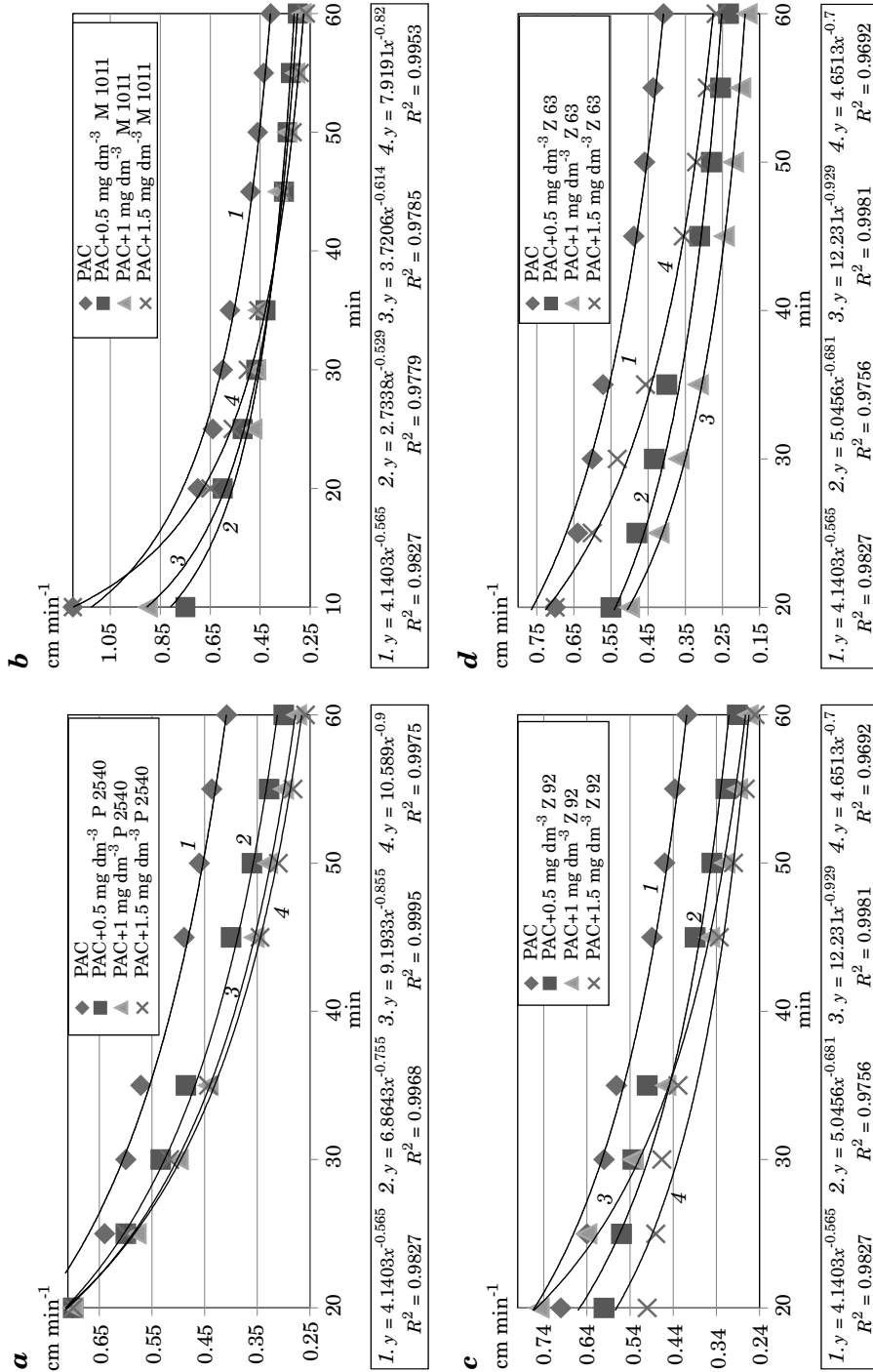


Fig. 1. Sludge volume gain over time during coagulation with 15 mg dm⁻³ PAC and a, b – anionic, c, d – cationic polymer

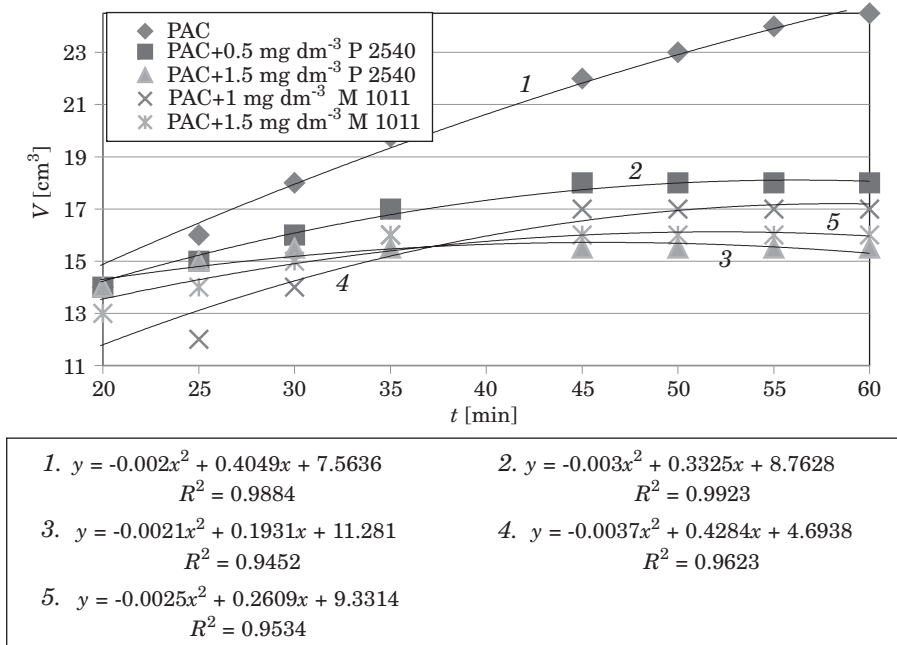


Fig. 2. Characteristics of sludge sedimentation (with 15 mg dm^{-3} PAC and anionic flocculants)

Figure 2 presents the separation characteristics of flocs with 15 mg dm^{-3} PAC and selected doses of anionic flocculants. It was found that the doses of 0.5 mg dm^{-3} and 1.5 mg dm^{-3} of P 2540 flocculant ensure a reduction in the sludge volume by 27% and 37%, respectively. The fall time of the flocs obtained with P 2540 was shortened from 60 (only PAC) to about 30–45 min. A similar result was obtained with an anionic flocculant – M 1011. The highest applied doses of 1.5 mg dm^{-3} was found to be optimal. However, the resulting flocs fell slightly slower than those obtained with the cationic flocculants. It could be assumed that these flocs were hydrated to a greater degree. The entire sedimentation process was completed in periods from 35 min (1.5 mg dm^{-3}) to 45 min (1 mg dm^{-3}). Other authors (ØDEGAARD 1992) reported that the application of PAC results in the formation of flocs with a predominating local positive charge which can potentially serve as a site of flocculant particle bonding.

Figure 3 characteristics of sludge sedimentation with 15 mg dm^{-3} PAC and cationic flocculants.

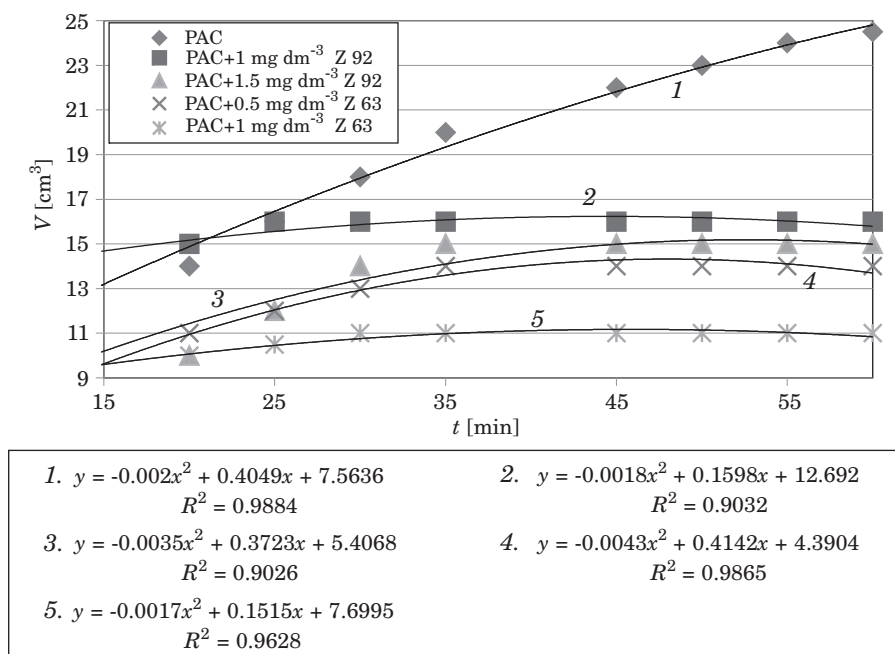


Fig. 3. Characteristics of sludge sedimentation (with 15 mg dm⁻³ PAC and cationic flocculants)

The data presented in Figure 3 show the sludge sedimentation effect obtained in the coagulation/flocculation of pulp and paper wastewater with 15 mg Al dm⁻³ (PAC) and the most effective doses of cationic flocculants. In all cases, the flocs' falling process was completed within 30–35 min. An addition of 1–1.5 mg dm⁻³ of Z 92 flocculant resulted in the reduction of sludge volume by 35–39% compared to a control sample (only coagulant). The highest effectiveness in sludge volume reduction, however, was obtained with the Z 63 cationic polymer. The optimum dose of 1.5 mg dm⁻³ reduced the volume of solid phase by 55.1% when compared with the control sample. The reduction in the sedimentation time by half (when compared with the samples treated only PAC) indicates the high effectiveness of both Z 92 and Z 63 flocculants. Due to their adsorption and bridging properties, polymers may enhance orthokinetic flocculation (KOWAL 2009).

Cationic flocculants have a destabilising activity due to the adsorption processes, charge neutralising and bridging by polymers. Simple anions and polycations are produced in the process of dissociation of a cationic polyelectrolyte in water. Organic polycation activity is similar to that of polyhydroxyocations of a coagulant, therefore, cationic polymers act similar to flocculants and coagulants applied together (ØDEGAARD et al. 1992).

The 35–55% reduction in the sludge volume obtained with cationic flocculants Z 92 and Z 63 was more beneficial than the 27–37% reduction reported for anionic flocculants P 2540 and M 1011. Similar results, i.e. a 42%-reduction in sludge volume obtained in the coagulation of breeding farm wastewater with anionic PAA applied in combination with FeSO_4 were obtained by Aguilar et al. (2005). Generally, the application of both the anionic and cationic flocculants in combination with PAC had a positive effect on the reduction of sedimentation time by approx. 40–50%. Both the cationic and anionic (flocculants ensured greater packing of sludge flocs by forming {Al(OH)₃}-cationic flocculant}-type and {anionic flocculant}-Al(OH)₃}-type structures which were more compact than the {Al(OH)₃}-org} structures (without flocculant).

Wastewater particles with predominating negative surface charge (DENTEL, GOSSET 1987) are destabilised by cationic organic flocculants through charge neutralisation, while anionic flocculants may initiate bridging, which could explain the slightly lower effectiveness of anionic flocculants when compared to cationic ones.

Based on the above results, it appears that the application of an inorganic coagulant in combination with a cationic organic flocculant such as Z 92 and Z 63 ensures a better flocs separation characteristic for pulp and paper wastewater. At the optimum dose of an inorganic coagulant, only a small addition of flocculant to a large degree reduced the sludge volume. In the present study, an increase in the sludge volume over that obtained in the control sample was not observed.

The reduction in the solid phase volume and the time of phase separation following a coagulation serves as a measure of efficient activity of a coagulant-flocculant system. Both a coagulant and a flocculant should be applied in optimum doses when used in combination. Excessive doses of coagulants may produce larger sludge volumes and reduced efficiency of the process. The application of optimum doses of a coagulant-flocculant pair ensures cost-efficiency and ecologically-friendly results.

Flocculant doses are much smaller than those of coagulants and practically do not usually exceed 2 mg dm^{-3} . However, for the currently reported high wastewater loads, the application of flocculants without an inorganic coagulant is not recommended for economic reasons.

Conclusions

1. Both the cationic and anionic flocculants considerably enhance the sedimentation of flocs produced in the coagulation process.

2. The best characteristic of phase separation was recorded for Z 63, whose 1 mg dm^{-3} dose reduced the sludge volume gain to the largest degree, i.e. to $I_S = 0.18 \text{ cm}^3 \text{ min}^{-1}$. An addition of cationic organic flocculants to inorganic coagulant decreased this value to $I_S = 0.4 \text{ cm}^3 \text{ min}^{-1}$ (for PAC), $I_S = 0.18\text{--}0.26 \text{ cm}^3 \text{ min}^{-1}$ (for Z 63) and $I_S = 0.25\text{--}0.29 \text{ cm}^3 \text{ min}^{-1}$ (for Z 92).

3. The applied anionic polyelectrolytes had a positive effect on phase separation, however, this influence was not as strong as that exerted by cationic polyelectrolytes. When applied in combination with PAC, P 2540 and M 1011 produced $I_S = 0.258\text{--}0.3 \text{ cm}^3 \text{ min}^{-1}$ and $I_S = 0.266\text{--}0.3 \text{ cm}^3 \text{ min}^{-1}$, respectively.

4. The obtained value of $I_S = 0.258\text{--}0.3 \text{ cm}^3 \text{ min}^{-1}$ following the application of P 2540 and $I_S = 0.266\text{--}0.3 \text{ cm}^3 \text{ min}^{-1}$ for M 1011 also had a positive effect on phase separation but not to as high a degree as the cationic flocculants.

5. The application of PAC in combination with cationic (Z 63 and Z 92, at a dose of $1\text{--}1.5 \text{ mg dm}^{-3}$) and anionic (1.5 mg dm^{-3} P2540 and $1\text{--}1.5 \text{ mg dm}^{-3}$ M 1011) flocculants shortened the sedimentation time to 30–35 min when compared with the samples with only an inorganic coagulant.

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**STIMULATION OF OVULATION IN AFRICAN CATFISH
CLARIAS GARIEPINUS (BURCHELL 1822)
FOLLOWING TREATMENT WITH CARP PITUITARY
HOMOGENATE, OVOPEL OR DAGIN**

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Key words: artificial spawning, stimulation of ovulation, carp pituitary homogenate, Ovopel, Dagin, African catfish *Clarias gariepinus*.

A b s t r a c t

Effects on the reproduction of *C. gariepinus* after ovulation stimulation with carp pituitary homogenate (CPH), Ovopel or Dagin were investigated. After the application of Ovopel all the females spawned while after CPH or Dagin treatment spawning was reduced to 83.3%. No statistically significant effect of the stimulator was found on the weight of eggs expressed in g and in % of female B.W. however, the highest mean values of these parameters were found after Dagin. A statistically significant effect of the stimulator was noted for the percentage of fertilization and living embryos after 24h and 28h incubation. The highest percentage of live embryos after a 28h incubation of eggs was found after the treatment with Dagin and the lowest after Ovopel; the difference between the means of these stimulators was statistically significant. The females ovulated 12h after Ovopel treatment yielded eggs of a higher weight and of significantly higher quality compared with those obtained 3h later. The relative effectiveness of reproduction, expressed as the number of live embryos per kg of female B.W., was highest after Dagin treatment.

**STYMULOWANIE OWULACJI U SUMA AFRYKAŃSKIEGO *CLARIAS GARIEPINUS*
(BURCHELL 1822) HOMOGENATEM PRZYSADKI KARPIA, OVOPELEM LUB DAGINEM**

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Słowa kluczowe: kontrolowany rozród, stymulowanie owulacji, homogenat przysadki karpia, Ovopel, Dagin, sum afrykański *Clarias gariepinus*.

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Abstrakt

Badano wyniki rozrodu suma afrykańskiego *C. gariepinus* po stymulowaniu owulacji homogenatem przysadki karpia (CPH), Ovopeltem lub Dagine. Po podaniu Ovopelu ikrę oddały wszystkie samice, a po zastosowaniu CPH lub Dagine 83.3% ryb. Nie odnotowano statystycznie istotnego wpływu stymulatora na masę pozyskanej ikry, wyrażonej zarówno w gramach, jak i w procencie masy ciała samic, ale najwyższe wartości tych parametrów wykazano po podaniu Dagine. Statystycznie istotny wpływ stymulatora owulacji odnotowano dla procentu zapłodnienia i żywych zarodków po 24 i 28 godz. inkubacji jaj. Najwyższy procent żywych zarodków po 28 godz. inkubacji jaj wykazano po podaniu Dagine, a najniższy po użyciu Ovopelu; różnica między średnimi dla tych stymulatorów była statystycznie istotna. Samice, które owulowały 12 godz. po podaniu Ovopelu, oddały ikrę w większej masie i istotnie lepszej jakości w porównaniu z ikrą pozyskaną od ryb, które owulowały 3 godziny później. Relatywna efektywność rozrodu, wyrażona jako liczba żywych zarodków na 1 kg masy ciała samic, była najwyższa po podaniu Dagine.

Introduction

The African catfish *Clarias gariepinus* is a species well established in European aquaculture (HUISMAN and RICHTER 1987, KUCZYŃSKI et al. 1999). In optimal conditions the growth of this fish is rapid and the production cycle can be shortened to seven months. The flesh of this species has high nutritional quality and good taste. In the temperate climatic zone in summer months, *C. gariepinus* can be stocked in fishing ponds as a species interesting for anglers. *C. gariepinus* is a model species for reproduction endocrinological investigations in Teleosts (VAN OORDT and GOOS 1987, RESINK et al. 1989, VAN WEERD et al. 1990). The above traits and the fact that *C. gariepinus* can mature and reproduce in captivity (HUET 1972, HOGENDOORN and VISMANS 1980) encourage the investigation of improvements to the reproduction biotechnology of this interesting fish species.

In carrying out the reproduction of fish in controlled conditions the basic aim is to obtain the highest possible yield of the best quality eggs and, hence, to produce the highest possible numbers of good quality hatches. To this end, attempts are made to find ovulation stimulators which ensure the best effects of the controlled reproduction. Also it is obvious that suitable maternal and paternal material should be used to obtain satisfactory results in stimulated fish propagation. In the case of the African catfish *C. gariepinus* different preparations were used to induce the maturation of oocytes and ovulation. Among these materials were acetone-dried powdered carp pituitary (HOGENDOORN and VISMANS 1980, ADAMEK 1993, DE GRAAF et al. 1995), crude pituitary extract of *Clarias albopunctatus* and frog *Rana elegans* (Inyang and Hettiarchchi 1994), 17α -hydroxy-progesterone (RICHTER et al. 1985, RICHTER et al. 1987), 11-desoxycorticosterone-acetate (RICHTER and VAN DEN HURK 1982), human chorionic gonadotropin-hCG (EDING et al. 1982; Inyang and Hettiarchchi 1994), hCG + Oxitocin (Hecht et al. 1982), Des Gly¹⁰[D-Ala⁶]LHRH-

ethylamide with pimozide (DE LEEUW et al. 1985, RICHTER et al. 1987, RESINK et al. 1989) or in combination with different drugs with anti-dopamine and anti-serotonin properties (GOOS et al. 1987, KOUŘIL et al. 1992). A list of the hormonal glycoproteins used to induce spawning in *C. gariepinus* was given by HAYLOR (1993).

The extended studies on the effects of controlled reproduction of *C. gariepinus* which started in 1996 at the Gołysz Institute of Ichthyobiology and Aquaculture (Polish Academy of Sciences) included numerous experiments using different ovulation stimulators of natural and synthetic origin. The natural spawning agents with which the fish were treated, were carp *Cyprinus carpio* pituitary homogenate (see BRZUSKA 2005a), bream *Abramis brama* pituitary homogenate (Brzuska et al. 1998b) and human chorionic gonadotropin hCG (BRZUSKA et al. 1998c, 1999, 2000). The synthetic spawning agents were preparations of: desGly¹⁰,[D-Ala⁶]-LHRH Ethylamide applied with dopaminergic inhibitor pimozide (BRZUSKA et al. 1998c, 1999) and [Tle⁶,ProN-Het⁹]mGnRH (Lecirelin) applied with dopaminergic inhibitor metoclopramide (BRZUSKA et al. 2004). The complex ovulation inducing preparations contained GnRH-a and a dopamine receptor blocker at the pituitary level used in aquaculture. The preparation Aquaspawn (Republic of South Africa) was also tested on the females of *C. gariepinus* at Gołysz Institute (BRZUSKA 2003) as well as Ovopel produced in Hungary (HORVÁTH et al. 1997) (BRZUSKA 2001b, 2002a,b, 2004, 2005a, BRZUSKA et al. 1998a, 2000).

The results of the successive experiment presented in this paper illustrate the progress in the studies on this interesting fish species carried out at Gołysz Institute. An experimental agent for inducing spawning in fish named Dagin (DRORI et al. 1994, KULIKOVSKY et al. 1996, "Dagin" – Instructions for use) was used in the present study. It has been successfully used for ovulation stimulation in different fish species e.g. in carp *Cyprinus carpio* (KOUŘIL et al. 2003a, BRZUSKA 1999, 2005b, 2006), in grass carp *Ctenopharyngodon idella* (KOUŘIL et al. 2003a) in tench *Tinca tinca* (KOUŘIL et al. 2003b) and in the European catfish *Silurus glanis* (BRZUSKA, unpubl. data). However, an attempt at ovulation stimulation with Dagin in pike *Esox lucius* failed (SZABÓ 2003).

Dagin combines a superactive salmon GnRH analogue [(D-Arg⁶,Pro⁹NET)-sGnRH] and the dopamine receptor antagonist, metoclopramide. Each dose, calculated per kg body weight of fish, contains 10 µg of the analogue and 20 mg metoclopramide (KULIKOVSKY et al. 1996). A diagram showing latency dependence on water temperature has been elaborated for Dagin, facilitating the proper timing of ovulation control (YARON et al. 2002). A study on the use of this preparation in *C. gariepinus* – a species not used previously in the tests with this preparation – was undertaken owing to the clear advantages of Dagin. Dagin is made of fully synthetic components and is therefore free of any pathogen which may reside in pituitaries of the

donor fish used in hypophysation. Ovulation induction by Dagin requires only a single injection and hence reduced stress to the females and less work compared with carp pituitary homogenate treatment. An important factor in hatchery conditions is that the preparation of this stimulator for injection is very simple since there is no need to pound it in a mortar, as is the case with carp pituitary or Ovopel. Neither is it necessary to weigh Dagin in order to calculate its proper dose. It is only necessary to dissolve the content of the dry matter in the vials (provided with the number of doses per body weight of the spawners given on the label) in a saline solution or water and inject it into the fish.

The aim of the present investigation was to compare the effectiveness of reproduction of African catfish *C. gariepinus* after the Dagin treatment with propagation results after CPH or Ovopel which are the most frequently used ovulation stimulators in this fish species. The investigation also concerned the dependence between the reproduction effects after ovulation stimulation with different spawning agents and the latent period.

Material and Methods

The experiment was carried out at the Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences in Gołysz. It included 18 females of the African catfish *C. gariepinus* of body weight varying from 1.50 kg to 2.53 kg. The fish, selected from a larger population of spawners, were divided into three groups of six (Table 1). The external signs of maturity (large and soft abdomen) were taken into consideration in selecting the females. They were placed in nine 3 m³ volume tanks with two females in each tank. Thus, the fish from each group were in three tanks. During the experiment the water temperature was maintained in the range of 24–25°C. After a 24h adaptation period the ovulation was stimulated with CPH in group I; with Ovopel in group II; and with Dagin in group III. The doses of the applied agents for spawning induction and the application method are given in Table 1.

Table 1
Number of females, substances used in stimulating ovulation, doses and method of application

Group	Number of females	Substances	Dose*
I	6	Carp pituitary	4 mg (i.p.)
II	6	Ovopel	1 pellet (i.p.)
III	6	Dagin	1 standard dose
Total	18		

* dose per kg body weight; i.p. intraperitoneally

1 pellet of Ovopel contains 18–20 µg of D-Ala⁶,Pro⁹NET-m GnRH and 18–20 mg of metoclopramide (HORVÁTH et al. 1997)

The control of ovulation began 10h after stimulation with the above three preparations and was continued every hour during the next five hours. The fish were checked for ovulation by gentle pressing of the abdomen.

Eggs yielded by stripping the females were weighed and fertilized from each fish separately with pooled milt taken from the macerated testes of three killed males (INYANG and HETTIARACHCHI 1994). The incubation of the eggs from each female was carried out in separate Weiss glasses 7L in volume. After a 12h incubation the percentage of egg fertilization and after 24h and 28h incubation the percentage of live embryos were calculated as follows: the mean percentage of fertilization and the mean percentage of live embryos were calculated for each fish separately from three samples of 100 eggs taken on a Petri dish. After the hatching of larvae the correctness of their development was observed and the percentage of deformed individuals was calculated.

The obtained data (descriptive statistics are given in Table 2) were subjected to an analysis of variance using the least-squares method (HARVEY 1987)

Table 2
Statistical characteristics of the experimental data. (\bar{x} , arithmetical mean; SD, standard deviation)

Variables	Descriptive statistics				
	<i>n</i>	\bar{x}	Minimum	Maximum	SD
Weight of females [kg]					
Group I	6	2.16	1.50	2.53	0.40
Group II	6	1.99	1.60	2.50	0.34
Group III	6	2.12	1.80	2.30	0.19
Weight of eggs [g]					
Group I	5	260.40	182.00	307.00	48.48
Group II	6	216.67	103.00	334.00	79.58
Group III	5	265.60	175.00	394.00	86.23
Weight of eggs [percentage of female body weight]					
Group I	5	11.97	11.46	12.53	0.40
Group II	6	10.76	6.44	13.36	2.51
Group III	5	12.44	9.05	17.90	3.57
Fertilized eggs after 12-h incubation [%]					
Group I	5	98.00	97.00	99.00	0.71
Group II	6	95.83	94.00	98.00	1.83
Group III	5	97.60	94.00	99.00	2.07
Live embryos after 24-h incubation [%]					
Group I	5	97.20	97.00	98.00	0.44
Group II	6	93.00	87.00	97.00	3.41
Group III	5	96.00	93.00	98.00	2.12
Live embryos after 28-h incubation [%]					
Group I	5	91.00	90.00	93.00	1.41
Group II	6	85.17	64.00	93.00	10.85
Group III	5	94.80	91.00	98.00	2.77

whose main classification factor was the ovulation stimulator. The investigated parameters were: the weight of the eggs expressed in grams and in the percentage of female body weight, the fertilization percentage after a 12h incubation and the percentage of live embryos after a 24h and 28h incubation of the eggs. Analysis of variance was carried out according to the following linear model:

$$Y_{ij} = \alpha + g_i + bW_{ij} + e_{ij} \quad (1)$$

where:

α – the theoretical general mean (with the assumption that $W_{ij} = 0$);

g_i – the effect of treatment (spawning agent) i ($i = 1...3$);

b – the regression on female body weight;

W_{ij} – the body weight of the female j ;

e_{ij} – the random error connected with the observation j .

Since the ovulation did not occur at the same time in all the females treated with Ovopel, the question arose whether the latency significantly affected the results of reproduction. An analysis of variance was carried out to answer this question, the main classification factor being the ovulation time. The following linear model was used:

$$Y_{ij} = \alpha + c_i + bW_{ij} + e_{ij} \quad (2)$$

where:

α – the theoretical general mean with the assumption that $W_{ij} = 0$;

c_i – the effect of time i on the ovulation ($i = 1...2$);

b – the regression on female body weight;

W_{ij} – the body weight of a female;

e_{ij} – the random error associated with the observation j .

In the present investigation we also attempted to resolve the problem of significant differences in reproduction effects in the case of females which ovulated after the same latent period but after the application of different preparations. Therefore, two separate analyses of variance for the ovulation time of 12h and 15h were carried out, using the least-squares method according to the following linear model:

$$Y_{ij} = \alpha + g_i + bW_{ij} + e_{ij} \quad (3)$$

where:

α – the theoretical general mean with the assumption that $W_{ij} = 0$;

g_i – the effect of ovulation stimulator i ($i = 1...2$);

b – the regression on female body weight;

W_{ij} – the body weight of a female;

e_{ij} – the random error associated with the observation j .

The significance of the effect of treatment on the investigated parameters was verified with the F-test while Duncan's multiple range test was used for analyzing the significance of differences between the means of the three investigated groups (Table 3). The estimated constants and the means of the least squares for the investigated parameters within the three groups are given in Table 3. The least-squares means, characterizing the effect of propagation associated with the time of ovulation, are given in Table 4. Phenotypic correlations between all the parameters were calculated separately for each group.

Relative effectiveness of the reproduction (expressed as the number of live embryos after 28 h incubation per kg female B.W.) for each ovulation stimulator was calculated as:

$$\text{RER} = \frac{ab}{100}$$

where:

a – number of eggs in the weight of eggs obtained per 1 kg female body weight;

b – mean percentage of live embryos after 28 h incubation of eggs

The calculation was carried out on the assumption that the mean weight of one *C. gariepinus* egg is 1.43 mg (VIVEEN et al. 1986).

Results

Percentage of females ovulating after hormonal stimulation

After the application of Ovopel, eggs were obtained from all the females while after CPH and Dagin from 83.3% of fish treated with these preparations.

Ovulation time

In the group of females treated with CPH in five females ovulation occurred 12h after its application. One female which did not spawn at that time was controlled 13, 14 and 15h after the CPH treatment. At the first two controls no information was obtained; after 15h the release of single degenerated eggs was found, showing the disturbed maturation process. Therefore, no further controls were carried out.

In the case of Dagin five females yielded eggs 15h after the injection. At that time in one fish which did not give eggs the release of single degenerated eggs

Table 3
 Constants (LSC) and least-squares means (LSM) for investigated reproduction parameters and results of Duncan's test

Classification factor	Weight of eggs [g]			Weight of eggs (% of female body weight)			Percentage of fertilized eggs after 12 h incubation			Percentage of living embryos after						
	$\alpha = 246.31$			$\alpha = 1.70$			$\alpha = 97.14$			24 h incubation			28 h incubation			
	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	
Ovulation stimulator																
CPH (group I)	-0.57	245.74 ^a	22.64	0.02	11.72 ^a	1.11	0.82	97.96 ^a	0.78	2.02	97.44 ^{Ab}	1.06	1.35	91.74 ^a	2.99	
Ovopel (group II)	-10.96	235.35 ^a	20.92	-0.64	11.07 ^a	1.03	-1.25	95.89 ^b	0.72	-2.72	92.70 ^{Ba}	0.98	-6.16	84.22 ^b	2.77	
Dagin (group III)	11.57	257.94 ^c	22.43	0.61	12.32 ^b	1.10	0.44	97.58 ^{ab}	0.77	0.71	96.13 ^b	1.05	4.81	95.19 ^a	2.97	
Regression/female body weight	172.47	172.47	42.64	2.83	2.83	2.09	0.51	0.51	1.47	-2.81	-2.81	2.00	-8.70	-8.70	5.63	

Group means designated by the same letters do not differ significantly from each other. Mean values marked with different letters are significantly different; with capital letters at $P \leq 0.01$ and with small letters at $P \leq 0.05$.

α – the theoretical general mean; SE – standard error of least-squares means; CPH – carp pituitary homogenate

in abundant ovarian fluid was observed and hence further controls were stopped.

In the group of females treated with Ovopel two terms of ovulation were noted; 50% of fish yielded eggs 12h after the injection of this preparation and after a further three hours the remaining 50% of females.

Effect of the ovulation stimulator on the weight and quality of obtained eggs

The values of the least squares means for weight of eggs expressed both in grams and in percentage of female body weight show that the highest weight of eggs was found in the group of females treated with Dagin and the lowest after Ovopel stimulation (257.94 g, 12.32% and 235.35 g, 11.07%, respectively; Table 3). However, the ovulation stimulator did not significantly determine these traits (Table 3). This main classification factor significantly affected investigated traits characterizing the quality of eggs ($P \leq 0.05$; $P \leq 0.01$; $P \leq 0.05$) (Table 3). After the incubation of 12h, 24h and also 28h the poorest quality characterized eggs yielded by females treated with Ovopel (95.89%, 92.70% and 84.22%, respectively; Table 3). The difference between the mean of this group and the mean of the groups after CPH as well as after the Dagin treatment was statistically significant for the percentage of live embryos both after the 24h and 28h incubation (Table 3). The highest percentage of live embryos after a 28h incubation of eggs was found for fish stimulated with Dagin (95.19%) however, it was not statistically significantly higher than the respective value noted for hypophysectomized females (91.74%) – Table 3.

Ovulation time and the weight and quality of eggs

No statistically significant difference was found between the means determining the weight of eggs – both expressed in grams and in the percentage of female body weight – obtained 12h and 15h after the application of Ovopel (Table 4). However, in the case of 12h latency, the mean values for these traits were higher by 28.35g and 1.86% (Table 4). The latent period significantly affected the percentage of fertilization and the percentage of live embryos after 24h and 28h incubation of eggs ($P \leq 0.05$; $P \leq 0.05$; $P \leq 0.05$; Table 4) while the means for these traits showed higher values for 12h latency compared with 15h latency (97.4%, 94.4%, 89.6% and 94.3%, 91.6%, 80.7%, respectively; Table 4).

Within the latency of 12 h the effect of the ovulation stimulator was only statistically significant ($P < 0.05$) with respect to the percentage of live embryos

Table 4
 Constants (LSC) and least-squares means (LSM) characterizing the effects of propagation associated with the time of ovulation

Classification factor	Weight of eggs [g]						Weight of eggs [% of female body weight]						Percentage of fertilized eggs after 12 h incubation						Percentage of living embryos after							
	Latency			Time of ovulation 12h			Time of ovulation 15h			Time of ovulation 12h			Time of ovulation 15h			24 h incubation			28 h incubation							
	α	LSC	LSM	SE	F	α	LSC	LSM	SE	F	α	LSC	LSM	SE	F	α	LSC	LSM	SE	F	α	LSC	LSM	SE	F	
Ovopel	216.67	14.18	230.84	19.93	-	10.76	0.98	11.74	1.11	-	95.83	1.51	97.35	0.62	-	93.00	4.44	89.61	3.59	-	85.17	4.44	89.61	3.59	-	
Time of ovulation 12h		-14.18	202.49	19.93	-		-0.98	9.78	1.11	-		-1.51	94.32	0.62	*		-4.44	80.72	3.59	*		-4.44	80.72	3.59	*	
Time of ovulation 15h																										
Carp pituitary	238.68	8.26	246.97	9.99	-	11.55	0.41	11.96	0.50	-	97.70	0.21	97.91	0.42	-	96.02	1.42	97.44	0.47	-	91.36	-0.42	90.93	0.89	-	
Ovopel		-8.26	230.42	13.21	-		-0.41	11.14	0.66	-		-0.21	97.49	0.56	-		-1.42	94.60	0.62	*		0.42	91.78	1.18	-	
Time of ovulation 15h																										
Ovopel	247.14	-13.46	233.68	38.36	-	11.47	-0.83	10.64	1.77	-	95.98	-1.59	94.39	1.04	-	93.49	-2.55	90.94	1.68	-	86.63	-8.48	78.16	3.88	-	
Dagin		13.46	260.59	29.67	-		0.83	12.30	1.37	-		1.59	97.56	0.81	*		2.55	96.04	1.30	*		8.48	95.11	3.00	**	

SE – standard error of least-squares means; α – the theoretical general mean * $P \leq 0.05$; ** $P \leq 0.01$

after 24h incubation of eggs. The least-squares mean for this trait was higher after the CPH treatment compared with the mean calculated for females treated with Ovopel (97.44% and 94.60%, respectively; Table 4).

Within the 15h latency the effect of the ovulation stimulator was statistically significant with respect to three investigated traits determining the quality of eggs ($P \leq 0.05$; $P \leq 0.05$; $P \leq 0.01$; Table 4). The least-squares means for the percentages of egg fertilization and live embryos after a 24h and 28h incubation showed higher values in the group of fish stimulated with Dagin compared with the respective values calculated for fish injected with Ovopel (97.56%, 96.04%, 95.11% and 94.39%, 90.94%, 78.16%, respectively; Table 4).

Dependences between the investigated parameters

The coefficient of correlation between the body weight of females and the weight of obtained eggs showed positive high values for fish after a CPH treatment and for females treated with Ovopel (+0.98 and +0.93, respectively) while its value for fish treated with Dagin was markedly lower (+0.52). The weight of spawning females was positively correlated with the percentage of live embryos after a 28h incubation of eggs both in the group of fish stimulated with CPH (+0.62) and in the group treated with Dagin (+0.71) while after the application of Ovopel the correlation coefficient between these parameters showed a negative value (-0.80). Similarly, in the group of fish stimulated with Ovopel, the coefficient of correlation between the weight of eggs expressed in grams and the percentage of live embryos after a 28h incubation of eggs only showed negative values. The index of correlation between the fertilization percentage and the percentage of live embryos after a 24h incubation of eggs, as well as between the percentage of fertilization and the percentage of live embryos after a 28h incubation showed high positive values only for fish injected with Dagin (+0.91 and +0.90, respectively). The percentage of live embryos after a 24h incubation of eggs and the percentage of live embryos after 28h was positively correlated both in the group of females stimulated with Ovopel and in the group of fish after the application of Dagin; the values of the respective coefficients were high (+0.93 and +0.92). The correlation between these parameters was negative (-0.40) for hypophysectomized fish.

Occurrence of deformed larvae

The occurrence of larvae with body deformations was found in three investigated groups. The mean percentage of deformed larvae was low in all the investigated groups treated with different preparations: in group I it was 6.20%; in group II – 7.06%; and in group III – 5.63%.

Relative effectiveness of reproduction after ovulation stimulation with CPH, Ovopel or Dagin

The lowest relative effectiveness of reproduction was found in the group of fish treated with Ovopel. The number of live embryos per kg female body weight after the application of this preparation was 65,551. The highest relative effectiveness of reproduction was found in the group of fish treated with Dagin. The number of live embryos per kg body weight of females was 83,324. In the group of fish treated with CPH the number of live embryos per kg female body weight was 76,580. A statistically significant ($P \leq 0.01$) difference was noted only between values of RER for the Dagin and Ovopel treatment.

Discussion

The results obtained in the present experiment show that the application of Dagin resulted in satisfactory effects on reproduction. After using this preparation a high percentage of females ovulated, the weight of eggs was high and their quality after a 28h incubation exceeded the quality of eggs yielded by hypophysectomized females and by fish treated with Ovopel. It is particularly worth stressing that after the Dagin treatment the very high quality of eggs, expressed by the fertilization percentage after a 12h incubation, was maintained during the rest of the incubation. In this group the mean percentage of live embryos after a 28h incubation of eggs was lower only by 2.4% compared with the mean fertilization percentage. In the group of fish treated with CPH the respective values were approximately 6%. In the investigation carried out at the Gołysz Institute attention was paid to the fact that after some spawning agents applied to *C. gariiepinus* the quality of eggs decreased during their incubation. A decrease in the quality of *C. gariiepinus* eggs during incubation was observed after the application of desGly¹⁰[D-Ala⁶]-LHRH Ethylamide (50 µgkg⁻¹ body weight of females) (BRZUSKA et al. 1999), of [D-Tle⁶,ProN-HEt⁹]m GnRH (15 µgkg⁻¹) (BRZUSKA et al. 2004) and of the complex preparation-Aquaspawn (0.5 mLkg⁻¹) (BRZUSKA 2003).

The results presented here show that the most pronounced decrease in the quality of eggs (in the period between 12 and 28h of incubation) occurred in the group of females treated with Ovopel. A difference of 11.7% was noted between the mean percentage of fertilization and the mean percentage of live embryos after 28h incubation of eggs. The results of three previous experiments with Ovopel applied to *C. gariepinus* showed distinctly that in these experiments no serious decreases in quality occurred during the period of egg incubation (BRZUSKA 2004). An analysis of data obtained in an investigation conducted on a different species of the African catfish, i.e., *Heterobranchus longifilis* showed that, in the course of incubation, the quality of eggs yielded by females ovulating 14h after an Ovopel application deteriorated to a higher degree in comparison with decreases in the quality of eggs yielded by fish two hours earlier (BRZUSKA and ADAMEK 2008).

The striking fact is that after the application of Dagin all the females of *C. gariepinus* spawned at the same time. A synchronized ovulation induced in all the females by this spawning agent is an important positive trait determining its value for hatchery practice. The lack of ovulation synchronization in spawners hinders and disorganizes the work order in a hatchery at the time of a controlled fish reproduction.

The results of an investigation of the common carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella* (KOUŘIL et al. 2003a) clearly showed that, in these two species, the application of Dagin induced the synchronization of ovulation in all the treated females. In the common carp the time of stripping was 14h 30 min (water temperature 24°C) and in grass carp 16h (the water temperature 22.5°C). In tench, *Tinca tinca* treated with Dagin the latency period was much longer – up to 30h 30min (water temperature 22.5°C) and was the same in all the females which yielded eggs (KOUŘIL et al. 2003b). The effects of experiments with the common carp carried out at the Institute of Ichthyobiology and Aquaculture at Gołysz showed that this preparation did not synchronize ovulation in females from two Hungarian breeding strains: strain W (BRZUSKA 2005b) and strain 7 (BRZUSKA 2006). However, the ovulation synchronization after the Dagin treatment was recorded in common carp females of the Polish strain 6 (BRZUSKA 2005b). The time interval between the injection of Dagin and the initial egg release in the females of strain 6 (at 21.5°C) was 14h and was above one hour shorter as compared with the respective time interval reported by DRORI et al. (1994) and by YARON et al. (2002). The application of Dagin induced the ovulation in all the females of the European catfish *Silurus glanis* at the same time, i.e., 24h after the injection of this spawning agent (BRZUSKA, unpubl. data).

Synchronized ovulation also occurred in individuals treated with CPH while after the application of Ovopel two different terms of egg release were

recorded. We stress that a higher weight of eggs was obtained from females ovulating 12h after the treatment with Ovopel. The percentage of live embryos both after a 24h and 28h incubation developing in these eggs was significantly higher compared with the percentage of live embryos developing in eggs obtained from females ovulating three hours later. These results may suggest that females which ovulated earlier were characterized by a higher physiological readiness for ovulation induction using a preparation based on the stimulation of the endogenous gonadotropin from the pituitary of fish treated with a synthetic hypothalamic hormone. In previous experiments carried out with females of this fish species stimulated with Ovopel the synchronization of ovulation was noted in all the investigated fish both after the application of Ovopel at one dose (1 pellet kg^{-1}) or at two doses (1/5 + 1 pellet kg^{-1}) (BRZUSKA et al. 2000, BRZUSKA et al. 1998b, BRZUSKA 2002a,b, 2004). The lack of ovulation synchronization after an Ovopel treatment was found in the European catfish *Silurus glanis* (BRZUSKA 2001a) and in the African catfish *Heterobranchus longifilis* (BRZUSKA and ADAMEK 2008).

A very important piece of information obtained in the investigation of *C. gariepinus* is that after the application of Dagin the percentage of deformed larvae was not higher in comparison with the hypophysed group or that treated with Ovopel. The high percentage (>20%) of larvae hatched with body deformations was found in this fish species whose ovulation was stimulated with desGly¹⁰[D-Ala⁶]-LHRH (applied with pimozide) irrespective of the LHRH – a dose of 50 $\mu\text{g kg}^{-1}$ or 20 $\mu\text{g kg}^{-1}$ (BRZUSKA et al. 1998c). It is also important that eggs obtained from all the fish after an ovulation stimulation with Dagin were of a very good quality.

In summary, the results of controlled reproduction after the application of the tested preparations were satisfactory. In spite of the fact that the results of reproduction of *C. gariepinus* after ovulation stimulation with Dagin can be only regarded as preliminary, the best effects were found after the treatment with this preparation. In the group of fish treated with Dagin synchronization of ovulation was noted in five females in which the ovulation occurred. The highest mean weight of obtained eggs was found after the application of this stimulator, however, it did not significantly differ from the mean weight of eggs obtained from hypophysed females or those treated with Ovopel. The highest mean percentage of live embryos after 28h incubation of eggs was obtained after the application of Dagin, however, it differed significantly from the mean percentage of live embryos in the group of fish treated with Ovopel. The stimulation of ovulation with Dagin resulted in a higher relative effectiveness of propagation (the number of live embryos per kg female body weight) than the stimulation with carp pituitary homogenate or Ovopel.

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**THE ICHTHYOFAUNA OF THE REGULATED SECTION
OF THE NIDA RIVER (THE UPPER WKRA)
IN THE COMMUNE OF NIDZICA**

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Key words: river, Upper Wkra, Nida, ichthyofauna, degradation of river.

A b s t r a c t

The purpose of this study has been to characterize the ichthyofauna of the Upper Wkra River during the initial stage of the implementation and execution of revitalization of the Nida River in the commune of Nidzica. The study covered a nearly 5-km-long section of river flowing across the commune of Nidzica. Four sites of control catches were localized between the administrative border to the town of Nidzica and a water-raising fixed weir ($h=1.40$ m) in the village called Borowy Młyn. In the fish assemblage, dominated by three- and ninespined stickleback, nine species in total were observed. By referring to the available research results, it can be concluded that no significant quantitative or qualitative changes occurred in the structure of the ichthyofauna from 2001 to 2008. One of the factors which limit the process of natural regeneration of the fish population is the fact that the analyzed section of the river is cut off from the remaining part of the Wkra River and its tributaries.

**ICHTIOFAUNA UREGULOWANEGO ODCINKA RZEKI NIDY (GÓRNEJ WKRY)
W GMINIE NIDZICA**

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Słowa kluczowe: rzeka, Górna Wkra, Nida, ichtiofauna, degradacja rzeki.

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Abstrakt

Celem opracowania jest charakterystyka ichtiofauny uregulowanego odcinka górnej Wkry we wstępnym etapie wdrażania i realizacji programu rewitalizacji Nidy w powiecie nidzickim. Badaniami objęto prawie pięciokilometrowy odcinek rzeki położony w gminie Nidzica. Cztery stanowiska połowów kontrolnych zlokalizowano pomiędzy granicą administracyjną miasta Nidzica a stałym jazem piętrzącym ($h=1.40\text{m}$) w miejscowości Borowy Młyn. W zespole ryb zdominowanym przez ciernika i cierniczka stwierdzono występowanie łącznie dziewięciu gatunków ryb. Na tle dostępnych wyników badań w latach 2001–2008 w strukturze ichtiofauny nie nastąpiły istotne zmiany jakościowe i ilościowe. Jednym z czynników ograniczających proces naturalnej odbudowy ichtiofauny rzecznej jest odcięcie objętego badaniami odcinka rzeki od pozostałej części dorzecza Wkry.

Introduction

The species composition of ichthyofauna found in Polish rivers is mainly modelled by such man-made factors as the regulation of rivers, development of river shores, discharge of municipal and industrial wastewater (PENCZAK and MANN 1993) as well as the introduction of fish stocks composed of a variety of species, including foreign ones (WITKOWSKI 1996). Mechanisms involved in the modification of the number and biomass of river ichthyofauna in water courses, varied in their size and degree of environmental degradation, have been described by such authors as PENCZAK et al. (1992a). What is commonly observed is that sensitive species of high environmental requirements withdraw and are replaced by species adaptable to an unfavourably altered environment (WIŚNIEWOLSKI 1995).

The Wkra River originates in an area of drained peat bogs situated west of Kownatki Lake. Geographically, this area lies on Lubawa Hummock, which belongs to the Mława Hills. In its middle course, the Wkra flows through Raciąż Plain and next along the edge of Ciechanów Plateau (KONDRACKI 1998). The Wkra, which is a right tributary of the Narew River, is 249 km long and has a catchment basin covering 5,322.1 km². In its upper course, i.e. within the administrative district of Nidzica, the river is known as the Nida but in the administrative district of Działdowo, it is usually called the Działdówka, and it is not until it passes Żuromin that it is given the name the Wkra River (*Atlas hydrograficzny...* 1986). Towards Działdowo, the river flows through a peat-like valley and the catchment basin is characterized by rather uniform relief. From its sources to the borders of Nidzica, the Wkra serves as a collective draining ditch. In Nidzica, the river turns into a regulated watercourse and the last regulation works were performed in the 1960s. In some parts of the river, especially within the town of Nidzica, some periodical repairs are carried out on fascines and river embankments. However, in many places the remaining effects of the river regulation works are becoming less visible and the river turns into a semi-natural watercourse.

The observations of the ichthyofauna in the Wkra River were carried out in 2001 (PENCZAK et al. 2001). The qualitative composition of the ichthyofauna in some larger tributaries of the river was described by MARSZAŁ et al. (2005). Earlier, some other studies on the ichthyofauna in the Narew River and its tributaries had been completed (PENCZAK et al. 1990a, 1990b, 1991a, 1991b, 1992b, WITKOWSKI 1984a, 1984b). In all these papers, while analyzing the current qualitative and quantitative composition of the ichthyofauna in the Narew and Wkra Rivers, the researchers noticed processes of natural regeneration of fish populations in the rivers owing to improved quality of their waters and fish stockings. The research completed by PENCZAK et al. (2001) partly covered the section of the river located in the commune of Nidzica and, in general terms, described the then structure of the degraded ichthyofauna in the Upper Wkra (the Nida) River, which in the 1960s and 1980s was particularly heavily polluted.

The objective of the present study has been to characterize the ichthyofauna in the Upper Wkra during the implementation and execution of the revitalization programme for the Nida River in the administrative district of Nidzica.

Materials and Methods

The research on the ichthyofauna in the regulated section of the Upper Wkra River covered a nearly 5-km-long part of the river within the commune of Nidzica. Four sites of control catches were localized between the administrative border of the town of Nidzica and a water-raising fixed weir ($h=1.40$ m) in the village called Borowy Młyn. The sites were assigned the symbols from S_1 to S_4 . (Figure 1). This construction is completely impassable to fish migrating upstream the river. The characteristics of some morphological features of the river channel and the habitat conditions at the sites where electrofishing was performed were described on the basis of direct field measurements and set in Table 1. The initial points of the control catch sites are located around 1,200 meters from each other. Apart from the differences in the width and depth of the river as well as water flows, the habitat conditions along the regulated river section are modified by the presence of few elements typical of a seminatural river channel, i.e. washed-off river banks with damaged fascines, river bank outwashes or deeper holes in the river channel behind groups of submerged plants as well as single boulders. The quality of the habitats at the control catch sites was also shaped by the degree of river channel shading, expressed as a percentage of the share of banks shaded with tree canopy on both sides of the river. Submerged plants at the control sites were scarce and single groups

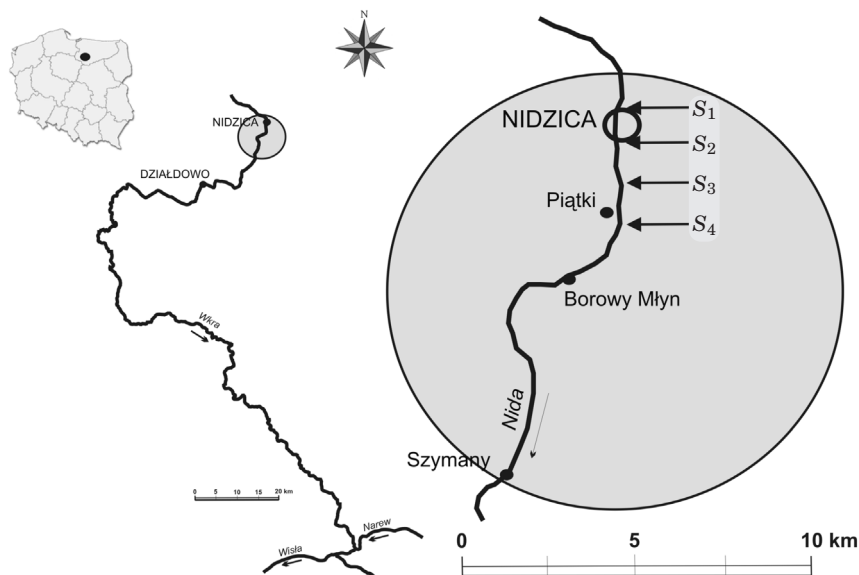


Fig. 1. The location of study area and electrofishing stations

Table 1
Characterization of the Nida River at the sites of electrofishing

Parameters	Electrofishing stations			
	S_1	S_2	S_3	S_4
Position of start point	N 53° 21.92' E 20° 25.38'	N 53° 21.29' E 20° 25.27'	N 53° 20.59' E 20° 24.93'	N 53° 20.48' E 20° 24.48'
Average width [m]	3.5	3.0	3.5	4.0
Average depth [m]	0.25	0.40	0.60	0.65
Maximum depth [m]	0.55	0.50	0.85	1.20
Maximum velocity [m s^{-1}]	0.35	0.40	0.20	0.30
Buttom substrate	sand	sand with gravel and stones	muddy sand	sand
Submerged plants	–	<i>Elodea canadensis</i> , filamentous algae	<i>Sagittaria sagittifolia</i>	<i>Elodea canadensis</i> , filamentous algae
Features of river channel	regulated with seminatural elements	regulated	regulated with seminatural elements	regulated with seminatural elements
Trees along banks [% of bank]	70	10	50	50
Adjacent area	meadows and pastures	meadows and pastures	bushes and wasteland	pastures and cultivated area

of such plants covered in total less than 20% of the bottom surface. In 2008, the ichthyofauna was examined twice, i.e. on 19th May (water temp. 14°C) and 5th September (water temp. 12°C). Control catches were carried out using two impulse sets IUP-12 powered with a 12V battery, by wading upstream simultaneously on both river banks, along 100-meter-long sections, with two anode-poles, which is in accord with the unified method (PENCZAK 1967). The analysis of the collected biological material included determination of the qualitative and quantitative composition of particular components of the ichthyofauna and their biomass. Having identified the species of each captured fish, its length and individual weight were measured. For our analysis of the structure and distribution of the ichthyofauna, commonly used biocenotic indices were applied (SZCZERBOWSKI 1972, MARSZAŁ et al. 2005), i.e. species dominance index

$$D = \frac{100n_i}{N}$$

where:

n_i – number of individuals of a given species in a sample;

N – number of all individual in this sample) and occurrence stability index.

$$C = \frac{100S_i}{S_n}$$

where:

S_i – number of sites where a given fish species was found;

S_n – total number of control catch sites.

The index of similarity of the effects of subsequent catches was calculated according to Steinhaus's formula (PERKAL 1958, MARCZEWSKI and STEINHAUS 1959), i.e.

$$W = \frac{2c}{a+b}$$

where:

a – number of species in the first catch;

b – number of species in the second catch;

c – number of species that are common in the first and second catch.

The general species diversity of the ichthyofauna at the control sites was expressed with Shannon-Wiener index (SHANNON 1948, KREBS 1997). The statistical significance of differences in the values of Shannon-Wiener index between the control sites was verified with t-test at the level $p < 0.05$.

Results

In the spring electrocatches, 229 fish in total were captured, weighing 415 g and representing seven species (Table 2). Over 95% of the fish belonged to the family of sticklebacks (*Gasterosteidae*). The dominant species in the ichthyofauna structure was the three-spined stickleback, *Gasterosteus aculeatus* L., ($D = 65.5\%$), which prevailed in number at three control catch sites. The total share of the nine-spined stickleback, *Pungitius pungitius* (L.), was 30.2%. Other fish species which exceeded a one-percent threshold of the dominance index were gudgeon, *Gobio gobio* (L.), present at one site ($D = 1.8\%$) and perch, *Perca fluviatilis* L., found at two sites ($D = 1.3\%$). The other species, i.e. tench *Tinca tinca* (L.), roach *Rutilus rutilus* (L.) and sunbleak *Leucaspius delineatus* (Heck.) were found as single individuals. During the electrofishing carried out in the first decade of September, neither the three above species nor gudgeon were captured at any of the control sites. In total, 1,001 fish individuals were captured of the aggregate weight of 1,307 g, and the structure of the ichthyofauna comprised five species. Three- and nine-spined sticklebacks occurred at all the sites ($C = 100\%$) and the dominance index values for these species were, respectively, 64.5% and 31.3%. The remaining three species, i.e. perch, ide *Leuciscus idus* (L.) and pike, *Esox lucius* L., were characterized by a 50% value of the occurrence stability index in the examined section of the river. In the first decade of September, the smallest value of the dominance index was assigned to pikeperch ($D = 0.3\%$). At the same time, this species was a dominant component of the biomass structure, reaching 37.2% (Figure 2) and the individual weight of the captured fish ranged from 33 g to 286 g. In May, the biomass structure was dominated by three- (36.2%) and nine-spined stickleback (25.1%). The average individual weight of the representatives of these species was, respectively 1.07 g (± 0.53) and 1.84 g (± 1.03). As the share of these species in the biomass of captured fish declined in September, being 30.5% for three- and 17.9% for nine-spined stickleback. The individual weight of representatives of these species likewise decreased, falling to 0.55 g (± 0.09) for three- and to 0.69 g (± 0.24) for nine-spined stickleback (Table 2). A similar tendency appeared for perch, whose share in the biomass structure from May to September decreased from 8.5% to 6.4% and the average individual weight in the same period diminished from 10.78 g (± 0.61) to 3.75 g (± 1.37). The average individual mass of ide, whose contribution to the biomass of the September catches was 8.0%, reached 5.96 g (± 2.44). With the observed differentiation of the qualitative and quantitative structure of the ichthyofauna in the control catches completed in May and in September, the indices of the similarity of species composition at particular sites of sample collection between the first and second electrofishing varied from 0.66 for sites S_1 and S_2 to 0.80 for site S_4 (Figure 3).

Tabela 2
The number of individuals and structure of ichthyofauna at control stations in 2008 with mean individuals weight (*B*), dominance index (*D*) and occurrence stability (*C*) of species

Fish species	May							September						
	<i>S</i> ₁	<i>S</i> ₂	<i>S</i> ₃	<i>S</i> ₄	<i>B</i> (±SD)	<i>B</i>	<i>C</i>	<i>S</i> ₁	<i>S</i> ₂	<i>S</i> ₃	<i>S</i> ₄	<i>B</i> (±SD)	<i>D</i>	<i>C</i>
	number of fish				[g]	[%]	[%]	number of fish				[g]	[%]	[%]
Three-spined stickleback	9	20	80	41	1.07 (±0.53)	65.5	100	16	239	30	361	0.55 (±0.09)	64.5	100
Nine-spined stickleback	34	11	12	12	1.84 (±1.03)	30.2	100	71	191	33	18	0.69 (±0.24)	31.3	100
Gudgeon	-	4	-	-	10.82 (±3.48)	1.8	25	-	-	-	-	-	0.0	0
Tench	-	-	1	-	38.14	0.4	25	-	-	-	-	-	0.0	0
Roach	-	1	-	-	40.11	0.4	25	-	-	-	-	-	0.0	0
Perch	-	1	2	-	10.78 (±0.61)	1.3	50	-	18	4	-	3.75 (±1.37)	2.2	50
Sunbleak	-	-	-	1	1.24	0.4	25	-	-	-	-	-	0.0	0
Ide	-	-	-	-	-	0.0	0	4	13	-	-	5.96 (±2.44)	1.7	50
Pike	-	-	-	-	-	0.0	0	1	-	2	-	162.06 (±126.58)	0.3	50
Total	43	37	95	54	-	100.0	-	92	461	69	379	-	100.0	-

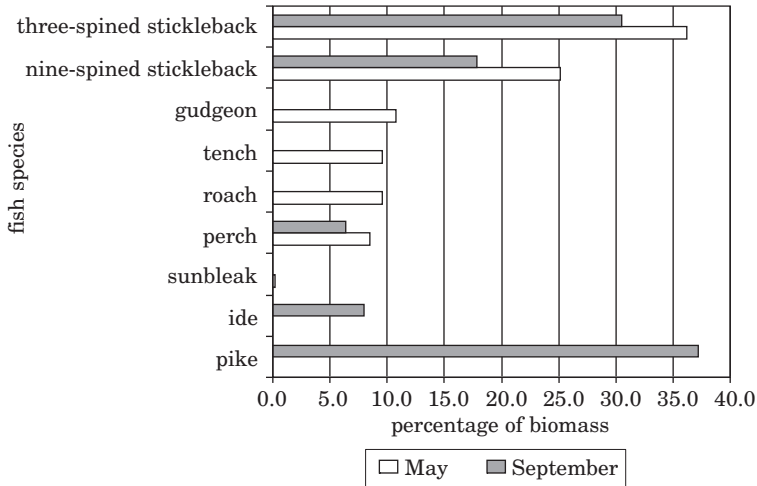


Fig. 2. The structure of the biomass of the ichthyofauna in the Nida River in the commune of Nidzica in 2008

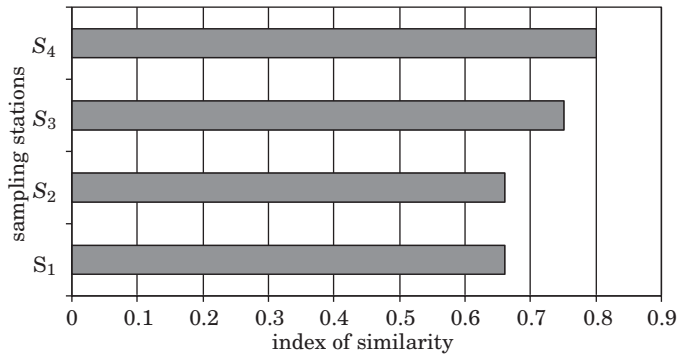


Fig. 3. The index of similarity of the species composition of the ichthyofauna at stations during two control catches (May and September) in 2008

In spring, the diversity of the ichthyofauna inhabiting the analyzed section of the river, expressed with Shannon-Wiener index was 0.83 and did not differ statistically from the value obtained during the September catches, when it reached 0.90 (t -test, $p < 0.05$). In the May sample, the highest species diversity of the ichthyofauna was recorded at site S_2 (1.13). At sites S_1 , S_3 and S_4 , this index was 0.51, 0.54 and 0.62, respectively, and statistically significant differences in its value were demonstrated only between site S_2 and the other ones (t -test, $p < 0.05$). During the September electrofishing, the highest values of Shannon-Wiener index were recorded at sites S_3 (0.98), S_2 (0.93) and S_1

(0.69). They differed statistically from the biodiversity index recorded at site S_4 , which was 0.19 (*t*-test, $p < 0.001$).

Discussion

In the section of the Nida River (the Upper Wkra River) chosen for a revitalization programme and covered by our study, nine fish species in total were found. The ones most characteristic for this section of the Wkra River are three-spine stickleback, nine-spine stickleback and perch, as well as gudgeon along this course of the river where the water flow was faster. Such species as tench, roach, pike and sunbleak should be described as very scarcely occurring ones. The presence of ide, found in September samples at the two sites of the fastest water flow (S_1 and S_2) is a direct outcome of the fish stockings carried out in the first decade of June 2008. Then, as part of the initiated revitalization treatments in the section of the Wkra River within the commune of Nidzica, 12,000 individuals of summer fry of ide weighing 0.3–0.4 g/individ. were introduced to the river. The average weight of nearly 6 g of the ide individuals captured in September proves that the ide fry introduced to the river habitat obtained good weight gains (SKRZYPCZAK et al. 2009).

The tendency for an increasing biodiversity index as the number of species increased and quantitative proportions between them levelled (Krebs 1997) became very evident at the sites where only three- and nine-spined sticklebacks were found (S_1 and S_4). An increase in the number of fishes in the September sample and a simultaneous decrease in their average individual weight reflect the fact that the control electrofishing catches contained juvenile individuals.

The dominance of nine-spined stickleback at site S_1 and that of three-spined stickleback at site S_2 , which repeated in the May and in the September samples, should be considered as a characteristic feature. Alongside the analysis of the number of fish and quantitative proportions between them, it may indicate higher adaptability of nine-spined stickleback to poorer habitats, hardly differentiated in habitat-specific features, e.g. lower water depth or sandy bottom free of vegetation. At the same time, the quantitative dominance of three-spined stickleback was the most evident at site S_4 , located below the point of discharge from the wastewater treatment plant in Piątki, where the quality of water in the river has been found to be much worse (SKRZYPCZAK et al. 2009). This proves that three-spined stickleback is more tolerant and adaptable to polluted habitats. High resistance of this species to pollution has been demonstrated by PENCZAK (1975). Numerous occurrence of three-spined stickleback in tributaries of the Wkra River at sites polluted with municipal

sewage and wastewater has been observed by MARSZAŁ *et al.* (2005). In their study of 2001, PENCZAK *et al.* (2001) observed a rapid increase in the number of three-spined sticklebacks and the evident dominance of this species at a site located just below the town of Nidzica and between Nidzica and Działdowo. In 2002, along this section the Wkra River carried waters beyond the water purity classification system because of the phosphorus concentration above the norm (WIOŚ 2003). In 2008, the water there was classified as class II in the water purity classification system (SKRZYPCZAK *et al.* 2009).

The recorded values of Shannon-Wiener index on the two sampling dates (0.83 and 0.90) confirm low fish species diversity. Nonetheless, the ichthyofauna contained more species than was demonstrated by PENCZAK *et al.* (2001). In 2001, these authors observed only sporadic occurrence of three-spined stickleback and roach below Nidzica, i.e. near site S_1 , and four species, such as three-spined stickleback (very numerous), nine-spined stickleback (numerous), sunbleak and prussian carp between Nidzica and the village of Piątki (near sites S_2 , S_3 and S_4). Higher species diversity in 2008, alongside the lack of direct fish stockings in the Nida River within this river section as well as the improving quality of water, may reflect the phenomenon of self-regeneration of the ichthyofauna. On the other hand, since the river is impassable to fish in Borowy Młyn and the communal course of the river is isolated from the fish populations inhabiting the middle and lower course of the river, the species structure of fish inhabiting the Nida may be affected by fish ponds in the villages of Załuski and Borowy Młyn. This hypothesis can be supported by the presence of such species as tench, prussian carp and pike. However, it can be surprising that with the good quality of river water no presence of roach was found in the structure of the analyzed ichthyofauna. Many observations suggest (SCHIEMER and WIESER 1992) that this species is resistant to anthropogenic changes and, like other representatives of adaptable stagnophilous fish (e.g. perch, bleak, bream), it can rebuild its population (KRUK *et al.* 2001).

Conclusions

1. The ichthyofauna of the Upper Wkra River in the administrative district of Nidzica, dominated by sticklebacks, is poor in the number and quality of fish, thus being typical of largely degraded river habitats.

2. The available results of relevant research carried out in 2001–2008 suggest that the structure of the ichthyofauna did not change significantly in the number or quality of fish. As the purity of water in the Nida flowing through the district of Nidzica has improved, among the factors which restrict

the process of natural regeneration of the river ichthyofauna is the water-raising weir, which completely isolates the analyzed section of the river from the remaining part of the Wkra and its tributaries.

3. The dominance of sticklebacks fish of low value, as well as a low biodiversity index of the ichthyofauna justify the revitalization programme which covers the upper part of the Wkra River, with an aim of recreating the river ichthyofauna structure.

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OPTIMIZATION OF ARTIFICIAL REPRODUCTION OF ASP, *ASPIUS ASPIUS* (L.) UNDER CONTROLLED CONDITIONS

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Key words: asp, artificial reproduction, wild cyprinids, Ovopel, Ovaprim.

Abstract

Artificial reproduction of asp under controlled conditions was done using two different spawning agents based on GnRH analogues and dopamine antagonists (Ovopel and Ovaprim). Fish in the Ovopel and Ovaprim and combined treatment groups were treated with a dose equivalent to 1.2 pellets (0.2 and 1.0), 0.5 cm³ liquid (0.1 and 0.4) and 0.2 pellets and 0.4 cm³ liquid per kg of body weight respectively. The highest percentage of ovulation (100%) and embryo-survival to the eyed-egg-stage (81.3%) was recorded after the application of a combination of Ovopel and Ovaprim in comparison with other groups. Fish from the control group did not ovulate. The latency time was shorter in the groups where Ovopel and Ovopel with Ovaprim was applied (40) than in Ovaprim group (42–44 hrs). The obtained results indicates that combination of Ovopel with Ovaprim might be successfully used for artificial reproduction of asp.

OPTYMALIZACJA KONTROLOWANEGO ROZRODU BOLENIA, *ASPIUS ASPIUS* (L.) W WARUNKACH KONTROLOWANYCH

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Słowa kluczowe: boleń, rozród kontrolowany, ryby karpowate, Ovaprim, Ovopel.

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Abstrakt

Kontrolowany rozród bolenia przeprowadzono z zastosowaniem dwóch różnych preparatów opartych na analogach GnRH i inhibitorach dopaminy (Ovopel i Ovaprim). U ryb z grupy kontrolnej nie stwierdzono owulacji. Najwyższy odsetek owulacji (100%) oraz przeżywalność embrionów do stadium zaoczkowania (81.3%) zaobserwowano po zastosowaniu kombinacji Ovopelu i Ovaprimu. Krótszy czas owulacji odnotowano w grupach, w których aplikowano Ovopel oraz Ovopel z Ovaprimem (40 godzin) niż w grupie, w której zastosowano tylko Ovaprim (42–44 godziny). Uzyskane wyniki wskazują, że Ovopel w kombinacji z Ovaprimem mogą być z powodzeniem używane w kontrolowanym rozrodzie bolenia.

Introduction

Cyprinid aquaculture has been developing rapidly in the last two decades. The list of cultured species was still increasing and there are two main topics of production: for human consumption and for restocking. In Europe, such species as ide *Leuciscus idus* (KREJSZEFF et al. 2009), dace *Leuciscus leuciscus* (ŻARSKI et al. 2009), chub (KREJSZEFF et al. 2008, 2010) and asp *Aspius aspius* (TARGOŃSKA et al. 2010) are cultured in closed systems and ponds. Every year, millions of larvae and juveniles are put into lakes and rivers (WOJDA 2004). Quickly increasing the economic effectiveness of such kind of production is of critical importance (HAKUĆ-BŁAŻOWSKA 2009, 2010). But in all cases, the artificial reproduction has been the basis of production.

The reproduction of asp under controlled conditions was only possible after applying hormonal stimulation (TARGOŃSKA et al. 2008, 2010, FALAHATKAR et al. 2010). To date, different spawning agents have been tested in artificial reproduction of asp: carp pituitary homogenate (CPH) and commercial products containing GnRH analogues combined with dopamine inhibitors: Ovopel and Ovaprim. Obtaining results showed that hormonal stimulation was important not only in the case of females but also in the case of males (CEJKO et al. 2008). Recently, the combination of Ovopel and Ovaprim was found to have a high impact on artificial reproduction in wild cyprinids (ŻARSKI et al. 2009).

The aim of this study was to compare the spawning effectiveness of Ovopel, Ovaprim and their combination in artificial reproduction of asp.

Material and Methods

The spawners were collected during the winter (end of February) from Lake Mosąg (Olsztyn district). River Łyna are flowing through the Lake Mosąg (Dam Reservoir). Selected spawners were transported to the hatchery of the Department of Lake and River Fisheries, University of Warmia and Mazury in

Olsztyn. The size of spawners ranged from 1.7 to 2.4 kg for males and from 1.9 to 3.6 kg for females. The selected males and females (without visible damages) were kept in separate 1000 L tanks in the hatchery with controlled temperature and photoperiod (12 L : 12 D) (KUJAWA et al. 1999). The maximum fish load in the tanks was 25 kg m⁻³. The dissolved oxygen level was maintained above 6 ppm.

All fish were individually marked using floy tags and weighed. Oocytes from females were taken *in vivo* by catheterization and placed in Serra's solution (6:3:1, 70% ethanol, 40% formaldehyde and 99.5% acetic acid) for five minutes. After clarification of the cytoplasm, the position of the germinal vesicle was determined according to a four-stage scale described by BRZUSKA (1979) for common carp. All females had oocytes in 2nd maturation stage.

Fish were divided into four groups: a control group and three experimental ones. Number of females in each groups was presented in Table 1. In the hatchery, the temperature of water in fish tanks was gradually raised from 8 to 10°C. After 2–3 days of acclimation at 10°C, the fish from the experimental groups were treated with two commercial products: Ovopel (Unic-trade, Hungary) or Ovaprim (Syndel, Canada). One pellet of Ovopel typically contained 18–20 µg mammalian LHRH analogue (mLHRHa) [D-Ala⁶ Pro⁹Net-mLHRH] and 8–10 mg of the dopamine antagonist metoclopramide (HORVATH et al. 1997). Ovaprim contains 20 µg of salmon LHRH analogue (sLHRHa) [D-Arg⁶Pro⁹Net-sLH-RH] and 10 mg of the dopamine antagonist domperidon in 1 cm³ propylene glycol (PETER et al. 1993). Ovopel pellets were pulverized in a mortar and then dissolved in saline (0.25 cm³ 0.9% NaCl per one pellet). Ovaprim is ready to use in a liquid form. Hormones were applied intraperitoneally in double injections at the base of the ventral fin. The time between injections was 24 hours. Fish from the control group were injected with a sterile saline solution (0.5 cm³ kg⁻¹). Fish in the Ovopel and Ovaprim and combined treatment groups were treated with a dose equivalent to 1.2 pellets (0.2 and 1.0) , 0.5 cm³ liquid (0.1 and 0.4) and 0.2 pellets and 0.4 cm³ liquid per kg of body weight respectively. The males were not hormonally stimulated. The average weight of females is presented in Table 1. Before injection, fish were anaesthetized with 2-phenoxyethanol (0.5 cm³ dm⁻³) (Sigma-Aldrich, Germany). After hormonal treatment, the water temperature was raised to 11°C and to 12°C after a further 24 hours (TARGOŃSKA et al., 2008). Milt from males was collected using plastic 1 cm³ syringes and kept at 4°C. Females were checked every 2–4 hours between 36 and 48 hours post injection. Eggs were stripped into a plastic vessel and were fertilized using the “dry method”. Only those samples of milt which showed a motility of more than 70% of spermatozoa were used for fertilization. Three egg samples (100–150 eggs each) from each female were mixed with 0.05 mL of pooled milt

taken from at least three males. Eggs were incubated at 12°C on Petri dishes in a closed-water system and survival to the eyed-egg stage was observed. Oocyte samples from non-ovulated females were taken after the experiment and their maturity stage was recorded.

Table 1
Results (mean \pm SD) of artificial reproduction of asp under controlled conditions

Parameter/groups	Control	Ovopel	Ovaprim	Ovopel/ Ovaprim
Number of females	4	4	4	4
Mean weight (mean \pm SD) of females [kg]	2.3 \pm 0.2 ^a	2.4 \pm 0.3 ^a	2.3 \pm 0.3 ^a	2.2 \pm 0.4 ^a
Ovulation [%]	0	50	75	100
Oocyte-maturity-stage after stimulation in non-ovulated females	2/3	3/4	3/4	–
Range of latency time [hrs] (mean \pm SD)	–	40	42–44 (43.3 \pm 1.2)	40
Fecundity [eggs kg ⁻¹]	–	50124 + 2121 ^b	53172 + 2592 ^{ab}	54199 + 1524 ^a
Embryo survival to the eyed-egg-stage [%]	–	46.5 \pm 2.9 ^c	74.2 \pm 3.1 ^b	81.3 \pm 1.8 ^a

Data in the same row marked with different letters were significantly different ($P < 0.05$).

The control groups did not spawn and, therefore, no control data was included in the statistical analysis. All the data expressed as percent were subjected to arcsine transformation before being analysed statistically. The data for the fecundity were analysed by Kruskal-Wallis non-parametric test ($\alpha = 0.05$). The other data (mean weight of females and embryo survival to the eyed-egg-stage) were analysed by ANOVA. Where the analysis revealed statistically significant differences, a *post-hoc* Duncan test ($\alpha = 0.05$) was carried out.

Results and Discussion

The cultured and wild cyprinids need the application of hormonal agents for final gamete maturation (BABIÁK et al. 1998, BRZUSKA 2005), especially in the case of females (KUCHARCZYK et al. 1997, 2005, KREJSZEFF et al. 2008, 2010, PODHOREC, KOURIL 2009, TARGOŃSKA, KUCHARCZYK 2011). Without hormonal stimulation, only slight maturation of oocytes (germinal vesicle migration) was usually observed (KUCHARCZYK et al. 2005, TARGOŃSKA et al. 2010) – Table 1. In the present study only slightly oocytes maturation was noted in fish from control groups. Only in some cases of domesticated stock of cyprinids is it

possible to obtain eggs without hormonal stimulation under controlled conditions (KREJSZEFF et al. 2009, KUJAWA et al. 2011).

Ovulation rates in the present study were between 50 and 100%, independent of the type of hormonal stimulation (Table 1). This was similar to data obtained for asp by TARGOŃSKA et al. (2010). In wild cyprinids, the ovulation rates were different, e.g. for ide and dace they were usually between 90% and 100% (KREJSZEFF et al. 2009, ŹARSKI et al. 2009) but for chub (30–70%) they were much lower than for asp (KREJSZEFF et al. 2008, 2010). The differences in the fecundity between groups were small: the highest fecundity was noted after the application combined from Ovopel and Ovaprim and the smallest after Ovopel injections. In this case, the fecundity was higher than that described by ŚLIWIŃSKI (1998) for asp and similar to the data presented by TARGOŃSKA et al. (2008). But it is significant that ŚLIWIŃSKI (1998) worked on young and small females (average weight over 1 kg) cultured in carp ponds.

Latency time in the present study for asp females was between 40 and 44 hours after the resolving injection (Table 1). Exactly the same data was noted for this species by TARGOŃSKA et al. (2010) after the application of Ovopel and Ovaprim in single doses. Generally, the latency time was correlated with the type of applied hormonal stimulation, water temperature regimes and fish species. In wild, reophilic cyprinids, such as dace, ide or asp, the latency time was noted from 30 to 50 hrs. For asp, which were usually reproduced at a water temperature of 12–13°C, the latency was usually over 40 hours (TARGOŃSKA et al. 2008). Asp embryos survival rate to the eyed-egg-stage was the highest after the application of Ovopel and Ovaprim: 81.3% (Table 1). Lower survival was noted in Ovarpim group (74.2%) and the lowest in the Ovopel group (46.5%). Similar data was recorded by ŹARSKI et al. (2009) for dace and ide. It also resulted in the highest recorded asp embryo survival (TARGOŃSKA et al. 2008).

The data in the present study showed that the application of two commercial products, Ovopel and Ovaprim, influenced the artificial spawning effectiveness of asp under controlled conditions. This hormonal combination affected the highest spawning effectiveness in comparison to Ovopel and Ovaprim applied alone.

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QUALITY OF PEANUTS FROM THE OLSZTYN MARKET

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Key words: peanuts, quality, acid value, peroxide value, anizidine value, dienes and trienes, fatty acid composition.

Abstract

The aim of the study was to evaluate the quality of peanuts available on the Olsztyn market. The quality of researched peanuts was evaluated by determining degree hydrolysis and oxidation of oils and composition of fatty acids. Out of the nine peanut samples under examination, the content of primary and secondary products of oxidation was very high in eight of them, which could indicate their unsuitability for consumption. A high content of secondary products of oxidation proves an advanced degree of peanut deterioration, which, considering the good use-by date for seven out of nine samples, leads to the suspicion that the purchased peanuts were old and damaged, as indicated by the high values of lipid oxidation indices. While purchasing peanuts for consumption, the customer is not able to learn their nutritional value, since it is provided laconically and very sparingly. The use-by date printed for peanuts turns out to be false since it is not accompanied by their quality.

We believe that consumers are entitled to know the required information about the products, e.g. the country of origin of the raw material, its freshness status, production method (whether the peanuts were roasted in siliques and later shelled and packed), the parameters of roasting and frying, the type of oil used, etc.

JAKOŚĆ ORZECHÓW ARACHIDOWYCH Z RYNKU OLSZTYŃSKIEGO

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Słowa kluczowe: orzechy arachidowe, jakość, liczba kwasowa, nadtlenkowa, anizydynowa, dieny i trieny, kwasy tłuszczowe.

Abstrakt

Celem badań była ocena jakości orzechów arachidowych dostępnych na rynku olsztyńskim. Jakość orzechów określono poprzez wyznaczenie stopnia hydrolizy i oksydacji oraz składu kwasów tłuszczowych. W 8 spośród 9 badanych próbek orzechów arachidowych zawartość pierwotnych i wtórnych produktów utleniania była bardzo wysoka, co może wskazywać na ich nieprzydatność do konsumpcji. Świadczy to o zaawansowanym stopniu zepsucia orzechów, co przy aktualnym terminie przydatności do spożycia 7 z 9 próbek, rodzi przypuszczenia o zakupie orzechów starych i uszkodzonych, na co wskazują wysokie wartości wskaźników utlenienia lipidów orzechów. Kupujący orzechy arachidowe do konsumpcji nie ma żadnej możliwości zapoznania się z ich wartością odżywczą, gdyż jest ona podana lakonicznie i nader oszczędnie. Zamieszczony termin przydatności do spożycia okazuje się kłamliwy, ponieważ nie idzie w parze z jakością orzechów.

Uważamy, iż konsument powinien wiedzieć, np. w jakim kraju kupiono surowiec i jaka była jego świeżość, czy prażenie wykonano na orzechach w łuszczynach, a potem je obłuskiwano i pakowano, jakie były parametry prażenia i smażenia, rodzaj zastosowanego oleju itp.

Introduction

Peanuts as a oil material, represent approximately 5.4% of world production of oilseeds (ROSIAK et al. 2009). The U.S., Argentina, Sudan, Senegal and Brazil are major producers of this material (ÖZCAN 2010). Peanuts are cultivated mainly for food purposes, eg, production of edible oil, peanut butter and various kinds of snacks (roasted, fried, with the addition of various ingredients and spices). They are also sold as fresh vegetables, canned, frozen, and baked in the shell (MESTRALLET et al. 2004). Furthermore, they are used in the cosmetic industry and for animal feed.

Peanuts are a product of high energy. Nuts have a high oil (19–25%) and protein (25–28%) content, but low carbohydrates (19–21%) and minerals (2.3–2.5%) content (AYOOLA et al. 2010, ÖZCAN 2010, GROSSO et al. 1995). High oil content with high shares of oleic acid and low linoleic acid makes that peanut oil has a high oxidative stability (BOLTON et al. 2002, MUGENDIA et al. 1998, KNAUFT et al. 1993, O'KEEFE et al. 1993).

Peanuts, as a raw material for production of snacks are often subjected to heat treatment, eg, roasting and frying, in order to impart desirable for consumer qualities of flavor. The use of high temperatures gives the desired taste and fragrance effects of the product, however, alter its nutritional value, through, inter alia, the distribution of heat-labile components and accelerate the oxidation process.

The increasing number of manufacturers of snacks and a growing interest in this type of products meant that the objective of assessing the quality of work was adopted nine samples of peanuts prevalent on the market in Olsztyn.

Material and Methods

The experimental material were nine samples of peanuts, therein, 3 samples of peanuts in siliquas (1 non-roasted sample and 2 roasted ones) and 6 samples of peanuts without siliquas (1 non-roasted sample, 2 roasted samples and 3 samples of fried peanuts) – Table 1.

Characterization of the experimental material

Table 1

No.	Presence of siliquas	Adopted heat treatment of peanuts			Shelf life
		no	roasting	frying	
1	+	+	–	–	no information
2	+	–	+	–	05.2011
3	+	–	+	–	10.2011
4	–	+	–	–	no information
5	–	–	+	–	05.2011
6	–	–	+	–	11.09.2011
7	–	–	–	+	02.2011
8	–	–	–	+	01.2011
9	–	–	–	+	20.05.2011

All peanut samples were bought in stores localized on the area of Olsztyn in term of June 2010.

Oils were extracted from the peanuts according to the method of Folch method (FOLCH et. al 1957). The quality of oils was evaluated by assaying: acid value acc. to *Oleje i tłuszcze...* PN-ISO 660:1998, peroxide value acc. to *Oleje i tłuszcze...* PN-ISO 3960:1996, anizidine value and Totox index acc. to *Tłuszcze roślinne...* PN-93/A-86926 and the share of dienes and trienes (Ultraviolet spectrophotometric... 1973). The fatty acids composition was estimated acc. *Analiza...* PN-EN-ISO-5508:1996, preparing methyl esters by the method described by ZADERNOWSKI and SOSULSKI (1978). The separation of methyl esters were carried out applying GC 8000 series FISON'S 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.

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 oils of peanut samples unvaried analysis of variance (ANOVA) with Tukey's test of $p \leq 0.05$ significance level was used. Moreover, there were determined Pearson correlation coefficients (r) between individual quality factors.

Results and Discussion

The lipid content in peanut samples was statistically diversified at the significance level of 0.05 and ranged from 43.80% (sample 6) to 48.67% (sample 4) – Table 2.

This study found significant differences in the fatty acid composition of lipids in the peanut samples (Table 3).

In all lipid samples, oleic acid dominated, accounting for 52.26 to 80.85% of the total fatty acids (Table 3). Lipids of non-roasted peanuts and peanuts roasted in siliques (samples 1–3) had both the most balanced and the lowest mean share of oleic acid (53.5%). The most diversified share of oleic acid was found in lipids of non-roasted peanuts (sample 4 – 53.96%) and of peanuts roasted without siliques (samples 5 and 6 – about 80.50% each). The total share of other monounsaturated acids (palmitoleic and eicosenoic) ranged from 2.32 to 5.66% of total fatty acids (Table 3).

The results of the analysis of the monounsaturated acid composition of lipids in the examined samples corresponded to the results obtained by RIVEROS *et al.* (2009), ISLEIB *et al.* (2006) and O'KEEFE *et al.* (1993).

Monounsaturated acids (oleic, eicosenoic and palmitoleic) are considered to be health-promoting, since by reducing the levels of triglycerides and the LDL cholesterol fraction they lower the risk of cardiovascular diseases (KRIS-ETHERTON *et al.* 1999).

As regards the share of oleic acid in lipids, four samples of high-oleic peanuts could be distinguished (samples 5–7 and 9, containing an approx. 80% share of this acid) and one sample of medium oleic peanuts (sample 8) (Table 3). Other peanut samples were characterised as having a standard (about 50%) share of oleic acid.

The second highest share was found for palmitic acid, accounting for 6.45 to 26.08% of total acids (Table 3). A much larger (about 25%) share of this acid was found in lipids of peanuts considered to be standard in terms of oleic acid share (samples 1–4). On the other hand, lipids of high-oleic peanuts contained less than 10% palmitic acid.

Table 2
Lipid content [%] of peanuts

Peanut samples	Peanuts in siliquas			Peanuts without siliquas					
	1	2	3	4	5	6	7	8	9
Lipids content [%]	47.53 ^a ±0.28	47.42 ^a ±0.21	48.65 ^c ±0.22	48.67 ^c ±0.16	47.44 ^b ±0.16	43.80 ^d ±0.16	48.10 ^e ±0.12	46.31 ^b ±0.30	46.64 ^b ±0.17

Table 3
The share [%] of fatty acid of lipids from peanut samples

No.	Peanuts in siliquas								
	Palmitic acid [C _{16:0}]	Palmito-oleic acid [C _{16:1}]	Stearic acid [C _{18:0}]	Oleic acid [C _{18:1}]	Linoleic acid [C _{18:2}]	Linolenic acid [C _{18:3}]	Arachidic acid [C _{20:0}]	Eicosanoic acid [C _{20:1}]	Behenic acid [C _{22:1}]
1	25.13 ^c ±0.12	2.93 ^{de} ±0.10	6.26 ^c ±0.06	53.04 ^d ±0.47	5.13 ^a ±0.01	trace	2.39 ^e ±0.13	1.61 ^d ±0.07	3.53 ^e ±0.02
2	24.08 ^b ±0.81	3.32 ^g ±0.08	6.08 ^c ±0.37	52.26 ^c ±0.34	7.02 ^f ±0.15	trace	2.51 ^f ±0.05	1.17 ^b ±0.04	3.58 ^e ±0.46
3	24.45 ^{bc} ±0.26	2.83 ^d ±0.16	6.02 ^c ±0.23	55.11 ^f ±0.03	5.29 ^{ab} ±0.01	trace	2.30 ^e ±0.16	1.26 ^{bc} ±0.11	2.76 ^f ±0.34
	Peanuts without siliquas								
4	26.08 ^f ±0.37	2.55 ^f ±0.15	5.32 ^d ±0.04	53.96 ^c ±0.23	6.40 ^b ±0.11	trace	1.93 ^d ±0.16	1.38 ^{bcd} ±0.07	2.41 ^{bcd} ±0.16
5	6.45 ^d ±0.21	0.56 ^{bc} ±0.02	2.13 ^g ±0.04	80.85 ^b ±0.09	4.63 ^g ±0.04	trace	1.27 ^{abc} ±0.04	2.19 ^a ±0.04	1.94 ^a ±0.01
6	7.44 ^e ±0.26	0.75 ^c ±0.06	2.39 ^e ±0.11	80.23 ^b ±0.24	3.13 ^d ±0.06	trace	1.13 ^a ±0.04	2.76 ^c ±0.05	2.19 ^{ab} ±0.08
7	10.13 ^e ±0.15	0.00 ^b ±0.00	2.33 ^e ±0.09	76.13 ^b ±0.08	5.37 ^{bc} ±0.04	trace	1.08 ^a ±0.03	2.32 ^a ±0.13	2.65 ^{ad} ±0.01
8	17.12 ^b ±0.19	3.26 ^f ±0.11	2.33 ^e ±0.26	65.20 ^b ±0.59	4.05 ^f ±0.14	trace	1.84 ^d ±0.08	2.40 ^a ±0.17	3.24 ^a ±0.01
9	7.32 ^e ±0.28	1.19 ^f ±0.11	3.05 ^b ±0.04	78.87 ^a ±0.63	3.73 ^c ±0.15	trace	1.44 ^e ±0.03	2.13 ^a ±0.04	2.28 ^{abc} ±0.06

The share of stearic acid, ranging from 2.13 to 6.26%, was in direct proportion to the share of palmitic acid (Table 3).

The total share of other saturated acids (arachidic and behenic) accounted for 3.21 to 6.09% of total fatty acids, whereas the share of behenic acid was higher than arachidic acid (Table 3).

Until recently, long-chain saturated fatty acids were perceived as hyperlipidemic and/or hypercholesterolemic, but the current research has contributed to changing this view. CATER *et al.* (2001), in examining the effect of the intake of oil enriched with stearic acid on the changes to the cholesterol level, found that it had a neutral cholesterolemic effect. BONANOME *et al.* (1988), in examining the metabolic effects of a high-stearic diet, found that stearic acid reduced the cholesterol content and increased the content of oleic acid in serum triglycerides. The opinions concerning the effects of palmitic acid on the cholesterol level are varied. CATER *et al.* (1997) found that this acid had a greater effect on the increase in the cholesterol level than medium-chain fatty acids (caproic and caprylic). DENKE *et al.* (1992) proved that palmitic acid improved the concentration of total cholesterol and LDL more than lauric acid. Behenic acid is regarded as an acid which increases the total cholesterol level (CATER *et al.* 2001).

Polyunsaturated fatty acids in lipids of peanut samples were represented only by linolenic acid. The share of this acid ranged from 3.13 to 7.02% of total fatty acids (Table 3).

The study revealed low, although significantly diversified, acid values of lipids in the examined peanuts (Table 4). This diversity was not related to the type of peanuts (in siliques, without siliques) or the processing method applied (roasting, frying). Curiously enough, the peanuts with the longest use-by date at the time of purchase (about 16 months) – Table 1, roasted in siliques (sample 3) and without siliques (sample 6), were characterized by higher acid values, 2.29 and 2.75 mg KOH/g, respectively, as compared to other samples (Table 4). On the other hand, a sample of fried peanuts stored for a longer time (sample 7) was characterized by a low level of this value (Table 4). AYOOLA *et al.* (2010), in examining the effect of peanut heat treatment on quality changes concerning peanut fat, found that fat extracted from peanuts subjected to roasting was characterized by a higher acid value as compared to fat extracted from raw peanuts. ÖZCAN (2010), in examining fat extracted from seven different varieties of raw peanuts, found lower acid values than those obtained in this study, ranging from 0.86–1.11 mg KOH/g of fat.

The peroxide values of peanut lipids ranged from 1.25 to 14.49 mEq O₂/kg of fat (Table 4). Generally, higher values of this characteristics, 14.49–12.18 mEq O₂/kg of fat, were found for lipids of samples 1 and 2 (peanuts in siliques, non-roasted and roasted) and samples 4 (non-roasted peanuts without siliques). Higher peroxide values of lipids of these peanuts could be related to

poor quality of the raw material and/or improper storage conditions. Low values of this characteristic, 1.25 and 2.48 mEq O₂/kg of fat, were found for samples 5 and 7, respectively (peanuts without siliques, roasted and fried). According to the *Tłuszcze jadalne...* ZN-94/SGO-01, the acceptable peroxide value is 10 mEq O₂/kg of fat. As regards peanut lipids, samples 1–2, 4 and 9 were characterized by a significantly higher level of this value. TAŃSKA et al. (2003), in examining, among others, the quality of roasted peanuts and peanuts in mixtures of dried fruit and nuts, found a similar content of peroxides in lipids of the samples under analysis. BRADDOCK et al. (1995), on the basis of research concerning roasted nuts, found that a peroxide value amounting to about 10 mEq O₂/kg was an indicator of a highly advanced fat oxidation process. According to those authors, peanuts with a peroxide value exceeding 20 mEq O₂/kg fat, are not suitable for consumption. AYoola et al. (2010), in examining the effect of temperature on quality changes of peanut, found that peanut roasting increased the peroxide value. According to MOZINGO et al. (2004), the rate of lipid oxidation depends on the composition of fatty acids. The above quoted study proved that the rate of oil oxidation occurred more slowly in case of highly oleic varieties, in which the share of this acid amounted to about 80%. The results of the current study also support this thesis. Lipids obtained from roasted peanuts (samples 5 and 6) and from fried peanuts (samples 7 and 9) without siliques, which had about 80% share of oleic acid, demonstrated the lowest peroxide values (Table 3, Table 4). Production of peanut oils and snacks from highly oleic nuts is conducive to slowing down the fat oxidation process and, as reported by BRADDOCK et al. (1995), the use-by date of those products can be twice as long.

Statistical analysis showed that the peroxide value was inversely proportional to the share of oleic acid in lipids of the examined samples and was directly proportional to the share of palmitic, stearic, arachidic and behenic acids (Table 5).

Table 5
Significant correlation between individual quality factors of peanut lipids (Pearson correlation coefficients – *r*)

Features	Peroxide value (mEq O ₂ /kg oil)	Anisidine value
Palmitic acid [C _{16:0}]	0.81	–
Stearic acid [C _{18:0}]	0.87	0.69
Oleic acid [C _{18:1}]	-0.66	–
Arachidic acid [C _{20:0}]	0.87	0.60
Eicosanoic acid [C _{20:1}]	–	-0.62
Behenic acid [C _{22:1}]	0.73	–

The anisidine value, indicating the content of secondary products of oxidation (Jerzewska 1991), was statistically differentiated and its value ranged widely – from 8.59 to 76.72. Out of all lipid samples, the highest anisidine value was found for lipids of sample 4, which also had a high peroxide value and the highest content of conjugated dienes. The lowest anisidine value (8.59) was found for lipids of sample 5, which also had the lowest peroxide value and the lowest content of conjugated dienes and trienes (Table 4). Lipids of non-roasted peanuts and peanuts roasted in siliques were characterized by similar anisidine values (averaging approx. 33.8). The anisidine values of fried peanuts ranged from 18.94 to 36.38 (Table 4).

Statistical analysis also showed that the anisidine value was inversely proportional to the share of eicosenoic acid in lipids of all samples under examination, and this value increased in proportion to the increase in the share of stearic and arachidic acid (Table 5).

The value of total oxidation index, Totox, was within a broad range between 11.09 and 101.08 (Table 4). The maximum acceptable value of the Totox index calculated pursuant to *Oleje i tłuszcze...* PN-A-86908:2000 is 18. Out of all peanut samples, this requirement was satisfied only for sample 5, the lipids of which were characterized by a Totox value of 11.09. Lipids of other peanut samples were characterized by values significantly higher than the acceptable Totox index, therefore they should be regarded as unsuitable for consumption. ALLEN and HAMILTON (after JERZEWSKA 1991) report that the value of the Totox index for edible oil of good quality should not exceed 10.

The content of dienes in lipids of the examined peanuts ranged from 0.10 to 0.31% and was not statistically differentiated (Table 4). Generally, higher contents of those compounds were found in lipids of roasted and fried peanuts. This could result from the application of high temperatures in their manufacturing process, which, as MIYAGAWA et al. (1991) and JERZEWSKA et al. (1998) claim, stimulates transformation of polyunsaturated fatty acids to conjugated compounds. TAŃSKA et al. (2003), in examining roasted peanuts and peanuts separated from a mixture of dried fruit and nuts, found a higher content of those compounds (approx. 0.6%).

The content of conjugated trienes in lipids of the examined peanuts was low, as it did not exceed 0.005% (Table 4). TAŃSKA et al. (2003) found about a three times larger share of these compounds in lipids of roasted peanuts. According to YURAWECZ (after JERZEWSKA et al. 1998), conjugated trienes of linolenic acid are considered to be controversial compounds.

The results of this research indicate that the majority of the peanut samples under analysis can pose a health risk due to a high level of primary and secondary products of oxidation. Assuming a potential daily intake

of about 100 g of peanuts (a typical unit packaging), and about 50% fat content of peanuts, the amount of peroxides supplied to the body ranges from 0.06 to 0.69 mEq O₂/100 g of nuts and the amount of secondary products of oxidation ranges from 408 to 3734 [100*A/100 g of nuts] (Table 6).

Table 6

Discriminate of lipids technological value contained in 100 g of peanuts

	1	2	3	4	5	6	7	8	9
Content of peroxides [mEq O ₂ /100 g nuts]	0.69	0.65	0.45	0.59	0.06	0.22	0.12	0.22	0.27
Content of aldehydes [100*A/100 g nuts]	1684	1444	1721	3734	408	1211	1750	1439	883

Conclusions

1. Out of the nine peanut samples under examination, the content of primary and secondary products of oxidation was very high in eight of them, which could indicate their unsuitability for consumption.

2. A high content of secondary products of oxidation proves an advanced degree of peanut deterioration, which, considering the good use-by date for seven out of nine samples, leads to the suspicion that the purchased peanuts were old and damaged, as indicated by the high values of lipid oxidation indices.

3. While purchasing peanuts for consumption, the customer is not able to learn their nutritional value, since it is provided laconically and very sparingly. The use-by date printed for peanuts turns out to be false since it is not accompanied by their quality.

4. We believe that consumers are entitled to know the required information about the products, e.g. the country of origin of the raw material, its freshness status, production method (whether the peanuts were roasted in siliques and later shelled and packed), the parameters of roasting and frying, the type of oil used, etc.

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ELLAGIC ACID CONTENT IN FRUITS OF SELECTED STRAWBERRY CULTIVARS

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Key words: ellagic acid, phenolic acids, strawberries, cultivars.

Abstract

Ellagic acid is a biologically active compound, regarded as preventive against various diseases and demonstrates anticarcinogenic and antioxidant effects. In Poland, one of the five largest strawberry producers, the supply of these fruits is significant; therefore exploration of this subject and dissemination of the knowledge concerning their nutritional values and health-related properties is important. The aim of the study was to determine the content of ellagic acid in the most popular, selected strawberry cultivars in Poland.

Fruits of the strawberry cultivars under examination differed significantly in ellagic acid content. The content of the analysed component ranged from 452.5 mg kg⁻¹ fresh weight (4,575.6 mg kg⁻¹ dry matter) in fruits of the 'Heros' cultivar to 1,193.8 mg kg⁻¹ fresh weight (14,215.0 mg kg⁻¹ d.m.) in fruits of the 'Camarosa' cultivar. The highly diversified content of ellagic acid in fruits of individual cultivars, grown in similar soil and weather conditions and subjected to the same agricultural treatments, indicates that the content of the analysed phenolic acid depends not only on the fruit species, but also on the cultivar.

ZAWARTOŚĆ KWASU ELAGOWEGO W OWOCACH WYBRANYCH ODMIAN TRUSKAWEK

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Słowa kluczowe: kwas elagowy, kwasy fenolowe, truskawki, odmiany.

Abstrakt

Kwas elagowy jest związkiem biologicznie aktywnym, uznanym za prewencyjny dla wielu chorób, wykazującym m.in. działanie antykancerogenne i antyoksydacyjne. W Polsce, należącej do pięciu największych producentów truskawek na świecie, podaż tych owoców jest znaczna, istotne jest więc zgłębianie i propagowanie wiedzy dotyczącej ich wartości odżywczych czy prozdrowotnych. Celem pracy było określenie zawartości kwasu elagowego w wybranych najpospolitszych w kraju odmianach truskawek.

Owoce badanych odmian truskawek różniły się istotnie pod względem zawartości kwasu elagowego. Zawartość badanego składnika wynosiła od 452,5 mg kg⁻¹ św.m. (4575,6 mg kg⁻¹ s.m.), w owocach odmiany 'Heros' do 1193,8 mg kg⁻¹ św.m. (14215,0 mg kg⁻¹ s.m.), w owocach odmiany 'Camarosa'. Wysoce zróżnicowane zawartości kwasu elagowego w owocach poszczególnych odmian, uprawianych w zbliżonych warunkach glebowo-klimatycznych i poddanych tym samym zabiegom agrotechnicznym, pozwalają domniemywać, iż zawartość badanego kwasu fenolowego jest zależna nie tylko od gatunku owoców, ale również od odmiany.

Introduction

Ellagic acid is one of the increasingly often analysed and demanded biologically active compounds, such as phenoloacids (BUDRYN and NEBESNY 2006). Their most frequently listed, numerous nutritional benefits particularly include their participation in free radical scavenging, chelation of metal ions, changing enzyme activity and availability of proteins, counteracting coronary heart disease, cancer formation, inflammatory conditions and diabetes. Additionally, they display proven synergistic interaction with other biologically active compounds (BUDRYN and NEBESNY 2006 after SVILAAS et al. 2004).

A particular interest in the content of ellagic acid results from the fact that this component is regarded as preventive against many diseases (DA SILVA PINTO et al. 2008, IPPOUSHI et al. 2009). The anticarcinogenic effect of ellagic acid in strawberries is displayed, among others, by a change in the activity of enzymes participating in metabolic activation of a carcinogen (FJAERAA and NÅNBERG 2009, SZUMIŁO 2009). Ellagic acid blocks hepatic cytochrome P-450, which initiates bioactivation of carcinogens (OSZMIAŃSKI and LAMER-ZARAWSKA 1996). It was also found to inhibit cancers caused by various types of carcinogenic compounds, such as polycyclic aromatic hydrocarbons, N-nitrosamines, aflatoxin B₁ and aromatic amines (IPPOUSHI et al. 2009 after HANNUM 2004, HEUR et al. 1992, MANDAL et al. 1987).

Ellagic acid in strawberry fruit demonstrates potential health-related benefits for chronic pancreatitis (SCALBERT et al. 2005, SUZUKI et al. 2009). This component also reduces the development of neutropenia in patients suffering from prostate cancer (FALSAPERLA et al. 2005). It was established that consumption of the pomegranate fruit extract, rich in ellagic acid, had a protective and mitigating effect on skin pigmentation after exposure to UV radiation (KASAI et al. 2006).

Poland is one of the five largest strawberry producers in the world. In terms of production volume, this fruit is second in Poland, just after apples. Depending on the year, between 5 and 8 kg of strawberries are produced per inhabitant (ZURAWICZ 1994). In view of the facts mentioned above, we are an important supplier of this fruit; therefore it is important to explore and disseminate the knowledge concerning its nutritive values and health-related properties.

Ellagic acid is a gallic acid dimer, found in many indirect forms differing in solubility or reactivity both in the world of plants and in the world of animals (DA SILVA PINTO et al. 2008 after MAAS and GALETTA 1991). In strawberry fruits, it can be found both in a free and in a bound form. However, most ellagic acid found in berries takes the form of ellagitannins esterified with glucose (BUDRYN and NEBESNY 2006 after AZUMA et al. 2005). In order to release them, an acid hydrolysis is carried out, which provides a basis for determining the total content of ellagic acid in fruits (DANIEL et al. 1989).

The literature provides various data concerning the content of ellagic acid in strawberry fruits, depending on the analytic method applied (HÄKKINEN et al. 2000, WILSON and HAGERMAN 1990, CARPITA 1983, DA SILVA PINTO 2008). Additionally, research conducted by various groups has confirmed the diversity in ellagic acid content in fruits of different strawberry cultivars (AMAKURA et al. 2000, DA SILVA PINTO et al. 2008, HÄKKINEN et al. 2000, MAAS and GALETTA 1991, SKUPIEŃ and OSZMIANŃSKI 2004). However, these cultivars are not common in the Polish market. Thus, the aim of the study was to compare the content of ellagic acid in fruits of the most common strawberry cultivars in Poland.

Materials and Methods

Research material included fruits of eleven strawberry cultivars ('Camarosa', 'Dukat', 'Elsanta', 'Heros', 'Honeoye', 'Kama', 'Kent', 'Onebor', 'Polka', 'Senga Sengana', 'Thuriga'), gathered in the season of 2008. The fruits originated from experimental plots established on a commodity plantation in Jaroty near Olsztyn. Strawberries were planted on a plateau with a south-facing slope, on quality class III loamy soil.

Fruits of each of the cultivars were picked at their collective ripeness stage, at 3–4 day intervals, from fruits selected at random for each cultivar. Fruits, after gathering from the plantation, were selected and de-stemmed, frozen and stored at -20°C ($\pm 2^\circ\text{C}$) before analysis for four weeks.

Fruit dry weight was determined by gravimetric method (*Przetwory owocowe...* PN-90/A-75101/03). Extraction and hydrolysis was conducted

according to the method described by HÄKKINEN et al. (2000). A sample of carefully fragmented fruits (5.0 g) was placed in a 100 ml flat-bottomed flask. Next, 25 ml of methanol, 15 ml of distilled water and 10 ml of 6M hydrochloric acid were added. The mixture was placed in a laboratory heating mantle under a reflux condenser. The process of extraction was carried out at 85°C for 20 hours.

The obtained extract was then filtered through a Schott G4 funnel. 10 ml was taken from the total amount of the filtrate and concentrated to a dry substance using a Büchi R-210 type vacuum evaporator, not exceeding 35°C. The remaining part was solved in 2 ml of methanol and centrifuged for 10 min at 10,000 ref./min. in an Eppendorf test tube. The obtained sample was analysed by high performance liquid chromatography (HPLC). The chromatographic set applied in the analysis, Agilent Technologies Series 1200, was equipped with photodiode detector of the same series. The analysis was carried out according to the method described by AMAROWICZ and WEIDNER (2001).

Separation was performed on the LiChrospher 100, RP-18 column, 250 x 4.6 mm (5 µm). As a mobile phase, the following mixture was used: water, acetonitrile and acetic acid, in a 88:10:02 ratio. A quantitative analysis was carried out on the basis of a standard curve using the ellagic acid reference standard (Sigma).

Values reported in this study are the mean values from three replications. The significance of differences between mean results, at the significance level of $\alpha=0.05$, was calculated with the use of computer software Statistica PL9, applying a Tukey test.

Results and Discussion

Strawberry cultivars differed significantly in their ellagic acid contents (Table 1), which ranged from 452.5 mg kg⁻¹ fresh weight (4,575.6 mg kg⁻¹ dry matter) in fruit of the 'Heros' cultivar, to 1,193.8 mg kg⁻¹ f.w. (14,215.0 mg kg⁻¹ d.m.) in fruits of the 'Camarosa' cultivar.

The content of ellagic acid (Figure 1) in the fresh weight of the pulp did not statistically differ from the traditionally cultivated 'Senga Sengana' cultivar. The exception were the 'Camarosa' and 'Elsanta' cultivars, in which the content of the analysed acid was almost twice that of its mean content in the examined cultivars of strawberries. A significantly lower value in comparison to most cultivars was obtained for fruits of the 'Heros' cultivar.

An analysis of the content of ellagic acid in the dry matter of fruits showed that 'Camarosa' and 'Elsanta' cultivars had significantly higher contents in relation to other examined cultivars. In those two cultivars, the content of ellagic acid considerably exceeded 10,000 mg kg⁻¹ d.m. This relation was also

Table 1

Ellagic acid content in strawberry fruits

Cultivar	Ellagic acid	
	[mg kg ⁻¹ fresh weight]	[mg kg ⁻¹ dry matter]
Camarosa	1193.8 ± 22.1 ^c	14215.0 ± 156.4 ^g
Dukat	516.8 ± 7.2 ^{ab}	6374.7 ± 31.8 ^{bc}
Elsanta	1034.1 ± 25.7 ^d	10964.8 ± 149.9 ^f
Heros	452.5 ± 20.6 ^a	4575.6 ± 158.1 ^a
Honeoye	635.6 ± 2.9 ^c	7802.7 ± 7.3 ^d
Kama	540.8 ± 12.6 ^b	6794.6 ± 100.7 ^c
Kent	528.1 ± 9.4 ^b	6449.9 ± 71.6 ^{bc}
Onebor	534.0 ± 13.9 ^b	7552.2 ± 110.9 ^d
Polka	561.7 ± 2.4 ^{bc}	6038.7 ± 24.4 ^b
Senga Sengana	580.0 ± 5.3 ^{bc}	8767.1 ± 122.0 ^e
Thuriga	553.7 ± 16.0 ^b	6113.1 ± 148.3 ^b

Values in the same column marked with the same letters do not differ significantly ($p < 0.05$).

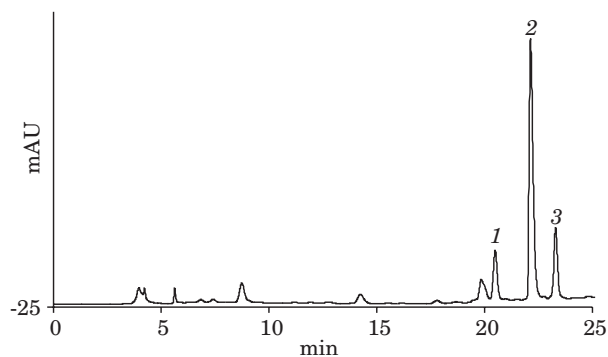


Fig. 1. Chromatogram of polyphenolic compounds in strawberry fruits: 1, 3 – ellagic acid derivatives, 2 – ellagic acid

observed in reference to the dry matter of fruits. Among the examined cultivars, the mean content of ellagic acid in the dry matter of fruits was established in fruits of 'Senga Sengana', 'Honeoye' and 'Onebor' cultivars. The content of the examined component in the above mentioned cultivars ranged from 7,552.2 to 8,767.1 mg kg⁻¹ d.m. The values obtained for fruits of other cultivars did not exceed 7,000 mg kg⁻¹ d.m.

Due to the multitude of forms in which ellagic acid is found and factors affecting its extraction, it is very difficult to compare the results obtained in data provided in literature. A similar content of the examined acid is reported by MAAS and GALETTA (1991) and SKUPIEŃ and OSZMIAŃSKI

(2004). A lower differentiation of the total content of ellagic acid in selected six cultivars of strawberries cultivated in Finland was found by HAKKINEN et al. (2000). The total content of ellagic acid ranged there between 400 and 520 mg kg⁻¹ fresh weight.

Conclusions

1. Fruits of the strawberry cultivars under examination significantly differed in the content of ellagic acid, both in fresh weight and in dry matter. The highest content of the examined acid, determined for the 'Camarosa' cultivar, was almost three times higher than the lowest one, reported for the 'Heros' cultivar.

2. The highly diversified content of ellagic acid in fruits of individual cultivars, grown in similar soil and weather conditions and subjected to the same agricultural treatments, indicates that the content of the analysed phenolic acid depends not only on the fruit species, but also on the cultivar.

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FATTY ACIDS COMPOSITION IN STORAGE FAT OF EXPORT SLAUGHTER HORSES

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Key words: fatty acids composition, storage fat, slaughter horses.

Abstract

The aim of the study was to evaluate the fatty acids composition of storage fat of slaughter horses. Samples of cervical and peri-intestinal fat taken from horses of different genders and weights in a slaughterhouse were used as the study material. The fat was esterified by the method described by Peisker. Separation and analysis of the fatty acids was conducted by gas chromatography with a flame ionisation detector. The samples were found to contain fatty acids with chains of 14 to 20 carbon atoms, mainly palmitic, myristic, oleinic, palmitoleic and linolenic acids. Small differences were found between groups of horses classed by body weight. The experiment confirmed that the composition of horse fat in terms of mono- and polyunsaturated acid content is interesting from a nutritional point of view. Cervical fat contained average 49% of monounsaturated fatty acids and polyunsaturated fatty acids in horses in mass to 550 kg and mass above 550 kg 12.57% and 10.79%, respectively.

SKŁAD KWAŚÓW TŁUSZCZOWYCH TŁUSZCZU ZAPASOWEGO EKSPORTOWYCH KONI RZEŻNYCH

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Słowa kluczowe: skład kwasów tłuszczowych, tłuszcz zapasowy, konie rzeźne.

Abstrakt

Celem badań była ocena składu kwasów tłuszczowych tłuszczu zapasowego koni rzeźnych. Badaniem objęto tłuszcz karkowy i okołojelitowy pobierany od koni różniących się płcią i wagą w ubojni eksportowej. Wydzielony tłuszcz poddawano estryfikacji metodą opisaną przez Peiskera.

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Rozdział i analizę składu kwasów tłuszczowych przeprowadzono metodą chromatografii gazowej z detektorem płomieniowo-jonizacyjnym. Wykazano występowanie kwasów tłuszczowych o długości łańcucha od 14 do 20 atomów węgla, głównie palmitynowego, mirystynowego, oleinowego, palmitooleinowego i linolenowego. Stwierdzono niewielkie różnice w przypadku koni podzielonych według płci i masy ciała. W badaniach potwierdzono, że tłuszcz koński charakteryzuje się interesującym pod względem wartości żywieniowej składem jedno- i wielonienasyconych kwasów tłuszczowych. W tłuszczu karkowym stwierdzono średnio około 49% jednonienasyconych kwasów tłuszczowych niezależnie od masy ciała koni, natomiast kwasów wielonienasyconych w tłuszczu koni o masie do 550 kg – 12,57%, a u zwierząt o masie powyżej 550 kg – 10,79%,.

Introduction

Poland's accession into the European Union sparked huge interest in Polish products of animal origin, including beef and horsemeat. Although horsemeat and horse fat are not popular in Poland interest in the meat has been growing in recent years, which may be attributed to fears of prions (BSE) associated with beef (SMOCZYŃSKA et al. 2002) and relatively high levels of cholesterol in other types of meat (BAROWICZ 2000, PALEARI et al. 2003). Among European countries, the demand for horse meat is highest in Italy, Belgium, France and Germany (GILL 2005, ZIN, WOJCIECHOWSKA 1998). Horse breeding for meat is also economically justified due to the relatively high horsemeat prices. Slaughter horses and foals purchased in Poland are intended mainly for export (SMOCZYŃSKA et al. 2002). The amount and composition of the fat accumulated in the animal's body, especially storage fat, depends on the species, gender, manner of nutrition, style of life and age (JANKOWSKA et al. 1996, NOWACKA, JANICKI 2002, MARKIEWICZ et al. 1982, TOMCZYŃSKI et al. 1982, 1991, ZIN et al. 1999). On the other hand, the nutritional and biological value of the fat, including the content of unsaturated acids, is determined by the fatty acid profile with special attention paid to omega-3 and omega-6 acids (BAROWICZ and BREJTA 2000).

Considering the fact that the sensory and nutritional value of meat is determined by fat, this study aimed at determination of the fatty acids composition in storage fat of horses from an export slaughter house.

Material and Methods

Samples of horse storage fat (cervical and peri-renal) taken at a slaughterhouse from export horses purchased all over Poland were used as the study material. Samples of fat were taken from randomly chosen animals in two-week intervals. Altogether, 6 samples of cervical and peri-renal fat were taken from animals of different genders. The fatty acid profile was determined after

classing the samples by gender and weight (animals below and above 550 kg). Before the analysis, samples were stored at -18°C .

The fat from finely-cut tissue was melted at 95°C and dried with anhydrous sodium sulphate, and methyl esters of fatty acids were obtained by esterification in an acidic environment according to Peisker (ŻEGLARSKA et al. 1991). Separation and analysis of the fatty acids was performed by gas chromatography under the following conditions: gas chromatograph PYE UNICAM with a flame-ionisation detector (separation conditions: glass column, length 210 cm, internal diameter 4 mm). Stationary phase – 10% diethylene glycol succinate on a Chromosorb W 60/80 mesh. Separation in an isothermal cycle at 195°C . Carrier gas – argon, flow rate $60\text{ cm}^3/\text{min}$. Evaporator temperature – 300°C .

Peaks were identified by comparison with retention times of reference standards of methyl esters of fatty acids of known composition. Quantitative calculations were performed with PU 4815 software. Statistical analysis was performed with Statistica 8.0 software.

Results and Discussion

The percentage content of fatty acids in cervical and peri-organ fat of horses broken down by gender is presented in Table 1 and Table 2. The samples were shown to contain fatty acids with carbon chains of 14 to 20 carbon atoms. Among the saturated acids, myristic (C:14), palmitic (C16:0) and stearic (C18:0) acids were determined, as well as certain amounts of acids with an odd number of carbon atoms – C15:0 and C17:0. In studies conducted by JANKOWSKA et al. (1996) and ROBB et al. (1972), horse fat was found to contain lauric acid (C12:0); MARKIEWICZ et al. (1982) found capric acid (C10:0). The samples were also found to contain some mono-unsaturated acids: palmitoleic (C16:1), oleic acid (C18:1) and certain amounts of the C20:1 acid. The group of polyenic acids was found to be represented by – C18:2; C18:3 and C20:2 acids. The tissues used in the study contained the same fatty acids, but they differed in terms of the quantitative composition. In particular, they contained more palmitic (by about 3%), stearic and myristic acids in peri-renal fat and lower levels of palmitoleic, oleic, linoleic and linolenic acids as compared to cervical fat. The differences resulted in a higher level of unsaturated fatty acids in cervical fat as compared to peri-intestinal fat. The findings of a study conducted by MARKIEWICZ et al. (1982) and JANKOWSKA et al. (1996) were similar to those obtained in this study. However, the linoleic acid content in this study was nearly twice lower than in the studies conducted by those authors. On the other hand, the fat of the horses used in the experiment contained much less

linoleic acid and slightly more myristic and palmitic acids than that used in the study conducted by Robb et al. (1972). Comparison of the composition of storage fat of mares with such fat from geldings reveals slightly lower levels of saturated fatty acids and higher levels of unsaturated fatty acids in the fat from geldings. The differences proved very small and were found mainly in cervical fat. The total saturated, monounsaturated and polyunsaturated fatty acids in storage fat of horses (classed according to weight) are shown in Table 3. The results for the two horse groups are slightly different. Cervical fat contained more saturated fatty acids in samples taken from animals with weights exceeding 550 kg, while the same fat of horses with weights in excess of 550 kg contained slightly more mono- and polyunsaturated fatty acids. Opposite results were obtained for peri-renal fat.

Table 1
Fatty acids composition in storage (cervical) fat of horses with regard to horses' sex (mare and gelding) [%]

Fatty acids	Mare				Gelding			
	X	SD	V	R	X	SD	V	R
C _{14:0}	5.67	0.19	3.35	5.48–5.98	5.29	0.22	4.16	5.02–5.61
C _{14:1}	0.74	0.12	16.22	0.54–0.90	0.82	0.10	12.20	0.65–0.93
C _{15:0}	0.58	0.06	10.34	0.48–0.64	0.58	0.06	10.34	0.47–0.64
C _{16:0}	29.13	4.12	14.14	21.28–32.31	28.42	4.23	14.88	21.14–33.6
C _{16:1}	9.53	0.25	2.62	9.15–9.80	10.92	1.28	11.72	9.85–13.30
C _{17:0}	0.56	0.05	8.93	0.50–0.62	0.37	0.07	18.92	0.29–0.45
C _{17:1}	0.74	0.09	12.16	0.62–0.87	0.61	0.07	11.48	0.50–0.70
C _{18:0}	3.90	0.08	2.05	3.80–4.02	3.40	0.08	2.35	3.29–3.50
C _{18:1}	36.74	0.53	1.44	36.00–37.24	37.32	1.45	3.89	36.0–39.9
C _{18:2}	6.15	0.09	1.46	6.0–6.24	6.08	0.11	1.81	5.90–6.20
C _{18:3}	5.26	0.13	2.47	5.02–5.36	5.09	0.42	8.25	4.25–5.36
C _{20:1}	0.99	0.08	8.08	0.90–1.11	0.94	0.08	8.42	0.84–1.04
C _{20:2}	0.06	0.01	16.67	0.04–0.08	0.00	0.00	0.00	0.00–0.00
Total SFA	39.84	12.03	30.20	0.56–29.13	38.06	11.81	31.03	0.37–28.42
Total MUFA	48.74	15.55	31.90	0.74–36.74	50.61	15.82	31.26	0.61–37.32
Total PUFA	11.47	3.29	28.68	0.06–6.15	11.17	3.26	28.42	0.00–6.08

Explanation: SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; X – average; SD – standard deviation; V – variation coefficient; R – range.

Table 2
Fatty acids composition in storage (peri-organ) fat of horses with regard to horses' sex (mare and gelding) [%]

Fatty acids	Mare				Gelding			
	X	SD	V	R	X	SD	V	R
C _{14:0}	6.34	0.29	4.57	6.00–6.82	6.59	0.31	4.70	6.32–7.16
C _{14:1}	0.58	0.11	18.97	0.48–0.78	0.66	0.06	9.09	0.59–0.69
C _{15:0}	0.62	0.10	16.13	0.50–0.78	0.52	0.06	11.54	0.42–0.60
C _{16:0}	32.88	3.93	11.95	26.14–37.81	32.50	2.78	8.55	27.15–35.12
C _{16:1}	6.79	0.50	7.36	6.25–7.39	7.11	0.17	2.39	6.87–7.31
C _{17:0}	0.62	0.07	11.29	0.52–0.70	0.58	0.14	24.14	0.40–0.74
C _{17:1}	0.62	0.16	25.81	0.30–0.72	0.64	0.07	10.94	0.53–0.71
C _{18:0}	5.53	0.09	1.63	5.42–5.62	5.50	0.27	4.91	5.30–6.0
C _{18:1}	35.32	0.38	1.08	35.12–36.05	35.32	0.39	1.10	34.80–35.84
C _{18:2}	5.05	0.09	1.78	4.90–5.12	5.86	0.05	0.85	5.80–5.94
C _{18:3}	4.91	0.14	2.85	4.80–5.12	4.18	0.12	2.87	4.04–5.12
C _{20:1}	1.06	0.04	3.77	1.02–1.08	1.06	0.04	3.77	1.02–1.12
C _{20:2}	0.02	0.01	50.0	0.01–0.03	0.06	0.01	16.67	0.04–0.08
Total SFA	45.99	13.51	29.38	0.62–32.88	45.69	13.35	29.22	0.52–32.50
Total MUFA	44.37	15.01	33.83	0.62–35.32	44.79	14.99	33.47	0.66–35.32
Total PUFA	9.98	2.86	28.66	0.02–5.05	10.01	2.98	29.77	0.06–5.86

Explanations as in Table 1.

Table 3
Fatty acids composition in storage fat of horses with regard to horses' mass (average content) [%]

Fatty acids	Cervical fat		Peri-organ fat	
	mass to 550 kg	mass above 550 kg	mass to 550 kg	mass above 550 kg
Total SFA	38.01 ^a	39.90 ^a	47.07 ^b	45.13 ^b
Total MUFA	49.42 ^b	49.30 ^b	43.04 ^a	44.30 ^a
Total PUFA	12.57 ^b	10.79 ^b	9.87 ^a	10.05 ^a

Explanation: SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Conclusions

The experiment confirmed that the fatty acid composition of horse fat is interesting from a nutritional point of view. Cervical fat was rich in unsaturated fatty acids. It contained average 49% of monounsaturated fatty acids and polyunsaturated fatty acids in horses in mass to 550 kg and mass above 550 kg 12.57% and 10.79%, respectively.

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**THE EFFECT OF OZONE AND VITAMINS C AND E
ON THE ACTIVITY OF 17- β -HYDROXYSTEROID
DEHYDROGENASE AND ALKALINE PHOSPHATASE,
AND TESTOSTERONE CONCENTRATIONS
IN MALE RATS**

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Key words: ozone, vitamin E, vitamin C, rats, testes.

Abstract

The objective of this study was to determine the effect of oxidative stress caused by exposure to ozone on the activity of 17- β -hydroxysteroid dehydrogenase and alkaline phosphatase, and testosterone concentrations in male rats, and to investigate the possible protective effect of easily available antioxidants such as vitamins E and C. The experiment was conducted on adult Wistar-Hannover rats. One group of animals was exposed to ozone without vitamin cover, and the remaining animals were administered vitamins E and C in various combinations and doses. Ozone exposure in the group of rats not receiving vitamin injections caused oxidative stress manifested by elevated MDA concentrations in the blood plasma and testicular tissue. An increase in MDA levels was also observed in the group of animals administered vitamins, excluding the animals receiving low- and average-dose combinations of vitamins E and C. A drop in the activity of 17- β -hydroxysteroid dehydrogenase was reported in animals exposed to ozone, but this effect was not noted in the groups exposed to ozone and receiving vitamins. The lowest blood testosterone levels were observed in rats exposed to ozone and in the groups receiving low- and average-dose combinations of vitamins E and C.

**WPLYW OZONU ORAZ WITAMINY E I C NA AKTYWNOŚĆ DEHYDROGENAZY
17- β -HYDROKSYSTEROIDOWEJ I ALKALICZNEJ FOSFATAZY ORAZ KONCENTRACJĘ
TESTOSTERONU U SAMCÓW SZCZURÓW**

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Słowa kluczowe: ozon, witamina E, witamina C, szczury, jądra.

A b s t r a k t

Celem pracy było określenie wpływu stresu oksydacyjnego powodowanego ekspozycją na ozon na aktywność dehydrogenazy 17- β -hydroksysteroidowej i alkalicznej fosfatazy oraz koncentrację testosteronu u samców szczurów, jak również ewentualne ochronne oddziaływanie łatwo dostępnych antyoksydantów, takich jak witamina E i C. Doświadczenie przeprowadzono na dorosłych szczurach samcach szczepu Wistar/Hannover. Część zwierząt była ozonowana bez osłony witaminowej, pozostałe otrzymywały witaminy E i C w różnych kombinacjach i dawkach. Ekspozycja szczurów na ozon, bez iniekcji witamin, wywołała stan stresu oksydacyjnego, objawiający się wzrostem koncentracji MDA zarówno w osoczu, jak i w tkance jąder. Wzrost poziomu MDA nastąpił również w grupach zwierząt otrzymujących witaminy, z wyjątkiem szczurów dostających niskie i średnie dawki witaminy E i C łącznie. U zwierząt ozonowanych zaobserwowano spadek aktywności dehydrogenazy 17- β -hydroksysteroidowej, czego nie zanotowano w grupach ozonowanych z witaminami. Koncentracja testosteronu we krwi była najniższa u szczurów eksponowanych na ozon i w grupach otrzymujących średnie i wysokie dawki witaminy E i C łącznie.

Introduction

Ozone is one of the key air pollutants. Its toxicity can be attributed mainly to interactions with polyunsaturated fatty acids, which stimulates the formation of free radicals and other compounds such as hydrogen peroxide, protein oxidation products, lipid hydroperoxides and the highly toxic malondialdehyde (PRYOR et al. 1991). Ozone is a highly reactive gas that penetrates the tissue-air boundary, but ozone-induced changes have also been determined in other organs (PRYOR 1992). The above effects are attributed not only to ozone's direct oxidative activity (which is principally restricted to lungs), but also its ability to activate free radical-initiated cascade reactions and produce toxic peroxidation compounds (ESKEW et al. 1986, TOMSITS et al. 1994).

Living organisms deploy various mechanisms of protection against oxidative stress, including with the involvement of vitamins E and C. Vitamin E's protective effect on the lipid environment is manifested by two processes. The first relies on direct interactions with ozone which deprive this gas of its oxidative effects, and second involves the free radical scavenging mechanism to inhibit the autooxidation of polyunsaturated fatty acids (LIEBLER et al. 1993, ZIELIŃSKI 1997). Vitamin C is a water-soluble antioxidant. It interacts directly with peroxides, free radicals and atomic oxygen in an aquatic environment to protect low density lipoproteins (LDLs) and serum lipids against oxidative damage (PACKER et al. 1979).

There is a scarcity of published data on ozone's effects on the male reproductive system, including testes. The results of previous research have demonstrated a drop in testosterone levels and the activity of 17- β -hydroxysteroid dehydrogenase in rats subjected to long-term ozone exposure (JEDLIŃSKA-KRAKOWSKA 1998). Pathological changes in the spermatogenic

epithelium were also observed (JEDLIŃSKA-KRAKOWSKA et al. 2006). In view of the mechanism of ozone-induced toxicity, the objective of this study was to investigate the effectiveness of popular antioxidants, such as vitamins E and C, in preventing the harmful consequences of oxidative stress.

Materials and Methods

The experiment was conducted on 96 Wistar-Hannover rats aged five months (at the beginning of the study), with average body weight of 370 ± 10 g. The animals were divided into 12 groups ($n = 8$ in each group):

- I (Con) – control;
- II (Con PBS) – control animals receiving PBS injections i.m.

All animals from groups 3–12 were exposed to 0.5 ppm ozone for 50 days. Daily exposure time was 5 h (8.30 a.m. to 1.30 p.m.). The air in the exposure chamber was exchanged every two hours to prevent excessive CO₂ accumulation. Ozone concentrations were restored to the original level after every exchange. The animals from groups 3–12 received vitamin E and C injections i.m. at the following doses:

III – (1.5E) – 1.5 mg vit. E; IV – (4.5E) – 4.5 mg vit. E; V – (15E) – 15 mg vit. E; VI – (3C) – 3 mg vit. C; VII – (9C) – 9 mg vit. C; VIII – (50C) – 50 mg vit. C; IX – (1.5E 3C) – 1.5 mg vit. E and 3 mg vit. C; X – (4.5E 9C) – 4.5 mg vit. E and 9 mg vit. C; XI – (15E 50C) – 15 mg vit. E and 50 mg vit. C; XII – (Oz) – ozone exposure only.

Ozone was supplied from air compressed in the IMPOZ-4 ozone generator (Institute of Precision Mechanics, Warsaw) to a chamber sealed with chemically neutral polyethylene film, where it was spontaneously mixed with air. Ozone concentrations in the exposure chamber were controlled by iodometry (SALTZMAN et al. 1959). During exposure, the animals had ad libitum access to water, but feed was not administered owing to ozone's oxidative effect. Outside the daily exposure regime of 5 h, all animals were kept under identical conditions as regards air composition, temperature and diet.

After the experiment, blood samples were obtained by heart puncture from all rats anesthetized with halothane (Narcotan, Leciva, Czech Republic) until completely bled. Blood samples were centrifuged at 4°C, 3000 rpm, for 10 minutes. Blood plasma was separated and stored at -22°C for the determination of vitamin E and C levels, testosterone and malondialdehyde (MDA) concentrations (WARD et al. 1985). Directly after bleeding, gonadal fragments were excised to measure MDA, vitamin E and C levels (RETTENMAIER et al. 1992, SANDOR et al. 1989, Omaye et al. 1979). MDA concentrations were determined in a 20% saline homogenate of testicular tissue.

Gonadal sections were also used to prepare frozen specimens in a cryostat (CRYOCAT, Reichert Young). The activity of alkaline phosphatase (AP) was determined in the specimens by Gomori's histochemical technique, and the activity of 17- β -hydroxysteroid dehydrogenase – by the method proposed by Levy, Deane and Rubin. The activity levels of the studied enzymes were estimated using computer image analysis (System for Image Processing and Analysis LUCIA). The optical density of the image produced by the studied reaction was measured in the specimens and expressed on a scale of 0 (black – high activity) to 62 (white – low activity).

The results were processed statistically to calculate the arithmetic means, the standard error of the mean (SEM) and the significance of differences relative to the control group, using Student's *t*-test (Statgraphic application, Statistical Graphic System).

Results and Discussion

A significant increase in malondialdehyde concentrations in the blood was noted in all animals exposed to ozone, regardless of the applied vitamin dose and combination. Elevated MDA levels in testicular tissue were observed in rats exposed to ozone as well as in the animals exposed to ozone and receiving vitamin C and E injections separately, regardless of the dose. In the groups administered low-dose combinations of both vitamins, MDA concentrations were comparable to control group values. The above could be attributed to the synergistic effect of the studied antioxidants where the produced tocopheryl radicals are reduced by vitamin C, thus reinstating the original structure of vitamin E (BARTOSZ 2009). In the group of rats exposed to ozone as well as animals exposed to ozone and receiving high doses of vitamin E and C, MDA concentrations were significantly elevated. Authors differ in their opinions regarding the antioxidative and prooxidative effects of ascorbic acid. When administered at higher doses, vitamin C demonstrates prooxidative activity, it contributes to the formation of hydroxyl radicals (Fenton's reaction), oxidative stress and elevated malondialdehyde levels (CARR et al. 1999, NAIDU 2003, JEDLIŃSKA-KRAKOWSKA 2006).

Vitamin C concentrations in the blood plasma were marked by a significant increase in the group of rats receiving the highest vitamin C doses as well as average- and low-dose combinations of vitamins E and C (Table 1). Despite significant variations between groups, the increase in vitamin E levels was statistically non-significant owing to high SEM values. Ascorbic acid is a water-soluble substance that does not demonstrate a cumulative effect, nonetheless, an insignificant increase was noted in testicular tissue. A significant increase

in vitamin E levels was noted in the testes of all ozone-exposed rats not receiving vitamins, in animals receiving vitamin E, animals receiving both vitamins as well as in rats administered the highest doses of vitamin C. Vitamin E may be mobilized from other parts of the body by organs that are directly exposed to ozone-induced oxidative stress, provided that it is present in sufficient quantities. The above applies mainly to lungs. The same mobilization mechanism may apply to other antioxidants, subject to the type of stress (ELSAIED et al. 1993). High levels of vitamin E in the testes of rats exposed to ozone and receiving high doses of vitamin E, high doses of vitamin C as well as high-dose combinations of both vitamins may be related to an increased demand for antioxidants (in view of the free radical cascade initiated by ozone and the properties of ascorbic acid) as well as the mutual interactions between the studied vitamins (PRYOR et al. 1991, BARTOSZ 2009).

Table 1
Concentration of vitamin E and C, and MDA in testes and blood plasma (\pm SEM)

Group	Vitamin C		Vitamin E		MDA	
	[μ g/ml]	[μ g/g]	[μ g/100 ml]	[μ g/100 g]	[μ M/l]	
	blood plasma	testes	blood plasma	testes	blood plasma	testes
I Con	44 \pm 8.9	84.5 \pm 20.47	424 \pm 224	70.1 \pm 16.5	7.35 \pm 0.84	1.88 \pm 0.2
II Con PBS	34.8 \pm 7.46	79.5 \pm 22.87	246 \pm 36.9	20.6 \pm 13.1**	7.92 \pm 1.02	2.15 \pm 0.33
III 1.5E	44.8 \pm 7.78	92.3 \pm 8.62	680 \pm 566	896 \pm 241**	14.8 \pm 1.77**	4.39 \pm 0.37**
IV 4.5E	46.6 \pm 4.82	98.4 \pm 9.3	408 \pm 65	166 \pm 55**	15.46 \pm 2.12**	4.47 \pm 0.5**
V 15E	40.2 \pm 4.66	104.9 \pm 9.61	409 \pm 145	225 \pm 74**	12.8 \pm 0.86**	2.78 \pm 0.22*
VI 3C	49.1 \pm 7.75	90.7 \pm 8.47	237 \pm 45	99 \pm 24	13.4 \pm 1.13**	2.98 \pm 0.21*
VII 9C	50.7 \pm 6.42	99.2 \pm 3.51	1260 \pm 1321	78 \pm 17	15.4 \pm 2.48**	3.53 \pm 0.47**
VIII 50C	60.1 \pm 4.07**	99.2 \pm 9.38	2195 \pm 2896	127 \pm 27**	16.53 \pm 1.52**	2.73 \pm 0.25*
IX 1.5E 3C	38.4 \pm 3.62	102.4 \pm 9.24	457 \pm 141	124 \pm 36*	12.32 \pm 1.06**	1.70 \pm 0.4
X 4.5E 9C	55.7 \pm 2.62**	91.2 \pm 7.5	278 \pm 59	743 \pm 198**	11.47 \pm 0.85**	1.95 \pm 0.42
XI 15E 50C	58.2 \pm 3.6**	90.8 \pm 9.67	3696 \pm 3412	843 \pm 357**	12.0 \pm 1.23**	4.82 \pm 1.26**
XII Oz	53.4 \pm 3.2	91.8 \pm 10.22	359 \pm 58	780 \pm 152**	12.28 \pm 1.47**	4.37 \pm 0.33**

** $p \leq 0.01$ * $p \leq 0.05$ significance in relation to the control group (Con)

The activity of alkaline phosphatase in testicular tissue was not marked by significant variations in comparison with the control group, and the lowest, statistically non-significant values were noted in rats exposed to ozone without vitamin cover (Table 2). Oxidative stress is generally accompanied by a drop in AP activity (OYAGBEMI et al. 2010) which it is an indicator of the quality and biological value of semen. The results of a previous study have demonstrated that although ozone exposure does not have a negative impact on sperm

morphology, it reduces sperm concentrations (JEDLIŃSKA-KRAKOWSKA et al. 2006). The activity of 17- β -hydroxysteroid dehydrogenase decreased only in rats exposed to ozone and not receiving vitamins. The lowest blood testosterone levels were also noted in this group of animals. Testosterone concentrations were marked by a significant drop also in the group of rats receiving average- and low-dose combinations of vitamin E and C. The above can be attributed to the fact that ozone-induced oxidative stress disrupts steroidogenesis in testes, thus lowering testosterone levels (JEDLIŃSKA-KRAKOWSKA et al. 2007).

Table 2
The activity of alkaline phosphatase and 17- β -hydroxysteroid dehydrogenase in testes, and concentration of testosterone in blood plasma (\pm SEM)

Group	17- β -hydroxysteroid dehydrogenase (the optical density)*	Alkaline phosphatase (the optical density)*	Testosterone [ng ml ⁻¹]
I Con	32.86 \pm 1.42	21.57 \pm 0.75	0.94 \pm 0.12
II Con PBS	36.38 \pm 1.09	23.75 \pm 1.68	0.95 \pm 0.08
III 1.5E	35.25 \pm 0.96	17.00 \pm 1.54	1.10 \pm 0.11
IV 4.5E	35.12 \pm 0.77	21.85 \pm 1.92	0.93 \pm 0.11
V 15E	36.43 \pm 1.78	18.50 \pm 1.48	0.87 \pm 0.18
VI 3C	34.8 \pm 1.12	19.25 \pm 0.94	0.83 \pm 0.02
VII 9C	38.14 \pm 2.20	21.42 \pm 1.17	0.84 \pm 0.08
VIII 50C	36.13 \pm 1.21	19.10 \pm 1.55	0.72 \pm 0.06
IX 1.5E 3C	36.75 \pm 1.39	18.25 \pm 1.90	0.74 \pm 0.09
X 4.5E 9C	35.63 \pm 0.85	20.00 \pm 1.46	0.59 \pm 0.09*
XI 15E 50C	35.88 \pm 1.21	19.50 \pm 1.43	0.69 \pm 0.14*
XII Oz	37.75 \pm 1.00*	22.88 \pm 1.22	0.42 \pm 0.07**

** $p \leq 0.01$ * $p \leq 0.05$ significance in relation to the control group (Con)

* The optical density was expressed on a scale of 0 (black – high activity) to 62 (white – low activity).

The results of this study suggest that neither vitamin E nor vitamin C effectively counteracted oxidative stress induced by ozone. The activity of 17- β -hydroxysteroid dehydrogenase in animals receiving the investigated vitamins was comparable to the levels noted in the control group, and so were testosterone concentrations (with the exception of animals receiving high-dose combinations of vitamins E and C).

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