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THE EFFECT OF MULTI-COMPONENT FERTILIZERS ON THE YIELD AND MINERAL COMPOSITION OF WINTER RYE*

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Key words: winter rye, yield, macronutrients, multi-component fertilizers, uptake.

Abstract

The objective of this study was to determine the effect of mixed multi-component fertilizers, Amofosmag 4 and Amofosmag 3, on winter rye yield, and the content and uptake of macronutrients. A three-year field experiment (2008–2010) 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 tested crop was winter rye (*Secale Cereale L.*) cv. Dańkowskie Diament. The obtained results showed that fertilization multi-component fertilizers, especially Amofosmag 3 has the effect of increasing the yield of grain and straw of winter rye as compared to simple fertilizer. The concentrations of the analyzed macronutrients in winter rye grain and straw varied insignificantly between fertilization treatments. Simple and multi-component fertilizers exerted a comparable effect on the mineral composition of the test crop. Significant differences were observed in this respect between successive years of the study. The highest total uptake of nitrogen, potassium and calcium by rye plants was noted in treatments with simple fertilizers, and somewhat higher phosphorus uptake was observed in the Amofosmag 4 treatment. Total magnesium uptake was similar in all treatments.

WPŁYW NAWOZÓW WIELOSKŁADNIKOWYCH NA PLONOWANIE I SKŁAD MINERALNY ŻYTA OZIMEGO

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Słowa kluczowe: żyto ozime, plon, makroelementy, nawozy wieloskładnikowe, pobranie.

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Abstrakt

Celem pracy była ocena wpływu nawozów wieloskładnikowych Amofosmagu 4 i Amofosmagu 3 na plon, zawartość i pobranie makroskładników przez żyto ozime. Trzyletnie doświadczenie polowe (2008–2010) przeprowadzono w Ośrodku Dydaktyczno-Doświadczalnym w Tomaszkanie należącym do Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Rośliną testowaną było żyto ozime odmiany 'Dańkowskie Diament'. Na podstawie otrzymanych wyników stwierdzono, że nawożenie nawozami wieloskładnikowymi, a szczególnie Amofosmagiem 3, miało wpływ na zwiększenie plonu ziarna i słomy żyta ozimego w porównaniu z nawozami jednoskładnikowymi. Zawartość badanych makroelementów w życie w poszczególnych obiektach nawozowych była na ogół zbliżona, zastosowane nawozy działały równorzędnie. Różnice w składzie chemicznym żyta wystąpiły w poszczególnych latach badań. Największe łączne pobranie azotu, potasu i wapnia przez żyto ozime stwierdzono w obiektach z nawozami jednoskładnikowymi, natomiast fosforu – w obiekcie z Amofosmagiem 4. Łączne pobranie magnezu przez żyto było zbliżone we wszystkich obiektach nawozowych.

Introduction

Multi-component fertilizers should be selected based on a thorough analysis of a variety of factors such as the plant species, expected yield, macronutrient availability, time and mode of application. Mixed fertilizers have to be applied in a rational manner, and their rates should be determined in view of crop yield, crop quality and fertilizer efficiency, so as to maximize the benefits of fertilizer use. A wide variety and range of multi-component fertilizers are currently available on the Polish market. The products differ with respect to quality, price, chemical composition and form, including the varying proportions of three primary macronutrients, N, P and K, and secondary nutrients, S, Mg and Ca, which helps consumers make the optimal choice (GRZEBISZ 2000). The use of multi-component mineral fertilizers, supplying a balanced mixture of major nutrients, allows to prevent environmental pollution and address environmental concerns in agricultural ecosystems (ŁABUDA 1994, LIPIŃSKI and BEDNAREK 1998).

The objective of this study was to determine the effect of mixed multi-component fertilizers, Amofosmag 4 and Amofosmag 3, on winter rye yield, and the content and uptake of macronutrients. The applied fertilizers provided the test crop with essential nutrients in optimal proportions.

Materials and Methods

A three-year field experiment (2008–2010) was carried out in a randomized block design at the Research and Experimental Station in Tomaszkanie, at the University of Warmia and Mazury in Olsztyn. The experiment, which comprised three fertilization treatments in four replications: control treatment (simple fertilizers), Amofosmag 4 and Amofosmag 3, 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 winter rye (*Secale Cereale L.*) cv. Dańkowskie Diament. The preceding plants were winter triticale. 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
2008	6.2	72	207	28
2009	7.0	84	149	35
2010	5.7	70	244	96

Based on the average levels of available phosphorus in the soil, 350 kg ha⁻¹ Amofosmag 3 (NPKMg 3:14:20:2 + 22% CaO + 9% SO₃: 10,5 kg N, 21,5 kg P, 58 kg K, 55 kg Ca, 4 kg Mg, 12,5 kg S on pure ingredient basis) and Amofosmag 4 (NPKMg 4:15:15:2 + 24% CaO + 9% SO₃: 12 kg N, 23 P, 43,5 kg K, 60 kg Ca, 4 kg Mg, 12,5 kg S 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 presowing: 14 kg N in the form of urea, 23 kg P in the form of triple superphosphate 46% and 43,5 kg K kg ha⁻¹ in the form of potash salt 60%.

Samples of winter rye 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 the growing season of 2008 differed insignificantly from the long-term average (Table 2). Precipitation total in May and June was substantially lower than the multiannual average for 1970–2000,

which could have reduced the growth rate of winter rye plants. In 2009, mean monthly temperatures were similar to the long-term average. April was very dry, while high precipitation levels were noted in June. According to CHMIELEWSKI (1992), and CHMIELEWSKI and KÖHN (2000), a constant supply of water and moderate temperatures in winter and early spring support the growth and yield of winter cereals (high coefficients of productive tillering). In 2010, air temperatures during the growing season were slightly above the long-term average. Precipitation total in May was over 2.5-fold higher than the long period average for 1970–2000. Weather conditions could have affected the yield of winter rye.

Table 2
Weather conditions in 2008–2010 – data provided by the Meteorological Station in Tomaszkowo

Month	Mean monthly temperature [°C]				Precipitation total [mm]			
	2008	2009	2010	1970–2000	2008	2009	2010	1970–2000
April	7.7	9.4	8.1	6.9	31.4	4.8	18.2	36.1
May	12.3	12.4	12.0	12.7	27.0	52.9	131.9	51.9
June	16.9	14.9	16.4	15.9	32.7	136.9	84.8	79.3
July	18.5	20.4	21.1	17.7	57.7	48.3	80.4	73.8
August	18.4	17.6	19.3	17.2	102.1	19.3	95.3	67.1
September	15.1	14.2	12.0	12.5	22.9	25.7	40.5	59.0

In 2008, the yield of winter rye grain ranged from 5.64 to 6.73 t ha⁻¹, and it was significantly affected by the type of fertilizers (Table 3). The highest average yield of winter rye grain was noted in the Amofosmag 3 treatment. Straw yield was not significantly influenced by the fertilizers applied in the study. In experiments conducted by SADOWSKI and RYCHCIK (2010), and SKUODIENĖ and NEKROŠIENĖ (2009), the average winter rye yield reached 5.47 t ha⁻¹ and 3.82 t ha⁻¹, respectively. In the second year of the study, the yield of winter rye grain varied from 5.96 to 5.97 t ha⁻¹, which was the lowest level noted throughout the experimental period. Precipitation total in April was very low, which could have reduced the number and size of rye ears. The experimental factors had no significant effect on straw yield. As demonstrated by CHMIELEWSKI and KÖHN (2000), and ALARU et al. (2003), cereal grains are highly sensitive to weather conditions. In the third year of the experiment (2010), Amofosmag 3 had the most beneficial influence on rye grain yield, which was found to increase by around 6%, compared with the control treatment. Rye straw yield was affected by the applied fertilizers to a lower degree. In a study by OLESEN et al. (2009), the average yield of winter cereals ranged from 3.2 to 5.1 t ha⁻¹.

Table 3

Winter rye yield after the application of Amofosmag 4 and Amofosmag 3 [t ha⁻¹]

Treatment	Grain				Straw			
	2008	2009	2010	mean for <i>a</i>	2008	2009	2010	mean for <i>a</i>
NPK	5.64	5.96	7.09	6.23	7.06	8.28	8.71	8.02
Amofosmag 4	5.96	5.94	7.23	6.38	7.58	7.81	8.98	8.12
Amofosmag 3	6.73	5.97	7.49	6.73	9.28	7.64	9.37	8.76
Mean for <i>b</i>	6.11	5.96	7.27		7.97	7.91	9.02	
LSD _{p = 0.05} for <i>a</i>	0.30				n.s.			
<i>b</i>	0.32				0.75			
<i>ab</i>	n.s.				0.73			

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

The results of the present study show that Amofosmag 3 caused an approximately 8% and 9% increase (on average) in the yield of rye grain and straw, respectively, compared with simple fertilizers. An increase in the yield of different cereal species in response to the application of mixed fertilizers was also reported by ZAWARTKA and SKWIERAWSKA (2004), and NOGALSKA et al. (2010, 2011), whereas in an experiment by WINIARSKI et al. (2002) the yield-forming effects of multi-component and simple fertilizers were comparable.

Rye is grown as a food plant for human consumption and animal feed, therefore its macronutrient and micronutrient content is equally important as yield (BEDNAREK et al. 2006). In 2008, the nitrogen content of winter rye grain ranged from 10.62 to 11.50 g kg DM. A lower nitrogen content of rye kernels was observed in 2009, and the highest nitrogen concentrations in rye grain (15.28 g kg⁻¹ DM) were noted in 2010. The applied fertilizers had no significant effect on changes in the nitrogen levels in rye grain. In a study by GROMOVA and POLACK (1995), the average nitrogen content of rye grain was 23.70 g kg⁻¹ DM. Nitrogen concentrations in winter rye straw were significantly affected by the type of fertilizers and the year of the study. A significantly lower nitrogen content of rye straw (on average) was reported following the application of Amofosmag 3. In 2008, rye grain contained significantly less phosphorus and potassium than in 2009 and 2010. In the second and third year of the experiment, rye kernels were more abundant in phosphorus and potassium (statistically significant differences). Multi-component fertilizers had no significant effect on changes in the concentrations of phosphorus and potassium in rye straw. The calcium content of winter rye grain was not determined by the type of fertilizers. Differences between treatments were minor, and the highest calcium concentrations in the test crop were noted in 2010. Both mixed

Table 4

Macronutrient content of winter rye after the application of Amofosmag 4 and Amofosmag 3
[g kg⁻¹ d.m.]

	Treatment	Grain				Straw			
		2008	2009	2010	mean for <i>a</i>	2008	2009	2010	mean for <i>a</i>
Nitrogen	10.62	10.62	8.98	15.95	11.85	3.16	3.46	6.65	4.42
	10.20	10.20	9.62	14.85	11.55	3.46	3.42	5.11	3.99
	11.50	11.50	8.66	15.06	11.74	3.21	3.17	3.87	3.41
Mean for <i>b</i>		10.77	9.08	15.28		3.27	3.35	5.21	
LSD _{p = 0.05} for <i>a</i>		n.s.				0.582			
<i>b</i>		1.012				0.520			
<i>ab</i>		n.s.				1.009			
Phosphorus	1.92	1.92	4.92	3.77	3.53	1.17	2.07	1.95	1.73
	1.99	1.99	5.51	3.66	3.72	1.08	2.13	1.98	1.73
	1.88	1.88	2.71	3.38	2.65	1.00	2.19	2.01	1.74
Mean for <i>b</i>		1.93	4.38	3.60		1.08	2.13	1.98	
LSD _{p = 0.05} for <i>a</i>		0.244				n.s.			
<i>b</i>		0.240				0.177			
<i>ab</i>		0.422				n.s.			
Potassium	3.07	3.07	5.47	4.24	4.25	15.15	11.90	13.55	13.52
	3.30	3.30	5.62	4.10	4.34	12.65	12.90	13.27	12.94
	2.82	2.82	5.57	4.10	4.16	11.32	12.72	11.32	11.78
Mean for <i>b</i>		3.06	5.55	4.14		13.04	12.50	12.71	
LSD _{p = 0.05} for <i>a</i>		n.s.				n.s.			
<i>b</i>		0.285				n.s.			
<i>ab</i>		n.s.				n.s.			
Calcium	0.58	0.58	0.54	0.71	0.61	5.26	3.22	3.86	4.11
	0.60	0.60	0.47	0.69	0.58	4.05	2.82	3.54	3.47
	0.51	0.51	0.46	0.74	0.57	4.58	2.42	3.44	3.48
Mean for <i>b</i>		0.56	0.49	0.71		4.63	2.82	3.61	
LSD _{p = 0.05} for <i>a</i>		n.s.				0.254			
<i>b</i>		0.051				0.280			
<i>ab</i>		n.s.				0.440			
Magnesium	0.77	0.77	0.84	0.94	0.85	0.37	0.43	0.44	0.41
	0.81	0.81	0.89	0.96	0.88	0.36	0.45	0.37	0.39
	0.72	0.72	0.84	0.95	0.83	0.41	0.44	0.32	0.39
Mean for <i>b</i>		0.76	0.85	0.95		0.38	0.44	0.37	
LSD _{p = 0.05} for <i>a</i>		n.s.				n.s.			
<i>b</i>		0.060				0.054			
<i>ab</i>		n.s.				n.s.			

Explanations as in Table 3

fertilizers contributed to a slight, but significant decrease in the calcium content of straw, compared with simple fertilizers. The magnesium content of winter rye remained stable throughout the experiment, and it was not significantly affected by the applied fertilizers. There were differences in

magnesium levels between the years of the study. The highest magnesium content of rye grain and straw was reported in 2010 and 2009, respectively. The results of chemical analyses of winter rye grain and straw show that the concentrations of the analyzed macronutrients varied between the years of the study, and that they were determined by weather conditions rather than by the applied fertilizers. The findings of other authors (FILIPEK 2001, TRAWCZYŃSKI and GRZEŚKIEWICZ 2006, NOGALSKA et al. 2010, 2011) indicate that multi-component fertilizers have no significant effect on the macronutrient content of test crops.

Macronutrient uptake was estimated based on the yield and macronutrient content of winter rye grain and straw. Nitrogen uptake by rye plants varied throughout the experimental period, and it reached the highest level in the third year of the study. Nitrogen uptake was correlated with rye yield and the percentage content of nitrogen in plants (Table 3 and Table 4). Phosphorus uptake levels were comparable in all treatments, and they tended to increase in response to Amofosmag 4. Phosphorus uptake varied considerably between years, due to differences in rye yield and the percentage content of macronutrients in plants. Potassium uptake by winter rye plants was highest in the third year of the experiment, in particular after the application of simple fertilizers. The highest calcium uptake was noted in the first year of the study, mostly due to a high calcium content of rye straw (Table 5). Magnesium uptake by rye plants was comparable in all treatments, regardless of the fertilizers applied. Differences in magnesium uptake were observed only between the years of the study. The highest total uptake of nitrogen, potassium and calcium was noted in treatments with simple fertilizers, and somewhat higher phosphorus uptake was observed in the Amofosmag 4 treatment (Figure 1). Total magnesium uptake was similar in all treatments. Partially different results were reported by NOGALSKA et al. (2010, 2011).

Table 5

Nutrient uptake by winter rye grain and straw [kg ha⁻¹]

Treatment	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
2008					
NPK	82.19	19.08	124.2	40.40	6.95
Amofosmag 4	87.01	20.04	115.3	34.26	7.54
Amofosmag 3	107.17	21.93	124.0	45.93	8.64
2009					
NPK	82.16	46.45	131.1	29.87	8.56
Amofosmag 4	83.77	49.35	134.1	24.81	8.79
Amofosmag 3	75.91	32.90	130.4	21.22	8.37
2009					
NPK	171.0	41.85	148.0	38.71	10.49
Amofosmag 4	153.2	44.24	130.2	36.76	10.26
Amofosmag 3	149.0	40.66	136.7	37.77	10.10

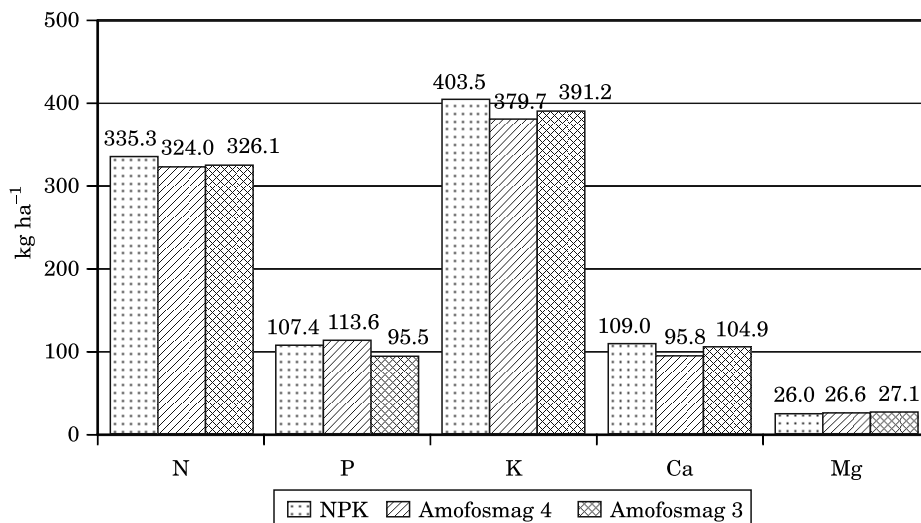


Fig. 1. Total macronutrient uptake by winter rye over a three-year experimental period

Conclusions

1. The most beneficial effect was reported for Amofosmag 3 which increased the yield of winter rye grain by 8% on average, compared with the control treatment.

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

3. The highest total uptake of nitrogen, potassium and calcium by rye plants was noted in treatments with simple fertilizers, and somewhat higher phosphorus uptake was observed in the Amofosmag 4 treatment. Total magnesium uptake was similar in all treatments.

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BEHAVIOURAL ACTIVITY IN PIGS AT HABITUAL TEST IN RELATION TO PRODUCTION TRAITS*

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Key words: young pig, behaviour test, feeding parameters.

Abstract

The aim of experiment was establish the relation between different behaviour activity and production results of young pigs. The observation were performed on 49 pigs of hybrid combination (Bu x La) x PIC weighing from 30 to 40 kg. It was used a twenty-minute habitual test in the one observation. During experiment were noted the following activities: number of squares that were walked through, duration of lying down, frequency of lying down and duration of standing. The animals were divided into groups for each habitual indicator separately by means of a quartile division. We monitored the following fattening indicators: average daily gains (ADG) and feed conversion ratio per one kilogram of gain (FCR). The group with the lowest number of squares walked through achieved the tendency to the highest ADG (1.038 kg) than other ones. The group with the highest number of squares walked through had tendency to the lowest FCR (2.900 kg). The group of the shorted durations of standing were achieved the tendency to highest ADG (1.065 kg) as well as to the lowest FCR (2.917 kg). Amount of lying down, the group of animals which hadn't been lying at all during the habitual test run achieved the lowest average daily gain (0.962 kg day⁻¹) and the highest fodder consumption per kilogram of gain (3.215 kg). The group of pigs with the longest time duration of lying down achieved the highest average daily gain (1.050 kg day⁻¹). The differences regarding the feeding indicators between the groups were not statistically significant. Within all the observed pigs the average daily gain significantly correlated ($P < 0.05$) with the time duration of standing ($r = -0.302$), as well as with the time duration of their lying down ($r = 0.301$).

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ZALEŻNOŚCI MIĘDZY AKTYWNOŚCIĄ ZACHOWANIA TUCZNIKÓW W TEŚCIE ADAPTACYJNYM A ICH WYNIKAMI W TUCZU

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Słowa kluczowe: świnie, test adaptacyjny, wyniki tuczne.

Abstract

Badania miały na celu określenie zależności między aktywnością zachowania a wynikami produkcyjnymi młodych tuczników. Badania wykonano na 49 mieszańcach pochodzących od loch ras Białej Szlachetnej (BU) x Landrace (La) krzyżowanych z knurami PIC (Pic Improvement Company) o początkowej masie ciała 30–40 kg. Obserwacje wykonywano przez 20 minut w godzinach od 7:00 do 11:00. Komora, w której testowano zwierzęta, składała się z 16 kwadratów. Podczas badań rejestrowano następujące rodzaje zachowań: liczbę przechodzonych kwadratów w kojcu, czas leżenia, częstość kładzenia się, czas trwania w pozycji stojącej. Wyniki tuczne (od 30 do 100 kg) obejmowały cechy średniego przyrostu dziennego (ADG) oraz wykorzystania paszy na 1 kg przyrostu masy ciała (FCR). Najmniej aktywne zwierzęta, które przechodziły przez najmniejszą liczbę kwadratów, osiągały nieco większe przyrostyienne od pozostałych grup (średnio od 5 do 28 g). Wykorzystanie paszy na 1 kg przyrostu w grupach było zbliżone z tendencją do osiągania niższego jej zużycia przez tuczniki najaktywniejsze ruchowo. Tendencję do osiągania największych przyrostów dziennych i najniższego zużycia paszy miały tuczniki, które najkrócej stały (odpowiednio 1,065 kg/dzień i 2,917 kg/kg). Grupa, która podczas testu nie leżała w ogóle, miała najniższe przyrosty dobowe i najgorzej wykorzystywała paszę (0,962 kg i 3,215 kg/kg). Z kolei tuczniki, które najdłużej leżały, osiągały najwyższe przyrosty (1,050 kg). Jednakże wszystkie wyniki nie różniły się statystycznie. Jedynie statystycznie istotne współczynniki korelacji zanotowano między wysokością dziennych przyrostów a czasem stania ($r = -0,302$) oraz z czasem leżenia ($r = 0,301$).

Introduction

The most important types of learning are as follows: habituation and sensitization, classical conditioning, instrumental and discrimination learning, and higher forms of animal learning (KOTTEROVÁ et al. 2008). Habituation is the simplest type of learning consisting in the fact that an animal can get used to a certain stimulus and stop responding to it if this stimulus is repeated too often. Habituation cannot be considered a sign of sensoral or muscle tiredness. It concerns the information that the memory records in relation to the insignificance and unimportance of certain stimuli or behaviour patterns. It is by negative learning which diminishes the ability to respond to a certain stimulus for a long time, whereas an animal in its central nervous system reacts quite normally to many other stimuli. This simple learning in not to respond to a certain stimulus, can be considered a kind of an adjustment by the

animal, which saves on its insignificant activities (VESELOVSKÝ 2005). The habituation phenomenon is used by the means of habituation tests in order to find out individual excitability and habituation differences in animals (KOTTEROVÁ et al. 2008). Such a habituation test is performed in a limited space that is isolated from all sights and sounds that could be disturbing (DEBRECÉNI et al. 2010). Behavioural indicators of pigs have a certain characteristic development that is always normal for assessing their individual differences. To determine individual differences in pig behaviour, more significant values have to be provided by the excitation level (occurrence of indicators) rather than by habituation speed (decrease in indicator frequency during the test run) (NOVÁČKÝ and LIDAY 1996). The excitation indicators don't remain stable in the long term if pigs are divided into individual excitable types because the reaction of pigs significantly changes as they age (from 30 kg to 90 kg) (DEBRECÉNI et al. 2010). According to CHALOUPKOVÁ et al. (2007) behavior of piglets before weaning did not affect behavior of fattening pigs. LORENCOVÁ et al. (2006), MLYNEK et al. (2001), observed the excitation in pigs during the habituation test, based on the motoric activities. MLYNEKOVÁ (2008) suggests to use, in case of need to differentiate between the excitation types of pigs for various experimental purposes, a twenty-minute habituation test in order to observe the following indicators of excitability: standing up, frequency of lying down, and the length of lying down at the weight of 30 kg. According to HAYNE and GONYOU (2006) for distinguishing among individuals is useful also the human approach test.

In connection with the given problem, the goal of our experiment was to evaluate the selected fattening parameters of pigs that were divided into groups with different degree of excitability, based on the observed ethological manifestations during the habituation test.

Material and Methods

The habituation observations were carried out in 49 pigs of the hybrid combination (Bu x La) x PIC with the live weight 30–40 kg in ECFA (Experimental Centre of Farm Animals). The habituation stall was isolated from the outdoor environment and had extensive lighting throughout. The habituation test is a modified test of the open field. The classical test of the open field evaluates natural behavior of an individual or a social group of animals in the open terrain. During the habituation test, an animal is observed in the bounded space, isolated from all the outdoor disturbing stimuli (in a habituation room). The one observation period was lasting 20 minutes. The floor of the habituation stall was markedly divided into 16 squares. The cameras were installed inside the room, from which the picture was

transmitted on the monitor in observation room. The observations were carried out in the morning from 7:00 to 11:00 o'clock a.m. We used the habituation test, or rather a twenty-minute modified test of the open area in our observations. The principle of the test was monitoring the various reactions to the new and unaccustomed pigs to their unknown surroundings. We were observing the following activities during the habituation test: number of squares walked through, duration of lying down, amount of lying down and duration of standing. We divided the animals into certain groups for each of the habituation indicators independently by means of the quartile division as follows (n-number of animals):

Number of squares walked through:

- P1 – a group with motoric activity < 55 squares ($n = 12$);
- P2 – a group with motoric activity from 55 to 102 squares ($n = 26$);
- P3 – a group with motoric activity > 102 squares ($n = 11$).

Duration of standing in s:

- P1 – a group with a standing length < 312.8 s ($n = 12$);
- P2 – a group with a standing length from 312.8 s to 617.52 s ($n = 25$);
- P3 – a group with a standing length > 617.52 s ($n = 12$).

Frequency of lying down:

- P1 – a group with the number of lying down < 1 ($n = 7$);
- P2 – a group with the number of lying down from 1 to 5 ($n = 32$);
- P3 – a group with the number of lying down > 5 ($n = 10$).

Duration of lying down in s:

- P1 – a group with the length of lying down < 150.26 s ($n = 12$);
- P2 – a group with the length of lying down from 150.26 s to 645.23 s ($n = 25$);
- P3 – a group with the length of lying down > 645.23 s ($n = 12$).

Within the fattening indicators of pigs, we observed the average daily gains and fodder consumption per one kg of gain of the stated weight ranged from 30 to 100 kg. Detailed mathematical/statistical data processing was realized in the statistical programme SAS version 9.1. To judge the statistically evidentiary differences between the groups, we used analysis of variance with testing of the contrasts from the Tukey (HSD) test. The Pearson coefficient index for calculating the correlation was used.

Results

There were quite significant individual differences in the observed indicators (Table 1). The variation coefficient values in the observed activities moved from 39.75% (moving through the number of squares) to 74.14% (number of lying down).

Table 1
Variation-statistical values of ethological indicators in the habituation test

(n = 49)	Number of squares	Duration of standing	Frequency of lying	Duration of lying
Min.	22.00	121.19	0.00	0.00
Max	191.00	936.20	9.00	913.37
\bar{x}	79.27	480.24	3.37	402.87
s	31.84	219.25	2.52	279.19
v%	39.75	45.18	74.14	68.59

Legend: n – number of animals, min. – minimum, max – maximum, \bar{x} – arithmetic mean, s – standard deviation, v% – coefficient of variation

The fattening pigs divided into groups based on the number of the squares walked through showed in the figure 1. The animals which walked through the lowest number of squares during the habituation test had the tendency to the highest average daily gains (1.038 kg), while the average daily gains of the individuals with the highest motoric activity had the lowest ones (1.010 kg).

Individual pigs that had the highest number of squares walked through during the habituation test also had tendency to the lowest fodder consumption. However the differences among the groups were not statistically significant.

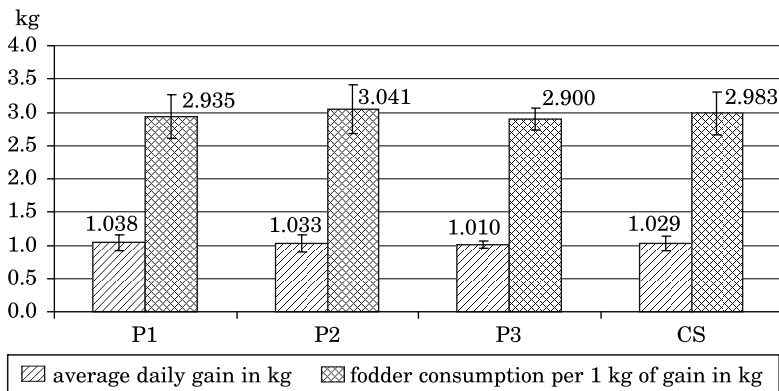


Fig. 1. Average values of the fattening indicators in the groups divided according to the number of squares walked through: P1 – the group with the number of squares walked through < 55, P2 – the group with the number of squares walked through from 55 to 102, P3 – the group with the number of squares walked through > 102, CS – together

The animals standing during the habituation test for the shortest period of time (Figure 2) achieved the highest average daily gains (1.065 kg day⁻¹), and at the same time the lowest fodder consumption per 1 kg of gain in the Test (2.917 kg). This fact can be explained in such a way that between the time

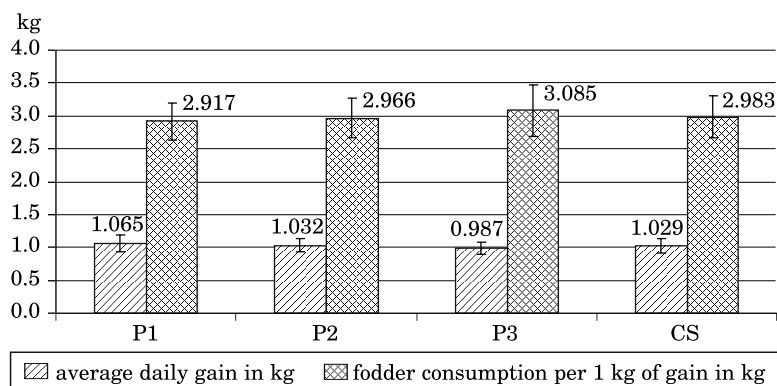


Fig. 2. Average values of the fattening parameters in the groups divided according to the duration of standing: P1 – the group with the duration of standing < 312.8 s, P2 – the group with the duration of standing from 312.8 s to 617.52 s, P3 – the group with the duration of standing > 617.52 s, CS – together

of the duration of standing and lying down there was a high negative correlation dependence ($r = -0.940$) – table 2, which means that individuals which were standing for a short period of time were lying for a longer period of time and could better use the received fodder for live weight formation. We haven't found out any statistically significant differences in feeding parameters among the groups divided according to the length of lying down.

Table 2
Correlation of the ethological indicators of pigs in the habituation test

Indicator	Duration of standing	Number of lying	Duration of lying
Number of squares	0.318 ⁺	0.213	-0.503 ⁺⁺
Duration of standing	–	-0.497 ⁺⁺	-0.940 ⁺⁺
Frequency of lying	–	–	0.386 ⁺⁺

⁺ – $P < 0.05$, ⁺⁺ – $P < 0.01$

It is possible to see from Figure 3 that group P2 has achieved the best fattening indicators in the animal division according to the numbers of those lying down, where it moved from 1 to 5. The group P1 represents the animals that didn't lie down at all during the habituation test. It means that the individuals reacted very sensitively to the unknown surroundings and it is very probable that they were reacting more to the stimuli of the outdoor environment during the fattening period, which had ended in worsened average daily gains ($0.962 \text{ kg day}^{-1}$) and in the fodder consumption per kg of gain (3.215 kg).

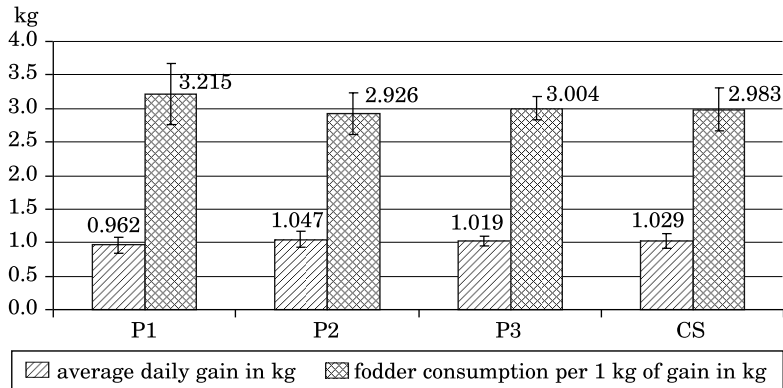


Fig. 3. Average values of the fattening parameters in the groups divided according to the number of lying down: P1 – the group with the number of lying down < 1, P2 – the group with the number of lying down from 1 to 5, P3 – the group with the number of lying down > 5, CS – together

According to the number of those lying down and to the above mentioned division, we can suppose that group P2 represents the animals with the lowest excitability, because a large number of those lying down followed by standing represented by the group P3 can also be a sign of the increased excitability of animals. We haven't found out any statistically significant differences among the groups despite the determined differences in the fattening indicators.

It suggests that the animals that had been lying during the habituation test for the longest period of time learned to react quickly to the unknown environment, which means that they habituated and adapted to the change of environment. This results in the fattening indicator value analysis (Figure 4)

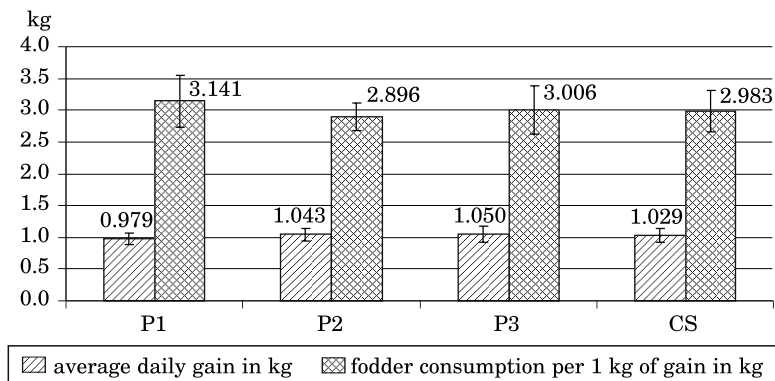


Fig. 4. Average values of the fattening results in the groups divided according to the duration of lying down: P1 – the group with the duration of lying down < 150.26 s, P2 – the group with duration of lying down from 150.26 s to 645.23 s, P3 – the group with the duration of lying down > 645.23 s, CS – together

that these individuals had the best average daily gains ($1.050 \text{ kg day}^{-1}$) in the test. The individuals lying during the test run for shorter than 150.26 s have achieved the worst fattening indicators. The differences among the groups were not statistically significant.

The correlations from the found data during the habituation test as well as from the data relating to their fattening, which is shown in Table 3. We have found out statistically significant ($P < 0.05$) negative correlation dependence between the length of standing and the average daily gain from 30 kg to 100 kg ($r = -0.302$).

Table 3
Correlation of the ethological indicators with the fattening results of pigs

Indicator	Average daily gain	Fodder consumption per 1 kg of gain
Number of squares	-0.116	0.023
Duration of standing	-0.302 ⁺	0.220
Frequency of lying	0.035	-0.133
Duration of lying	0.301 ⁺	-0.120

Legend: ⁺ – $P < 0.05$, ⁺⁺ – $P < 0.01$

The duration of standing correlated statistically significant with the number of the squares walked through ($r = 0.318$; $P < 0.05$). That's why we think that the individuals that were standing longer during the habituation test were the ones with higher excitability of the nervous system and have achieved worse results in the fattening parameters.

The duration of lying had statistically significant ($P < 0.05$) positive correlation dependence with the average daily gain during the test ($r = 0.301$). The length of lying down during the habituation test can be a kind of sign that an animal got used to the new environment, which means that it adapted to it.

It is very probable that these animals have better coped with unknown stimulus during the fattening process, as they could better get adapted to them and spend more time resting, which is a very important condition to achieve high average daily gains.

Discussion

Similar to DEBRECÉNI et al. (2010) results, in the own experiment have also used the quartile division for assessing the excitability of pigs and with regard to their production parameters into three groups. Also in this experiment the results were not statistically differentiated.

As it was mentioned in the methods the own experiment was realized at the weight of approximately 30–40 kg for the reason that animals show more activity at a lower weight. Another reason is that excitations based on ethological manifestations are more appropriate. VAN DE PERRE et al. (2011) find out most behaviour between 20 and 40 kg bodyweight of pigs and was decreasing over age. The issue of habituation tests was also dealt with by FÚSKA and BARBORÍK (2007), who observed pigs weighing between 25–30 kg and 90–100 kg. They found out that the individual pigs had been motorically more active at a lower body weight than at the weight of 90–100 kg. LORENCOVÁ et al. (2006), MLYNEK et al. (2001), MLYNEK et al. (2000), divided the animals according to the number of squares that had been walked through, which means the motoric activity, during the evaluation of the habituation tests. Like DEBRECÉNI et al. (2010), we have also used the quartile division for assessing the excitability of pigs and with regard to their production parameters, and we divided the animals into three groups.

MLYNEK et al. (2001) found out that the group with the lower motoric activity had a higher ADG (average daily gain) and lower fodder consumption per kg of gain, a higher VMP percent (valuable meaty parts) as well as a higher thigh weight. The differences of the average values among the groups were not statistically evidentiary. Nor did DEBRECÉNI et al. (2010) find out the statistically evidentiary relation of the motoric activity to the production and qualitative parameters, but they achieved higher average daily gains and lower feedstuff consumption per kg of gain in animals that showed lower motoric activity, but the difference between the groups was again not statistically evidentiary.

Comparing the feeding indicators from the view of motoric activity (number of squares walked through and the time of movement), we have found out similarly to the above mentioned authors that pigs with the lowest motoric activity had the highest average daily gains, but they did not have the lowest fodder consumption for the kg of gain. These differences were not statistically evidentiary.

MLYNEKOVÁ (2008) came to the conclusion that 30 kg animals that were lying down more times during the habituation test, achieved the highest ADG at the pre-fattening period ($P < 0.01$) as well as the consumption of the least amount of feedstuff to get kg of gain ($P < 0.01$). The animals have kept a similar tendency in ADG in the efficiency test ($P < 0.05$) and in the feedstuff consumption per kg of gain in the fattening test ($P < 0.01$).

In the own investigation the group of pigs with the low frequency of lying down from 1 to 5 (P2) achieved the highest average daily gain and the lowest feed conversion per 1 kg of body gain. The group P1 which did not lie down at all during the habituation test the worst feeding parameters were achieved. However also these differences were not statistically significant.

Our results are a fully agree with the statements of DEBRECÉNI'S et al. (2010), MLYNEKOVA'S (2008), NOVACK'S et al. (2002), MLYNEK'S et al. (2001), JASKULKE and MANTEUFFEL (2011), that motorical activity, sound manifestations and metabolic functions (urination, defecation) observed during the habituation test can serve to identify various neuro-reflexive types. Based on the statistical analysis, we haven't succeeded to confirm a statistically important relation of the motoric activity to production parameters of pigs. On the contrary, it has found out statistically important correlations of the passive ethological manifestations (duration of lying and duration of standing) with the average daily gains.

Conclusions

The important individual differences among animals by observing the ethological manifestations during the habituation test was founded. The values of the variation coefficient had been moving from 39.75% (number of squares) to 74.14% (number of lying down). We haven't found out any statistically significant differences in the feeding parameters among animals groups. However a negative correlation dependence between the duration of standing and the average daily gain ($r = -0.302$; $P < 0.05$) within the whole of animals were received. Also the duration of standing was positively correlated with the number of squares walked through ($r = 0.318$; $P < 0.05$). It indicates that the animals which were standing during the habituation test a longer period time reacted more sensitively on the environmental change and they achieved worse fattening results. On the contrary, the length of lying down positively correlated with the average daily gain ($r = 0.301$; $P < 0.05$). This investigation shows also that lying down during the habituation test for the longest periods of time had similar feed conversion and achieved tendency to higher average daily gains.

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A NEW STAND OF *OXYCOCCUS MICROCARPUS* TURCZ. EX RUPR. IN THE REGION OF WARMIA

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Key words: small cranberry *Oxycoccus microcarpus* Turcz. ex Rupr., new stand, rare species, endangered species, vulnerable species.

Abstract

Floristic research carried out in the region of Warmia and Mazury led to the discovery, on 16 May 2011, of a small cranberry stand in a forest, in a small raised bog with enclaves characteristic of transitional moors. The discussed stand is situated in the southern part of the Olsztyn Forest Division, district 499, in the vicinity of the eastern boundary of the Lake Košno landscape reserve.

NOWE STANOWISKO *OXYCOCCUS MICROCARPUS* TURCZ. EX RUPR. NA WARMII

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Słowa kluczowe: żurawina droбноowocowa *Oxycoccus microcarpus* Turcz. Ex Rupr., nowe stanowisko, gatunek rzadki, zagrożony i ustępujący.

Abstrakt

W trakcie badań florystycznych prowadzonych w województwie warmińsko-mazurskim 16.05.2011 r. stwierdzono występowanie żurawiny droбноowocowej. Zajmowała niewielkie śródleśne torfowisko wysokie mające enklawy charakterystyczne dla torfowisk przejściowych. Opisywane stanowisko jest położone w południowej części Nadleśnictwa Olsztyn, w granicach oddziału 499, przy wschodniej granicy Rezerwatu krajobrazowego jezioro Košno.

Introduction

The small cranberry *Oxycoccus microcarpus* Turcz. ex Rupr. (*Vaccinium microcarpum* Turcz. ex Rupr. Schmalh) is a dwarf shrub of the heath family (*Ericaceae*) which colonizes cool and temperate zones of the northern hemisphere. It belongs to a small group of glacial relic species. The small cranberry is found mostly in raised bogs and transitional moors, on acidic soils (pH below 5) with a high moisture content. It is considered to be a characteristic species of the alliance *Oxycocco-Empetrion hermaphroditi* which comprises plant communities typical of raised bogs in the sub-arctic and boreal zones of the European continent (MATUSZKIEWICZ 2001).

The small cranberry (*Oxycoccus microcarpus*) bears a resemblance to the bog cranberry *Oxycoccus palustris* Pers. (*O. quadripetalus* Gilib., *Vaccinium oxycoccus* L.). The two species are generally found in the same habitats, and they are often confused.

Oxycoccus microcarpus is significantly smaller and more delicate than *Oxycoccus palustris*. *O. palustris* has ovate or oblong-ovate leaves, round at the base with blunt tips, whereas the leaves of *O. microcarpus* are more triangular in shape, wider at the base and narrowing towards the end, with sharp tips. *O. microcarpus* has annual shoots and petioles that are smooth or sparsely covered with hairs. Bracteoles are located at mid-length of the petiole or near its base (ZARZYCKI 1963, RUTKOWSKI 2006).

In Poland, *Oxycoccus microcarpus* is a rare, vulnerable species (RUTKOWSKI 2006) which is threatened by extinction (ZARZYCKI and SZELAĞ 2006).

Results

The *Oxycoccus microcarpus* stand discovered on 16 May 2011 is situated in a forest, in a small raised bog whose fringe areas features enclaves characteristic of transitional moors. The bog is found in the southern part of the Olsztyn Forest Division, district 499, in the vicinity of the eastern boundary of Lake Kośno landscape reserve, between the villages of Łajs and Tylkowo (Figure 1 – stand marked with an asterisk).

The discussed bog occupies an area of 1.59 ha. It is surrounded by a 60-year-old *Serratulo-Pinetum* mixed coniferous forest (MATUSZKIEWICZ 1981, MATUSZKIEWICZ J.M. 2001) with a predominance of the Scots pine (*Pinus sylvestris*). The oldest trees, aged 120–140 years, occupy the south-most section of the complex. The edges of the peat bog are largely dried-up. The only open-water zones are two small ponds in the southern and north-eastern parts of the bog. Pine trees demonstrate signs of strong succession. *O. microcarpus*

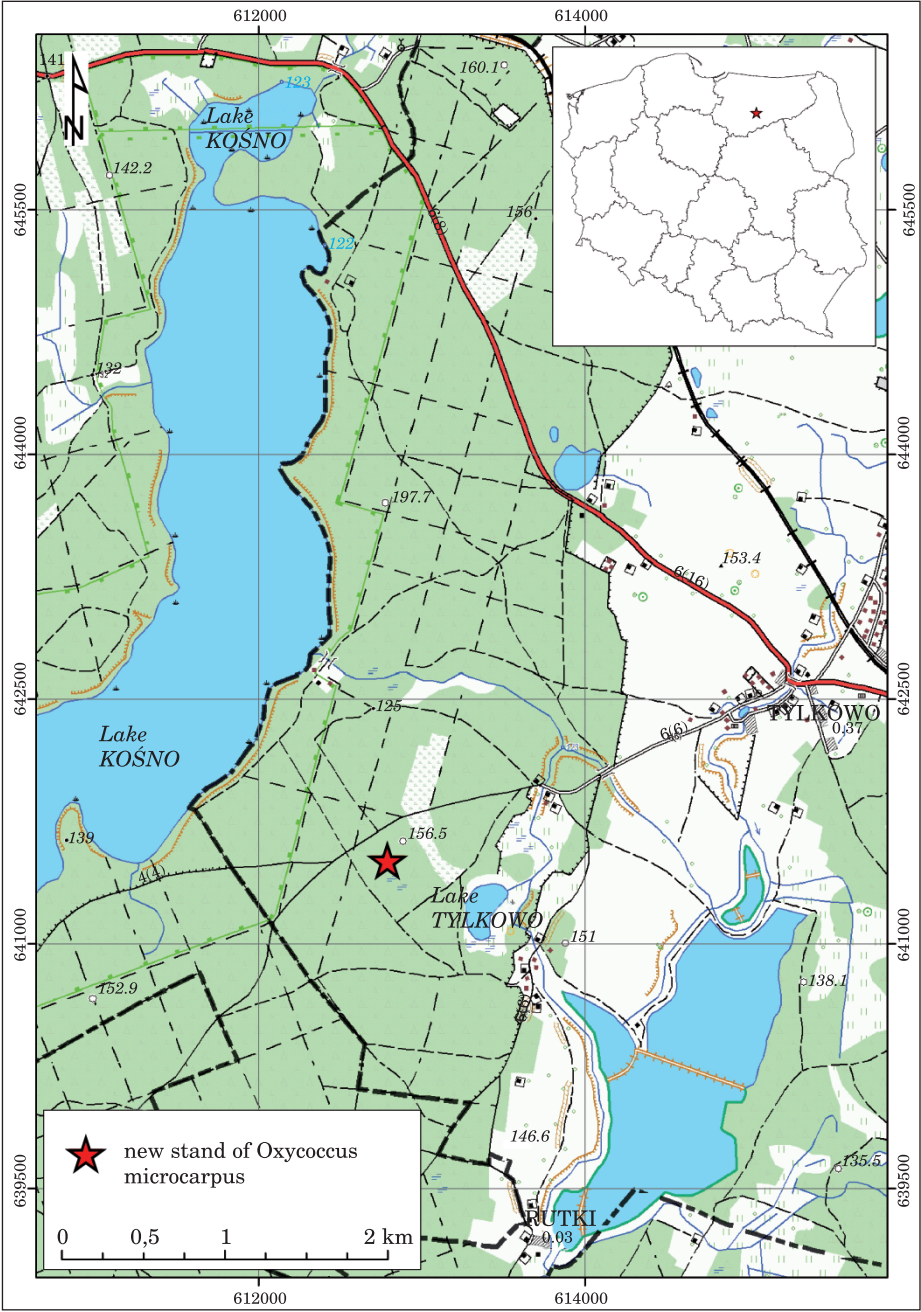


Fig. 1. New stand of *Oxycoccus microcarpus* localization

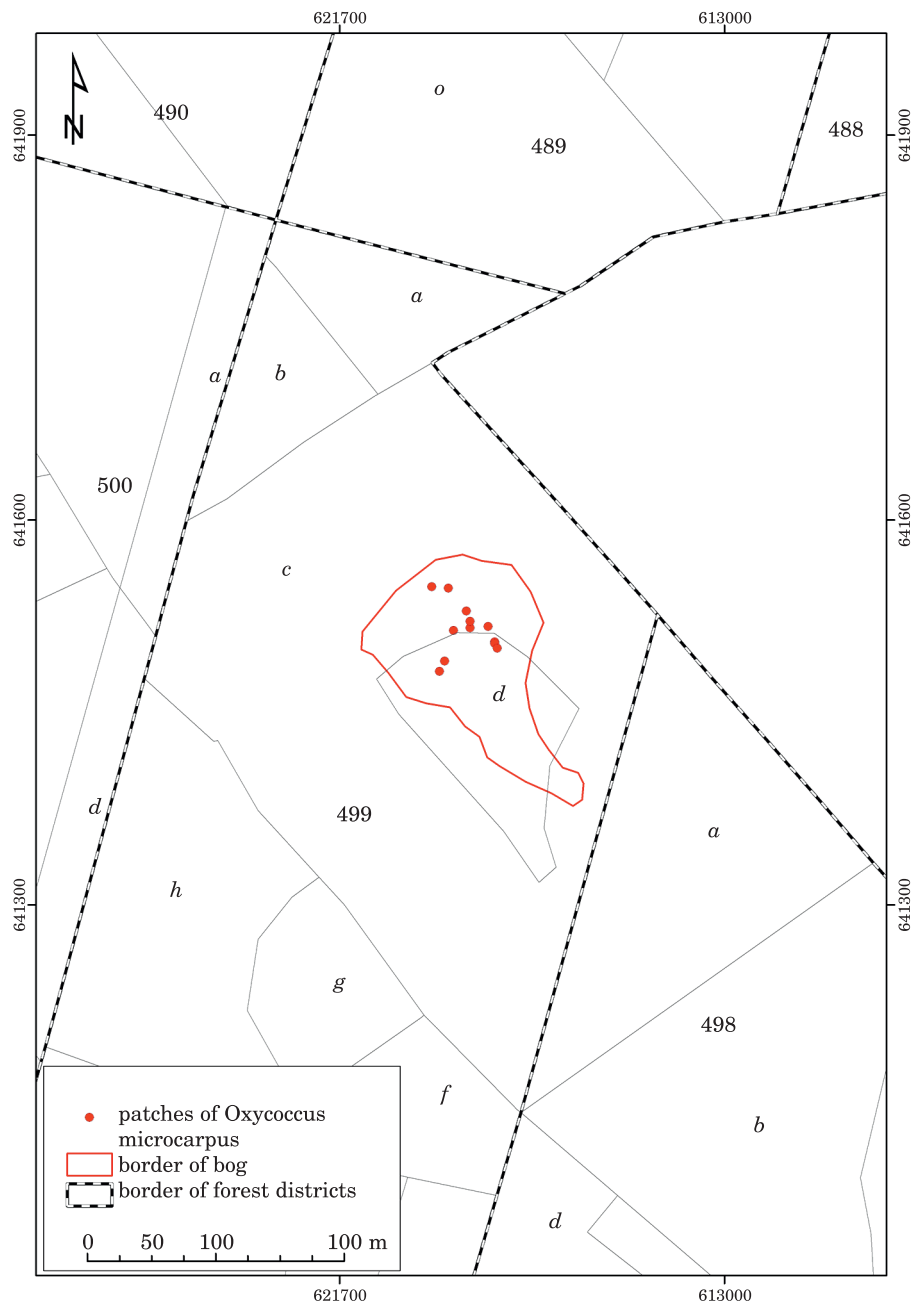


Fig. 2. Patches of *Oxycoccus microcarpus*

communities were found in 11 sites in northern and central parts of the bog (Figure 2). The small cranberry shows a preference for dried peat moss clusters raised around 30 cm above water level. In sites of the type, *O. microcarpus* creates dense communities that occupy an area of up to 2 m². The species colonizes its habitats by trailing in between individual plants of the bog cranberry (*O. palustris*), common sundew (*Drosera rotundifolia*) and tussock cotton-grass (*Eriophorum vaginatum*). Most individuals blossom and set fruit. Previous year's fruits were observed in several patches of *O. microcarpus*.

The habitats of *O. microcarpus* were colonized by four moss species: *Sphagnum fallax*, *Sphagnum magellanicum*, *Polytrichum strictum* and *Pleurozium schreberi*, two herbaceous plant species: *Eriophorum vaginatum* and *Drosera rotundifolia*, three dwarf shrub species: *Oxycoccus palustris*, *Andromeda polifolia* and *Vaccinium uliginosum* and juvenile forms of *Pinus sylvestris*. The fringe areas of the peat bog (in the east, north and west) are ecotones occupied by *Betula pubescens*, *Frangula alnus*, *Juniperus communis*, *Pinus sylvestris*, *Picea abies*, *Salix cinerea*, *Ledum palustre*, *Lycopodium annotinum*, *Vaccinium myrtillus*, *Carex rostrata*, *C. nigra*; *C. canescens*, *Dryopteris cathusiana* and *Scirpus sylvaticus*.

The described bog is the second confirmed habitat of the small cranberry *O. microcarpus* in the region of Warmia and Mazury. In 1998, a well-preserved population of the species from the Redykajny peatland reserve in the Olsztyn Municipal Forest was described by Dziedzic. The presence of small cranberry stands has also been reported from the Zakręt reserve in the Masurian Landscape Park and the Mechacz Wielki reserve in the Romnicka Forest Landscape Park (DĄBROWSKI et al. 1999). The above localities of *O. microcarpus* have not been confirmed recently, and they should be regularly monitored. According to Abromeit (ABROMEIT et al. 1898–1940) nearest, histological stands of this species are located in raised bogs situated in Russia (Kaliningrad oblast), near Fischhausen (presently called Rybaki), Cranz (presently called Koronowo), Friedland (presently called Frydland) and Schwentlund.

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**NEW, RARE AND NOTEWORTHY SPECIES
OF LICHENS AND LICHENICOLOUS FUNGI FROM
BIAŁOWIEŻA FOREST**

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Key words: lichenized fungi, lichenicolous fungi, new records, Białowieża National Park, Eastern Poland.

A b s t r a c t

Białowieża Forest is the best preserved piece of mixed deciduous forest on European lowland. Owing to large number of old trees and large amounts of dead wood this forest became the refuge for arboreal and xylophilous organisms. In this paper 26 particularly interesting species of lichens and lichenicolous fungi are presented. Nine of these species are very rare in Poland and seven of them are new to Białowieża Forest: *Lecanora compallens*, *Lichenomphalia umbellifera*, *Pachyphiale fagicola*, *Phaeosporobolus usneae*, *Rinodina degeliana*, *Trapelia corticola* and *Vouauxiella lichenicola*.

**NOWE, RZADKIE ORAZ INTERESUJĄCE GATUNKI POROSTÓW I GRZYBÓW
NAPOROSTOWYCH W PUŚCZY BIAŁOWIESKIEJ**

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Słowa kluczowe: grzyby zlichenizowane, grzyby naporostowe, rzadkie gatunki, nowe notowania, Białowieski Park Narodowy, Wschodnia Polska.

Abstrakt

Puszcza Białowieża jest najlepiej zachowanym fragmentem mieszanych lasów liściastych na niżu Europy. Ze względu na dużą liczbę starych drzew oraz dużą ilość martwego drewna las ten stał się ostoją dla wielu organizmów epifitycznych i ksylofitów. W artykule zaprezentowano 26 szczególnie interesujących gatunków porostów i grzybów naporostowych. Dziewięć gatunków jest bardzo rzadkich w Polsce, a siedem – nowych w Puszczy Białowieżskiej: *Lecanora compallens*, *Lichenomphalia umbellifera*, *Pachyphiale fagicola*, *Phaeosporobolus usneae*, *Rinodina degeliana*, *Trapelia corticola* i *Vouauxiella lichenicola*.

Introduction

Białowieża National Park is one of the oldest national parks in Poland. Its strictly protected core zone (4747 ha) covers the best preserved part of Białowieża Forest – the fragment of the most natural forest on lowland of Europe. It has primary character, which centuries ago extended over zone of deciduous and mixed forests. Over half of the National Park area (53%) is occupied by fertile oak-linden-hornbeam forest of the *Tilio-Carpinetum* type. Depending on habitat fertility and soil humidity woodstands of this forest type can be deciduous or mixed deciduous-spruce. Streams and river valleys are overgrown by streamside alder-ash forest *Fraxino-Alnetum* and periodically flooded land depressions by black alder bog forest *Ribeso nigri-Alnetum*. These wet deciduous forest communities cover about 15% of the National Park. The rest of the area is occupied by mixed and coniferous forests (PAWLACZYK 2009).

Białowieża National Park is characterized by a high biological diversity of animals (*Catalogue of the fauna...* 2001), plants (SOKOŁOWSKI 1995), macro-fungi (KUJAWA 2009) and lichens. In this area 268 species of lichens have been found so far (CIEŚLIŃSKI 2010).

In the last decade intensity of research on the distribution of lichens and lichenicolous fungi in Poland increased noticeably, using new methods and technologies. As a result, many new lichen species were described. Currently, in Białowieża Forest are known 450 taxons of lichens and about 40 species of lichenicolous fungi (CIEŚLIŃSKI 2003, 2010, KUKWA 2005a, KUKWA, CZARNOTA 2006, KUKWA, JABŁOŃSKA 2008, KUKWA et al. 2008, 2010, KUKWA, FLAKUS 2009).

During the recent studies conducted in Białowieża National Park several interesting species of lichens and lichenicolous fungi were found. Some of them were already known from the area, but only on single sites. Recent studies show that they are much more frequent. Among these are new species, not only for Białowieża National Park, but also for the whole complex of Białowieża Forest.

Materials and Methods

The material was collected during field studies conducted in 2010–2011 in various parts of Białowieża National Park (Białowieża Forest, NE Poland). All specimens were observed with stereomicroscope at first. The chemical analyses were carried out using thin layer chromatography (TLC) according to ORANGE et al. (2001).

In the list of species, the following abbreviations have been used: ‘BNP’ – Białowieża National Park, ‘f.s.’ – forest section. All recorded sites are located in the ATPOL grid square system (CIEŚLIŃSKI, FAŁTYNOWICZ 2003). Lichenicolous fungi are marked with an asterisk (*), saprobic fungus is marked with a cross (+), and taxa new to Białowieża Forest are marked with an exclamation point (!).

The phytosociological nomenclature of forest habitats in which species were found follows MATUSZKIEWICZ (2001), with exception of *Sphagno-Betuletum pubescentis* (SOKOŁOWSKI 1993). The nomenclature of lichen species follows SMITH et al. (2009), and lichenicolous fungi follows CZYŻEWSKA and KUKWA (2009). The specimens are deposited in the herbarium at the Jan Kochanowski University (KTC).

List of species

Cladonia norvegica Tønsberg & Holien

This species is similar to *Cladonia macilenta*. Its podetia are grey-blue, pointed at apices and often curved. Basal squamules are small, grey-blue, elongate, finely divided and rarely sparingly sorediate below (SMITH et al. 2009). Superficial red spots on basal squamules are characteristic of *Cladonia norvegica*. It grows mostly on soil and on the trunk of *Betula* and *Picea* in old, humid forest ecosystems.

The species is rarely reported from Poland. It belongs to indicators of lowland old-growth forests (CZYŻEWSKA, CIEŚLIŃSKI 2003). In the NE part of the country it has been reported from only three localities (CIEŚLIŃSKI 2003, CZYŻEWSKA et al. 2005). The species so far is known in Białowieża National Park from one site (KUKWA et al. 2008).

Specimens examined: BNP [Cg55]: on the base of trunk of *Betula pendula* in *Sphagno-Betuletum pubescentis*, f.s. no. 316B/D, 29.04.2011; on the base of trunk of *Betula pendula* in *Sphagno-Betuletum pubescentis*, f.s. no. 317C, 29.04.2011.

****Clypeococcum hypocenomycis* D. Hawksw.**

This species is quite frequent in Poland (CZYŻEWSKA, KUKWA 2009). It has also been reported in Białowieża National Park (KUKWA 2005a, KUKWA, CZARNOTA 2006).

Specimens examined: BNP [Cg55]: on thallus of *Hypocenomyce scalaris* growing on lignum in *Serratulo-Pinetum*, f.s. no. 256B, 11.05.2010; [Cg56]: on the thallus of *Hypocenomyce scalaris* growing on *Alnus glutinosa* in *Ribeso nigri-Alnetum*, f.s. no. 258B, 12.05.2010.

****Epicladonia sandstedei* (Zopf) D. Hawksw.**

This lichenicolous fungus grows on squamules and podetia of *Cladonia* species, and forming the characteristic galls containing punctate dark brown pycnidia.

This species is known from few localities in Białowieża Forest and Białowieża National Park (KUKWA, JABŁOŃSKA 2008, KUKWA et al. 2010).

Specimens examined: BNP [Cg55]: on podetia and squamules of *Cladonia coniocraea* growing on trunk of *Alnus glutinosa*, near Orlówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010; on squamules of *Cladonia coniocraea* growing on lignum in *Serratulo-Pinetum*, f.s. no. 256B, 11.05.2010.

***Fellhanera gyrophorica* Sérus., Coppins, Diederich & Scheidegger**

It is characterized by a corticolous thallus, which may also grow on corticolous mosses, forming large, and conspicuous patches up to 2–3 cm across (SÉRUSIAUX et al. 2001). Diagnostic features are always present superficial large pycnidia, pinkish to pale orange-brown. Pycnidia react with C+ red, because of production of gyrophoric acid.

The species is known so far in Białowieża National Park from several sites (CIEŚLIŃSKI 2003, SPARRIUS 2003). Recent studies indicate that it is much more common. It grows both at the younger and at the older specimens of trees. *Fellhanera gyrophorica* is also common species in the Pojezierze Olsztyńskie Lakeland (KUBIAK 2011). This species belongs to indicators of lowland old-growth forests in Poland (CZYŻEWSKA, CIEŚLIŃSKI 2003).

Specimens examined: BNP [Cg55]: on trunk of *Quercus* sp. in *Querco-Piceetum*, f.s. no. 314A, 14.05.2010; on trunks of *Alnus glutinosa*, *Fraxinus excelsior*, near Orlówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 342B, 10.05.2010; on trunk of *Tilia cordata* in *Tilio-Carpinetum*, f.s. no. 289C, 12.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 413A/C, 11.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 287C, 13.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 340D, 29.04.2011.

***Fuscidea arboricola* Coppins & Tønsberg**

So far the species is known only from northern Poland (KUKWA et al. 2008, KUBIAK et al. 2010). From area of Białowieża National Park it has been reported for the first time by SPARRIUS (2003).

Specimens examined: BNP [Cg55]: on trunk of *Alnus glutinosa*, near Hwoźna river in *Ribeso nigri-Alnetum*, f.s. no. 256A, 08.05.2010; on trunk of *Tilia cordata* in *Tilio-Carpinetum*, f.s. no. 342B, 10.05.2010.

***Lecanora compallens* Herk & Aptroot**

This sorediate lichen species forms immersed thallus with punctiform soralia, soon aggregating into irregular patches, usually covering most of the thallus. The yellow, to slightly mint-green, soredia react with K+ yellow to yellow-brown (SMITH et al. 2009).

Characteristic features of the species are the chemistry, with substances usnic acid and zeorin, detectable by TLC. *Lecanora compallens* may be confused with the morphologically similar *Lecanora expallens*, common sorediate lichen in Poland, but these two taxa can be easily distinguished by their chemistry.

The species has been distinguished recently in Poland. In northern Poland it has been reported by KOWALEWSKA and KUKWA (2003), and KUBIAK et al. (2010). The species is new to Białowieża Forest.

Specimens examined: BNP [Cg55]: on trunks of *Quercus robur*, *Tilia cordata*, *Carpinus betulus*, *Populus tremula* and *Fraxinus excelsior* in *Tilio-Carpinetum*, f.s. no. 372, 05.2009.

***Lecanora thysanophora* R.C. Harris**

This is a sorediate lichen species. Characteristic features of the species are the chemistry, with substances known as “thysanophora unknowns” detectable by TLC, and the conspicuous white, fibrous prothallus (compare KUKWA 2005b).

The species has been recorded for the first time in Białowieża National Park by KUKWA (2005b), as a result of taxonomic revision of herbarium historical materials of the *Haematomma ochroleucum* species. Recent researches conducted in Białowieża National Park indicate that the species is quite frequent both at the younger and at the older specimens of deciduous trees.

Specimens examined: BNP [Cg55]: on trunks of *Alnus glutinosa*, *Fraxinus excelsior*, near Orlówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010; on trunks of *Carpinus betulus* and *Tilia cordata* in *Tilio-Carpinetum*, f.s. no. 342B, 10.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 413A/C, 11.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 258B, 12.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 289C, 12.05.2010; on trunks of *Carpinus betulus* and *Tilia cordata* in *Tilio-Carpinetum*, f.s. no. 340D, 29.04.2011.

***Lecidella flavosorediata* (Vězda) Hertel & Leuckert**

This is common lichen in northern Poland (KUKWA et al. 2008, KUBIAK et al. 2010). From area of Białowieża Forest it has been reported for the first time from Białowieża village by KUKWA et al. (2008). New to Białowieża National Park.

Specimen examined: BNP [Cg55]: on trunk of *Tilia cordata* in *Tilio-Carpinetum*, f.s. no. 372, 05.2009.

Lepraria elobata Tønsberg

The species is rather common in Poland (KUKWA 2006). It has been reported previously in Białowieża National Park by KUKWA (2002). *Lepraria elobata* is common in Białowieża National Park and it grows on various species of trees.

Specimens examined: BNP [Cg55]: on trunk of *Fraxinus excelsior*, near Orłówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 342B, 10.05.2010; on trunks of *Carpinus betulus* and *Tilia cordata* in *Tilio-Carpinetum*, f.s. no. 287C, 13.05.2010; on trunk of *Tilia cordata* and *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 340D, 29.04.2011; [Cg56]: on trunk of *Alnus glutinosa*, near Hwoźna river in *Ribeso nigri-Alnetum*, f.s. no. 258B, 12.05.2010.

Lepraria vouauxii (Hue) R.C. Harris

The species is rather common in Poland (KUKWA 2006). It has been reported previously in Białowieża National Park by KUKWA (2002). *Lepraria vouauxii* is common in Białowieża National Park. It grows on various species of trees but prefers older specimens.

Specimens examined: BNP [Cg55]: on trunks of *Fraxinus excelsior* and *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 258B, 12.05.2010; on trunk of *Fraxinus excelsior*, near Orłówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010; in the crown of trees of *Fraxinus excelsior* and *Tilia cordata*, near Orłówka river in *Fraxino-Alnetum*, f.s. no. 340C, 09.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 342B, 10.05.2010; on trunks of *Carpinus betulus* and *Acer platanoides* in *Tilio-Carpinetum*, f.s. no. 413A/C, 11.05.2010; [Cg56]: on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 289C, 12.05.2010.

Lichenomphalia umbellifera (L.: Fr.) Redhead, Lutzoni, Moncalvo & Vigalys

The species is one of few lichens in Poland in which mycobiont is *Basidiomycota*. It is characterized by a dark green, granulate thallus *Botrydina*-type, which grows yellow-brown cap fruiting bodies (SMITH et al. 2009). *Lichenomphalia umbellifera* is known from north-eastern Poland (CZYŻEWSKA et al. 2005). It usually grows on soil, bryophytes, and on decaying stumps in shady places (FAŁTYNOWICZ 2003).

In Białowieża National Park the species has well developed fruiting bodies and grows in humid places on decaying wood. The species is new to Białowieża Forest.

Specimens examined: BNP [Cg55]: on lignum in *Sphagno-Betuletum pubescentis*, f.s. no. 316B/D, 29.04.2011; on lignum in *Sphagno-Betuletum pubescentis*, f.s. no. 317C, 29.04.2011; on lignum in *Tilio-Carpinetum*, f.s. no. 340A, 30.04.2011.

****Monodictys epilepraria* Kukwa & Diederich**

This lichenicolous fungus is very frequent in Białowieża National Park. So far it has been recorded from this area from several localities (KUKWA, CZARNOTA 2006, KUKWA, JABŁOŃSKA 2008).

Specimens examined: BNP [Cg55]: on thallus of *Lepraria incana* growing on trunk of *Quercus robur* in *Tilio-Carpinetum*, f.s. no. 225, 08.05.2010; on thallus of *Lepraria incana* growing on trunks of *Alnus glutinosa* and *Fraxinus excelsior*, near Orłówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010; on thallus of *Lepraria incana* growing on lignum in *Serratulo-Pinetum*, f.s. no. 256B, 11.05.2010; on thallus of *Lepraria incana* growing on trunk of *Picea abies* in *Tilio-Carpinetum*, f.s. no. 342B, 10.05.2010; on thallus of *Lepraria incana* growing on trunks of *Carpinus betulus* and *Tilia cordata* in *Tilio-Carpinetum*, f.s. no. 287C, 13.05.2010; on thallus of *Lepraria incana* growing on trunk of *Quercus robur* in *Querco-Piceetum*, f.s. no. 314A, 14.05.2010; [Cg56]: on thallus of *Lepraria incana* growing on trunk of *Alnus glutinosa*, near Hwoźna river in *Ribeso nigri-Alnetum*, f.s. no. 258B, 12.05.2010; on thallus of *Lepraria incana* growing on trunks of *Fraxinus excelsior* and *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 258B, 12.05.2010.

!*Pachyphiale fagicola* (Hepp) Zwackh

This species is characterized by a crustose, superficial but thin, green-grey thallus and often more crowded darker brown apothecia. It is easily identified by the presence of *Trentepohlia* photobiont, and the shape and size of ascospores (SMITH et al. 2009).

In the study area *Pachyphiale fagicola* was growing in the company of: *Hypogymnia physodes*, *Parmelia sulcata*, *Ropalospora viridis*, *Physcia tenella*, *Ph. stellaris*, *Ph. adscendens* and *Melanelixia fuliginosa* subsp. *glabratula*. This is a very rare species in Poland, which is placed in the category Vulnerable (VU) at "Polish Red List" (CIEŚLIŃSKI et al. 2006). The species is new to Białowieża National Park and to Białowieża Forest.

Specimen examined: BNP [Cg55]: in the crown of tree of *Fraxinus excelsior*, near Orłówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010.

****Phaeopyxis punctum* (A. Massal.) Rambold, Triebel & Coppins**

The species forms black apothecia on squamules of *Cladonia* species, and produces non-septate ascospores.

The species has already been reported in Białowieża National Park from a few localities (KUKWA 2005a, KUKWA, CZARNOTA 2006, KUKWA et al. 2010).

Specimens examined: BNP [Cg55]: on squamules of *Cladonia coniocraea* and *Cladonia* sp. growing on lignum in *Serratulo-Pinetum*, f.s. no. 256B, 11.05.2010.

!**Phaeosporobolus usneae* D. Hawksw. & Hafellner

The specimens agree with the description by HAWKSWORTH and HAFELLNER (1986), and produces conidia 6–12 celled, overall 15–25 µm diam, and individual cell is 4–6 µm diam. In examined material are also conidia deviated in size, up to 10 µm in diam, or number of cells, up to 30 cells.

So far only *Phaeosporobolus alpinus* has been reported from Białowieża National Park (KUKWA et al. 2010). In Poland *Phaeosporobolus usneae* has been found only from the Świętokrzyskie Mts., but from the historical herbarium materials of lichens (ŁUBEK 2009). The species is new to Białowieża National Park and to Białowieża Forest.

Specimens examined: BNP [Cg55]: on thalli of *Bryoria fuscescens* and *Evernia prunastri* growing in the crown of tree of *Quercus robur* in *Quercopiceetum*, f.s. no. 314A, 14.05.2010; on thalli of *Evernia prunastri* and *Ramalina farinacea* growing in the crown of tree of *Fraxinus excelsior*, near Orlówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010; on thalli of *Usnea filipendula* and *Ramalina farinacea* growing in the crown of tree of *Tilia cordata*, near Orlówka river in *Fraxino-Alnetum*, f.s. no. 340C, 09.05.2010.

**Phoma cytospora* (Vouaux) D. Hawksw.

The species has already been recorded in Białowieża National Park from one locality (KUKWA, FLAKUS 2009).

Specimens examined: BNP [Cg55]: on thallus of *Hypogymnia physodes* growing on lignum in *Serratulo-Pinetum*, f.s. no. 256B, 11.05.2010; [Cg56]: on thallus of *Hypogymnia physodes* growing on trunk of *Alnus glutinosa*, near Hwoźna river in *Ribeso nigri-Alnetum*, f.s. no. 258B, 12.05.2010.

Reichlingia leopoldii Diederich & Scheid.

The species previously described as a lichenicolous fungus growing on sterile powdery lichens, but later considered as lichenized fungus (*Hyphomycetes*) (APTROOT, SIPMAN 2001). From the north-eastern Poland *Reichlingia leopoldii* is known from Augustowska Forest and Biebrzański National Park (CZYŻEWSKA et al. 2005), and Pojezierze Olsztyńskie Lakeland (KUBIAK 2011). It is also known from Białowieża National Park (MOTIEJŪNAITĖ, CZYŻEWSKA 2008).

Specimens examined: BNP [Cg55]: on trunk of *Alnus glutinosa* in *Ribeso nigri-Alnetum*, f.s. no. 256A, near Hwoźna river, 08.05.2010; on trunks of *Fraxinus excelsior* and *Alnus glutinosa*, near Orlówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 413A/C, 11.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 342B, 10.05.2010; [Cg56]: on trunk of *Fraxinus excelsior* in *Tilio-Carpinetum*, f.s. no. 258B, 12.05.2010.

!Rinodina degeliana Coppins

The species is characterized by a whitish grey to greenish grey thallus, growing irregularly, and composed of compact or scattered areoles. Soralia of this species forming on the underside of raised areole margins, and they are concolourous with the thallus or lighter. Soralia react with PD+ intensive yellow, and with K+ yellow (SMITH et al. 2009).

Rinodina degeliana is currently known from 11 localities in northern and central parts of Poland (KUBIAK 2010). This is the first record of the species in whole Białowieża Forest.

Specimen examined: BNP [Cg55]: in the crown of tree of *Fraxinus excelsior*, near Orłówka river in *Fraxino-Alnetum*, f.s. no. 340C, 09.05.2010.

Ropalospora viridis (Tønsberg) Tønsberg

This sterile, crustose species is very similar to *Fuscidea pusilla*, but it is easily to diagnose using TLC, by the presence of perlatolic acid as major substance (SMITH et al. 2009).

The species has already been reported on a few localities in Białowieża National Park by CIEŚLIŃSKI (2003) and SPARRIUS (2003).

Specimens examined: BNP [Cg55]: on trunks of *Tilia cordata* and *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 342B, 10.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 289C, 12.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 287C, 13.05.2010; on trunk of *Carpinus betulus* in *Tilio-Carpinetum*, f.s. no. 369A, 14.05.2010.

+Sarea resinae (Fr.) Kunze

This saprobic fungus is rarely reported from Poland (FAŁTYNOWICZ 2003, KOSSOWSKA et al. 2007). In Białowieża National Park it is known only recently on two sites (KUKWA et al. 2008).

Specimen examined: BNP [Cg55]: on resine of *Picea abies* in *Serratulo-Pinetum*, f.s. no. 256B, 11.05.2010.

Sclerophora pallida (Pers.) Y.J. Yeo & Spooner

The species is distinguished, from other species of the *Sclerophora* genus, by the smaller size and pale yellow or grey colour of the apothecia, and presence on young apothecia of yellow pruina (SMITH et al. 2009).

This is a very rare species in Poland, which is placed in the category Critically Endangered (CR) at "Polish Red List" (CIEŚLIŃSKI et al. 2006). The species has been previously reported in Białowieża National Park only from one site (CIEŚLIŃSKI 2003). During the present study it has been found on old *Fraxinus excelsior*, where it was growing in the company of *Bacidia rubella* and *Lepraria vouauxii*.

Specimen examined: BNP [Cg55]: in the crown of tree of *Fraxinus excelsior*, near Orłówka river in *Fraxino-Alnetum*, f.s. no. 340C, 09.05.2010.

!Trapelia corticola Coppins & P. James

This is usually sterile species. It is characterized by a thin, effuse, green to greenish brown thallus consisting of scattered areoles. Soralia are greenish, usually punctiform and markedly convex. Like other species of genus *Trapelia*, it reacts with C+ red (SMITH et al. 2009).

In Poland the species has been reported mainly from southern parts of Poland (CZARNOŃ, KUKWA 2009), and only from one locality from northern Poland (ZALEWSKA 1998). It grows mostly on decaying wood, logs and stumps, and on the base of trees. The species prefers humid natural forest ecosystems. In some countries it is considered to be an indicator of ecological continuity of woodlands (COPPINS, COPPINS 2002).

Specimens found in Białowieża National Park were growing in humid places in fertile oak-linden-hornbeam forest, on the log in the company of other species: *Absconditella lignicola*, *Cladonia coniocraea*, *Placynthiella dasaea*, *P. icmalea* and *Micarea prasina*. These are the first known localities in Białowieża Forest.

Specimens examined: BNP [Cg55]: on log in *Tilio-Carpinetum calamagrostietosum*, f.s. no. 316/B, 29.04.2011; on log in *Tilio-Carpinetum*, f.s. no. 340D, 29.04.2011.

****Tremella cladoniae*** Diederich & M. S. Christ.

The species has already been reported in Białowieża National Park from a few localities (KUKWA 2005a, KUKWA, FLAKUS 2009).

Specimens examined: BNP [Cg55]: on squamules of *Cladonia* sp. growing on trunk of *Alnus glutinosa*, near Orlówka river in *Fraxino-Alnetum*, f.s. no. 314C, 09.05.2010; on squamules of *Cladonia* sp. growing on lignum in *Serratulo-Pinetum*, f.s. no. 256B, 11.05.2010.

****Tremella hypogymniae*** Diederich & M. S. Christ.

The species has already been reported in Białowieża National Park from a few localities (KUKWA 2005a, KUKWA, FLAKUS 2009).

Specimen examined: BNP [Cg55]: on thallus of *Hypogymnia physodes* growing on trunk of *Tilia cordata* in *Tilio-Carpinetum*, f.s. no. 287C, 13.05.2010.

****Tremella lichenicola*** Diederich

The species has already been recorded in Białowieża National Park from one locality (KUKWA 2005a).

Specimen examined: BNP [Cg55]: on thallus of *Mycoblastus fucatus* growing on trunk of *Alnus glutinosa*, near Hwoźna river in *Ribeso nigri-Alnetum*, f.s. no. 256A, 08.05.2010.

!Vouauxiella lichenicola*** (Linds.) Petr. & Syd.

This species is scattered in Poland (FAŁTYNOWICZ 2003). It grows on apothecia and on thalli of different species of *Lecanora* genus. In Białowieża National Park it was growing on thallus of *Lecanora pulicaris*. The species is new to Białowieża Forest.

Specimen examined: BNP [Cg56]: on thallus of *Lecanora pulicaris* growing on twigs of *Alnus glutinosa*, near Hwoźna river in Ribeso nigri-Alnetum, f.s. no. 258B, 12.05.2010.

Conclusions

Intensive lichenological studies carried in different regions of Poland, including Białowieża Forest, provide new data on the occurrence of lichens and lichenicolous fungi. Each new and verified site is very important for the knowledge on habitat preferences and distribution of the species in Poland.

Despite the long period of lichenological studies in Białowieża Forest the knowledge about distribution and ecology of rare lichen species is still unsatisfactory. In the present study we report five species of lichens new for biota of this forest complex. Most of them are rare species, or of inconspicuous size, and they are often overlooked in the field, such as: *Lichenomphalia umbellifera*, *Pachyphiale fagicola*, *Rinodina degeliana* and *Trapelia corticola*. These species merit particular attention because of its rarity in Poland. Given the present data, known number of lichen species in Białowieża National Park (CIEŚLIŃSKI 2010) increased to 274, and in Białowieża Forest – to 455.

Lichenicolous fungi are poorly explored in Białowieża Forest. We report in this paper two new species of this group: *Phaeosporobolus usneae* and *Vouauxiella lichenicola*, which is an important supplement to the knowledge of their species richness and occurrence in the region. Given all previous data in Białowieża National Park so far have been recorded 30 species of lichenicolous fungi.

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HYPERPARASITES OF *ERYSIPHALES* FUNGI IN THE URBAN ENVIRONMENT

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Key words: *Ampelomyces*, hyperparasites, fungicolous fungi, powdery mildew, transport pollution effects, anthropopressure.

Abstract

This manuscript presents data on the occurrence of hyperparasitic fungi colonizing the mycelium of selected species of *Erysiphales*: *Erysiphe alphitoides*, *E. hypophylla*, *E. palczewskii*, *Golovinomyces sordidus*, *Podosphaera fusca* and *Sawadaea tulasnei* in the urban environment. In the paper the effect of hyperparasites on the development of fungal hosts at diversified level of transport pollution is emphasized. Over a three-years experiment, the presence of hyperparasites was confirmed on all analyzed *Erysiphales* species, with prevailing species from the genus *Ampelomyces*. The representatives of other genera: *Alternaria*, *Aureobasidium*, *Cladosporium*, *Stemphylium* and *Tripospermum* were also observed on mycelium of *E. alphitoides* and *E. palczewskii*. The hyperparasites occurred only on stations situated at the main roads were found not to affect the extent of plant infection by fungi of the order *Erysiphales*, but reduced the number of chasmothecia.

NADPASOŻYTY GRZYBÓW Z RZĘDU *ERYSIPHALES* W ŚRODOWISKU MIEJSKIM

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Słowa kluczowe: *Ampelomyces*, nadpasożyty, mączniaki prawdziwe, zanieczyszczenia komunikacyjne, antropopresja.

Abstrakt

Praca dotyczy występowania nadpasożytów zasiedlających plechę wybranych grzybów z rzędu *Erysiphales*: *Erysiphe alphitoides*, *E. hypophylla*, *E. palczewskii*, *Golovinomyces sordidus*, *Podosphaera fusca* i *Sawadaea tulasnei* w warunkach miejskich. Oceniono wpływ nadpasożytów na rozwój grzybów-gospodarzy, ze zwróceniem szczególnej uwagi na zanieczyszczenia komunikacyjne. Podczas trzyletnich badań na wszystkich analizowanych gatunkach *Erysiphales* stwierdzono obecność nadpasożytów z przewagą gatunków z rodzaju *Ampelomyces*. Najwyższe porażenie mączniaków prawdziwych odnotowano na stanowiskach znajdujących się przy głównych szlakach komunikacyjnych. Na grzybni *E. alphitoides* i *E. palczewskii* odnotowano również występowanie grzybów z rodzajów *Alternaria*, *Aureobasidium*, *Cladosporium*, *Stemphylium* i *Tripodermium*, które występowały tylko w punktach zlokalizowanych do 50 m i 100 m.

Nadpasożyty nie wpływały na średni stopień porażenia roślin mączniakiem prawdziwym, natomiast redukowały liczbę owocników.

Introduction

Hyperparasitism is a phenomenon that plays a significant though underestimated role in the dynamics of fungi population in the natural environment (JEFFRIES 1995). However, studies on hyperparasites are mainly focused on the search for microorganisms effective in the biological fight against plant pathogens.

Results of many research addressing economically important phytopathogens – powdery mildews (*Erysiphales*), report on the occurrence on the mycelium of numerous antagonistic fungi, including: *Tilletiopsis* sp. Derx (URQUHART et al. 1994), *Sporothrix flocculosa* Traquair, Shaw & Jarvis (ELAD 1995), *Lecanicillium lecanii* (Zimm.) Zare & W. Gams [= *Verticillium lecanii* (Zimm.) Viégas] (ASKARY et al. 1998), *Phoma glomerata* (Corda) Wollenw. & Hochapfel (SULLIVAN, WHITE 2000), *Acremonium alternatum* Link (ROMERO et al. 2002), *Cladosporium uredinicola* Speg. (HEUCHERT et al. 2005) or fungi of the genus *Ampelomyces* Ces. ex. Schltdl. The latter, acknowledged to be endoparasites, have been recognized as the major antagonists of powdery mildews and thus have been the most frequent subject in this field of interest. Fungi of the genus *Ampelomyces* are widespread worldwide and were recorded on 66 host species of fungi, belonging to 9 genera (KISS et al. 2004). The ability of these antagonistic fungi to colonize the mycelium of many *Erysiphales* species, to restrict their sporulation as well as to inhibit the formation and development of chasmothecia, has been successfully used in practice. A fine example in this case is a biofungicide AQ10, applied in plant protection against powdery mildews (KISS et al. 2004).

Polish literature provides quite comprehensive information on the occurrence of fungi of the genus *Ampelomyces* on the powdery mildews. So far, the hyperparasites were recorded on 44 species of *Erysiphales* from 6 genera: *Erysiphe*, *Microsphaera*, *Podosphaera*, *Sphaerotheca*, *Uncinula* oraz *Oidium*

(MUŁENKO et al. 2008, erysiphalean nomenclature following Braun 1995). Most papers documented mainly the presence of *Ampelomyces* pycnidia on mycelium of different host species and occasionally reported either symptoms of infestation or the infected morphological structures. However, far too little attention has been paid to the ecology and ecophysiology of hyperparasites developing under natural conditions (ADAMSKA, CZERNIAWSKA 2010, CZERNIAWSKA 2005, CZERNIAWSKA et al. 2000, CZERNIAWSKA, ADAMSKA 2007, MUŁENKO, WOJDYŁO 2002, RUSZKIEWICZ-MICHALSKA 2006). Especially their effect on the development of host fungi in the natural environment is poorly understood (ADAMSKA, CZERNIAWSKA 2010, KISS 1997, 1998, MAJEWSKI 1971, RANKOVIČ 1997). The available information about an occurrence of the hyperparasites in urbicoenoses is insufficient (CZERNIAWSKA et al. 2011, MADEJ, ANTOSZCZYSZYN 1965, RUSZKIEWICZ-MICHALSKA 2010, RUSZKIEWICZ-MICHALSKA, MICHALSKI 2005, SCHMIDT, SCHOLLER 2002, SUCHARZEWSKA, DYNOWSKA 2005, SUCHARZEWSKA et al. 2011), especially the one covering the impact on the development of chasmothecia of *Erysiphales* members. Research works addressing this issue are usually focused on particular, economically important pathogens, for instance powdery mildew of grape – *Erysiphe necator* Schwein [= *Uncinula necator* (Schwein.) Burrill] (ANGELI et al. 2009, FALK et al. 1995, GADOURY, PEARSON 1988, FÜZI 2003).

This paper presents a part of results of study on life strategies of selected *Erysiphales* species carried out in the years 2000-2002 in the urban habitats diversified according to the level of transport pollution (SUCHARZEWSKA 2009, 2010, SUCHARZEWSKA, DYNOWSKA 2005). The aim of this study was to analyze the occurrence of hyperparasites and their effect on the development of selected fungi hosts in the above mentioned conditions.

Material and Methods

Six species of powdery mildews were selected for the study: *Erysiphe alphitoides* (Griffon et Maubl.) U. Braun et S. Takam. and *E. hypophylla* (Nevod.) U. Braun et Cunningt. on *Quercus robur* L., *E. palczewskii* (Jacz.) U. Braun et S. Takam. on *Caragana arborescens* Lam., *Golovinomyces sor-didus* (L. Junell) V.P. Heluta on *Plantago major* L., *Podosphaera fusca* (Fr.) U. Braun et Shishkoff on *Taraxacum officinale* F. H. Wigg. and *Sawadaea tulasnei* (Fuckel) Homma on *Acer platanoides* L.

The experiment was carried out in three vegetative seasons (2000–2002) in the city of Olsztyn and its vicinity north-eastern Poland. Research sites (175 localities), were situated at a range of distances from the main transport routes: up to 50 m, up to 100 m, up to 300 m, and >300 m used as a control

station. These distances were selected based on a study by LORENC-PLUCIŃSKA and BYCZYŃSKA (1997), which shows that the greatest value of exhaust gas concentration is recorded at a distance of 30–50 m away from the road and maintains at a level of up to 30%. The exhaust gas level drops to 10% at 200 m away from transport routes.

The material in the form of infested plants was collected every three weeks throughout the study period. In the case of *A. platanoides*, *C. arborescens* and *Q. robur* – single sample was defined as 25 leaves collected randomly from one host plant. In the case of *P. major* and *T. officinale* – single sample consisted of 10 leaves collected randomly from a 1 m² area covered with the host plant.

At the laboratory, the collected material was subjected to macro- and microscopic analyses:

1. To calculate the disease index (R) of hosts infected by fungi McKinney's formula (5° scale) – (MCKINNEY 1923) was used:

$$R = \frac{\Sigma (ab) \cdot 100\%}{N \cdot 4}$$

R_E – denotes disease index of plants infested by *Erysiphales* fungi while (R_A and R_H) concern the disease index of powdery mildews' mycelium infested by *Ampelomyces* species (exclusively) and by other hyperparasites (all species treated together), respectively.

$\Sigma (ab) \cdot 100\%$ – the sum of products obtained by multiplying the number of analyzed: organs of plants (a) by the degree of infection with parasite (b); in the case of hyperparasites the degree of infection is referred to the number of plants infected by powdery mildew host.

N – the total number of plant organs examined / the total number of organs of plants infected by powdery mildew,

4 – the highest degree of infection.

Degree of infestation was estimated according to a five-grade scale: 0 – no infection; 1 – up to 10% of leaf area; 2 – 11–25%; 3 – 26–50%; 4 – 51–100%.

2. Analyses were also conducted for the effect of hyperparasites on the number and development of chasmothecia:

a) in twenty samples selected at random from each *Erysiphales* species that proved to be hyperparasitized; the number of chasmothecia was set on randomly selected 1 cm² area of each leaf covered by powdery mildew mycelium in two variants: 1) with hyperparasites and 2) without them.

b) 10 mature chasmothecia (with dark peridium) selected at random from each sample were analyzed for:

- developmental stage of ascocarp appendages estimated according to a three-grade scale: **0** – lack of appendages, **I** – not fully developed and **II** – fully developed,
- the presence of asci and ascospores.

Olympus SZX9 stereomicroscope and Olympus BX41 light microscope were used to observe a mycelium, chasmothecia and hyperparasites' structures (pycnidia, conidiophores and conidia).

The final values provided in the Results section were computed based on an arithmetic mean determined for each fungi species, on a specified host plant. In the case of *E. albitoides* and *E. hypophylla*, the mean degree of infection was calculated as a total value for these species due to their co-occurrence (SUCHARZEWSKA 2009).

Fungi were determined using keys by BRAUN (1995), SALATA (1985), ELLIS and ELLIS (1985) and ELLIS (1971). The erysiphalean nomenclature was accepted after BRAUN and TAKAMATSU (2000) as well as BRAUN et al. (2003); other fungal names follow Index Fungorum (www.indexfungorum.org). The nomenclature of host plants was adopted after MIREK et al. (2002).

Results

In total, 867 samples of powdery mildews were collected. In 433 samples (50%) fungi of the genera (sensu lato treatment): *Alternaria* Nees, *Ampelomyces* Ces. ex Schltdl., *Aureobasidium* Viala, et G. Boyer, *Cladosporium* Link, *Stemphylium* Wallr. and *Tripospermum* Speg. were recorded.

Samples with fungi of the genus *Ampelomyces* had the highest percentage (92%) among the samples containing hyperparasites. They colonized powdery mildews' mycelium to a different extent. *Ampelomyces* fungi were dominant in samples of *Golovinomyces sordidus* – 48% and *Podosphaera fusca* – 45%. In the case of the other species, the percentage of *Ampelomyces* hyperparasites in samples ranged from: 25% in *E. palczewskii*, 19% in *S. tulasnei* and 15% in *E. hypophylla* to 2% in *E. albitoides*. The presence of the *Ampelomyces* fungi was observed not to affect the mean index of plants infection by *Erysiphales* species (R_E). In all cases, R_E values were higher than the disease indexes of powdery mildews mycelium infested by the analyzed hyperparasites (R_A and R_H) – Table 1. The highest disease index of mycelium (R_A) was noted at stations located up to 50 m and 100 m away from polluters. In contrast, at the control station and others located up to 300 m away, no hyperparasite or a low values of diseases index were observed.

Table 1
Mean index of infestation of host plants by powdery mildews and mean index of infestation of *Erysiphales* species by hyperparasites at various distances from roads

Species of <i>Erysiphales</i>		Distances from roads			
		up to 50 m	up to 100 m	up to 300 m	>300 m
<i>E. alphitoides</i>	R_E	44	52	36	34
	R_A	0	0	0	0
	R_H	34	22	0	0
<i>E. hypophylla</i>	R_E	44	52	36	34
	R_A	2	1	1	1
	R_H	0	0	0	0
<i>E. palczewskii</i>	R_E	55	68	47	14
	R_A	12	20	0	0
	R_H	25	0	0	0
<i>G. sordidus</i>	R_E	47	46	31	27
	R_A	42	31	18	17
	R_H	0	0	0	0
<i>P. fusca</i>	R_E	45	43	17	7
	R_A	39	38	7	3
	R_H	0	0	0	0
<i>S. tulasnei</i>	R_E	23	20	13	1
	R_A	16	19	1	0
	R_H	0	0	0	0

Explanations:

R_E – Mean index of host plants infestation (%) with powdery mildews; R_A – Mean index of powdery mildew infestation (%) with *Ampelomyces* species; R_H – Mean index of powdery mildew infestation (%) with hyperparasites from other genera.

Dark colored mycelium and conidiomata of the fungi from genera: *Alternaria*, *Aureobasidium*, *Cladosporium*, *Stemphylium* and *Tripospermum* were developed on the mycelium and chasmothecia of their host fungi. Hyperparasite' mycelium was also visible inside of appendages of *E. palczewskii* chasmothecia. In total, those hyperparasites constituted about 8% of all samples containing hyperparasites, predominating in samples of *Erysiphe palczewskii* (23%) and *E. alphitoides* (18%). In the case of the other *Erysiphales* species, the fungi occurred sporadically. Very often species of *Alternaria*, *Aureobasidium*, *Cladosporium*, *Stemphylium* and *Tripospermum* co-occurred on the host and were noted at stations located up to 50 and 100 m away from transport routes (Table 1).

The hyperparasites were found to affect the number of mature chasmothecia of powdery mildews. *Ampelomyces* species were observed to reduce the number of chasmothecia 7 times in case of *E. hypophylla* and 6 times in *E. palczewskii*, 5 times in *G. sordidus* and *P. fusca* as well as 3 times in *S. tulasnei* (Figure 1). Over 4-fold reduction in the mean number of chasmothecia was noted in *E. palczewskii*, and above 5-fold in *E. alphitoides* which mycelium was colonized by the other hyperparasites (Figure 1).

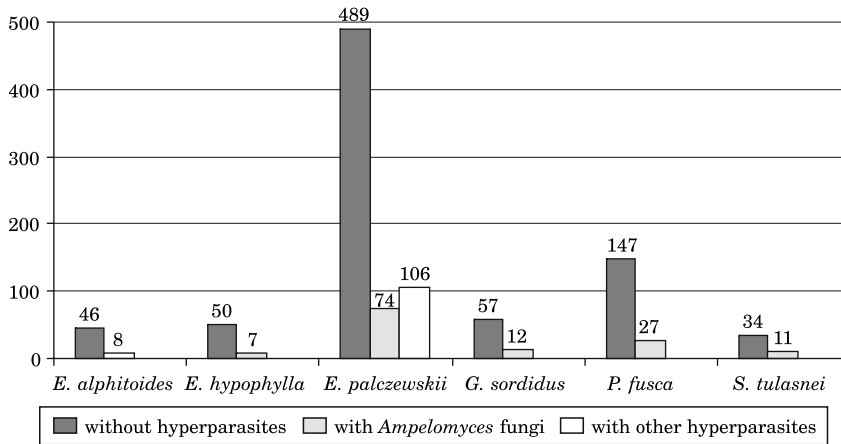


Fig. 1. The mean number of powdery mildews' ascocarps on 1 cm² of leaf surface without and with hyperparasites

The comparative analysis of mature chasmothecia morphology produced by infected and non-infected mycelium demonstrated no developmental disorders in the case of *Ampelomyces* fungi: mature chasmothecia had well developed appendages, asci and spores. In contrast, a negative effect on their development was observed in infection of *E. palczewskii* mycelium caused by other hyperparasitic fungi. A considerably lower percentage (32%) of appendages at II developmental stage was noted as compared to chasmothecia of the non-infected mycelium (91%). In addition, chasmothecia without asci and ascospores were more numerous present (11% of all samples) at the mycelium infected by parasites than at non-infected (1%).

Discussion

There is no group of living organisms that could avoid parasites (COMBES 1999). In the reported study, the presence of hyperparasites was recorded on mycelium of all of analyzed species of phytopathogenic fungi. Hyperparasites were most frequently the representatives of the genus *Ampelomyces*, widely regarded as the major antagonist of powdery mildews. Only the sporadic occurrence of *Ampelomyces* fungi on powdery mildew mycelium was reported in Polish literature covering investigations conducted in national parks and arboreta (ADAMSKA et al. 1999; CZERNIAWSKA et. al. 2000, MUŁENKO, WOJDYŁO 2002). MAJEWSKI (1971) suggested that *Ampelomyces quisqualis* Ces. is linked with the anthropogenic environment. MAJEWSKI (1971) based his opinion on the data and experience gathered during in-depth mycosociological research he

conducted in the Białowieża National Park. Despite intensive search those fungi was not found on powdery mildews in natural phytocoenosis. However *Ampelomyces* hyperparasites were often noted on numerous powdery mildew species in the cities of Szczecin and Łódź (MADEJ, ANTOSZCZYSZYN 1965, RUSZKIEWICZ-MICHALSKA, MICHALSKI 2005, RUSZKIEWICZ-MICHALSKA 2010). Our study confirms frequent occurrence of the hyperparasites in the urban environment.

The highest degree of infection by *Ampelomyces* hyperparasites proved at stations located closed to (50 m and 100 m) the main city transport routes resulted from a high disease index of the plants. In addition, *Ampelomyces* species occurred sporadically at stations with a low value of the index of plants infection. Our study demonstrates also that the mean index of host plants infection by powdery mildews was always higher than the mean index of *Erysiphales* mycelium infection by *Ampelomyces*. It indicates that the spread of their hosts in urban environment is not affected by the hyperparasites too drastically. The same conclusion has been drawn from the results of another of our studies dealing with hyperparasites of numerous *Erysiphales* species in urban environment (SUCHARZEWSKA et al. 2011).

Currently observed differences in the preference of *Ampelomyces* fungi to some species of host fungi may, presumably, result from the presumed genetical heterogeneity of the genus *Ampelomyces* considered by some authors to be rather a complex of taxa than a single species (KISS, VAJNA 1995, KISS, NAKASONE 1998). The differences in the mode of occurrence of those hyperparasites and disparate values of disease index were also observed by KISS (1998).

Results of the reported study addressing the effect of *Ampelomyces* fungi on the number of produced chasmothecia correspond with findings of GADOURY et al. (1991), FÜZI (2003) and ANGELI et al. (2009). The reduction in the number of chasmothecia recorded for all representatives of *Erysiphales* results from colonizing young chasmothecia and transforming them into own reproductive structures of the hyperparasite.

Apart from *Ampelomyces* fungi around forty of other natural antagonists of *Erysiphales* representatives are known (KISS 2003). Under laboratory conditions, some of these fungi (e.g. *Acremonium alternatum* and especially *Lecanicillium lecanii*) were observed to inhibit the growth of *Podosphaera fusca* [= *Sphaerotheca fusca* (Fr.) Blumer] more effectively than the *Ampelomyces* fungi (ROMERO et al. 2002). Various other fungi, e.g. species of *Alternaria*, *Botrytis*, *Candida*, *Cephalosporium*, *Cladosporium* and *Trichothecium* are commonly found within the mycelial colonies of the powdery mildews (BRAUN 1995). KISS (2003) reported colonization of chasmothecia of *Phyllactinia* spp. and reduction of their number by fungi from the genus

Cladosporium. The latter may occur on the leaf area together with other fungi such as *Aureobasidium pullulans*, forming a complex called 'Fumago vagans' (ELLIS and ELLIS 1985). In Poland representatives of the genus *Alternaria* and *Cladosporium* on mycelium of some *Erysiphales* species were observed by ADAMSKA and CZERNIAWSKA (2010) while PIĄTEK (2003) reported the occurrence of *Alternaria* sp. on *Phyllactinia fraxini* (D.C.) Fuss. On the same host species *Cladosporium uredinicola* and *Phoma glomerata* have been recently reported (RUSZKIEWICZ-MICHALSKA 2010). Complex 'Fumago vagans' was observed on the mycelium of *S. tulasnei*, in our study while no *Phoma* species was confirmed in the material studied.

The presence of anamorphic fungi of genera *Alternaria*, *Aureobasidium*, *Cladosporium*, *Stemphylium* and *Tripospermum* on the mycelium of *Erysiphe alphitoides* and *E. palczewskii* may be due to a high level of traffic pollution. Polish research on the occurrence of fungi in a variety of stressful conditions provide interesting information about a burst of activity of some phytopathogens. The intense development of fungi from the genus *Alternaria*, *Aureobasidium* and others on plants of industrially polluted sites was reported by DOMAŃSKI et al. (1977). Those fungi are referred to as saprotrophs that may infect of plants debilitated due to the detrimental effects of the environment. In our study those fungi disrupted development of *E. palczewskii* chasmothecia, that confirms their possibly negative impact. Presence of hyphae of antagonists inside of appendages of *E. palczewskii* chasmothecia points to internal interference – that according to JEFFRIES (1995) indicates invasive necrotrophy. A similar phenomenon was observed by HOCH and PROVIDENTI (1979) in *Sphaerotheca fuliginea* attacked by *Tilletiopsis* sp. This hyperparasite surrounded the hyphae of host causing their necrosis and penetrating them.

In summary, the reported study confirms the phenomenon of hyperparasitism on fungi of the order *Erysiphales* as widespread in the urban environment. The predomination of antagonistic fungi is found to be linked directly with the high degree of plants infestation by powdery mildews. This seems to enhance the development of antagonistic fungi which, in turn, significantly affect the powdery mildews population dynamics by reducing the number of vital chasmothecia.

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**BIOMONITORING IN THE REVITALIZATION
OF THE UPPER WKRA RIVER (NIDZICA COUNTY)
ON THE BASIS OF BENTHIC
MACROINVERTEBRATES**

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Key words: Wkra River, bioindication, BMWP-PL index, benthic invertebrates, revitalization.

Abstract

Water quality of the Nida River (NE Poland) was evaluated using the BMWP-PL biotic and diversity indices. Revitalization works were undertaken in the river during the study. Benthic macroinvertebrate samples were collected from seven different sites. Twenty-four taxa in total were recorded. Most of the studied sites (59%) were ranked into water quality class III, while 35% of sites were of poor water quality (class IV). Good water quality (class II) was found at only one site (the upper Nida section). The revitalization works did not significantly change values of the BMWP-PL index at the studied sites.

**BIOMONITORING REWITALIZOWANEGO ODCINKA GÓRNEJ WKRY
W GMINIE NIDZICA NA PODSTAWIE MAKROBEZKRĘGOWCÓW BENTOSOWYCH**

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Słowa kluczowe: Wkra, bioindykacja, indeks BMWP-PL, bezkręgowce bentosowe, rewitalizacja.

Abstrakt

Używając indeksu biotycznego BMWP-PL oraz indeksu różnorodności, oceniono jakość wód rzeki Nidy (północno-wschodnia Polska). W czasie trwania badań rzekę rewitalizowano. Na siedmiu stanowiskach pobierano próby makrobezkręgowców bentosowych. Zanotowano obecność 24 taksonów. Większość badanych stanowisk (59%) zakwalifikowano do III klasy czystości wód, natomiast 35% stanowisk miało złą ich jakość (IV klasa). Tylko na jednym stanowisku stwierdzono dobrą jakość wód (II klasa). Wartości indeksu BMWP-PL otrzymane dla prób przed urozmaicaniem dna i po tym zabiegu rewitalizacyjnym nie wykazały statystycznie istotnych różnic.

Introduction

In the 20th century, due to the industrial development, rivers and streams were altered by sewage pollution, regulation and straightening for land reclamation, and agricultural use or channelization for flood protection. Similar modifications have been performed on the Nida River. Its straightening caused reduction of invertebrate diversity in lotic habitats. Such modifications are sometimes considered more destructive for stream biodiversity than chemical pollution (ROGERS et al. 2002). Moreover, the industrial development and antiquated water treatment systems degraded water quality of the Wkra River in the 1960–90s. These processes reduced taxonomic diversity of the fish fauna to only 1–3 species in the upper part of the Wkra River in the 1960s (PENCZAK et al. 2001).

The Nida River and its banks underwent revitalization, which included planting trees and bushes, improving the riverbed heterogeneity and fish stockings aimed at raising the riverine ichthyofauna diversity. The main goal of the study is to present the recovery of the ecological function of the Wkra River by the reconstruction of its ecological corridors in the valley and to investigate potential effects of the diversity of fauna and flora (SKRZYPCZAK et al. 2009). The revitalization of a river can be achieved through various measures, which are carried out on large rivers (DAVIS 1997), small rivers and streams (WOOLSEY et al. 2006, KLIVAR 2000).

The European Water Framework Directive recognises the need to combine physicochemical, biological and geomorphological parameters for routine monitoring in freshwaters. Macroinvertebrates are the most frequently used group of organisms for assessment of the water quality in lotic habitats worldwide (ROSENBERG and RESH 1993, HERING et al. 2004). The use of these organisms as bioindicators has more advantages than those based on diatoms, fish or aquatic vegetation (METCALFE 1989). Biotic indices are the combined results of quantitative measurements of species diversity and qualitative information on the ecological sensitivity of individual taxa (CZERNAWSKA-KUSZA 2005).

The monitoring of the Nida River was conducted by the Voivodship Inspectorate for Environmental Protection in Olsztyn, and was based on physicochemical and biological features, chlorophyll *a* (*Raport...* 1999, *Raport...* 2009, 2010) and colonies of diatoms (*Raport...* 2011). The ecomorphological evaluation of river sections different in the degree of anthropogenic modification covered approximately the mid-course of the Wkra River (WASILEWICZ and OGŁĘDZKI 2006). Benthic invertebrates had not been previously examined.

The impact of the revitalization efforts on the diversity and density of the bottom fauna, and thus on the indices used to assess the water quality classes, may be either positive or negative. An example of a positive effect is the increased heterogeneity of the river bottom owing to the introduction of piles of stones, providing the bottom fauna with shelters and modifying the physical habitats (BECHARA et al. 1993, DOUGLAS and LAKE 1994). The planting of deciduous trees along the river banks results in the periodical enrichment of water with coarse particulate organic matter (CPOM), constituting shelters and valuable food sources for many species of macrozoobenthos (GRAÇA 2001, HIEBER and GESSNER 2002). On the other hand, fish stocking increases the predator pressure on the benthic invertebrates (FLECKER 1984, ARNEKLEIV and RADDUM 2001) and seems to be a negative effect of the revitalization on macroinvertebrates.

The main purpose of this study was to assess water quality in the upper catchment of the Nida River using the biotic and diversity indices. In addition, the potential impact of the revitalization efforts on the water quality indices was observed. We hypothesise that revitalisation works carried out in selected river sites will result in increased values of the water quality indices.

Study Area, Material and Methods

The Wkra River (total length 249 km) is a right-side tributary of the Narew River (the Vistula catchment). The section of the Wkra within the Nidzica County borders, called the Nida, was selected for the study.

The samples were collected in 2008 (sites nos 1–5), 2009 (2, 3, 6, 7), and 2010 (1, 2, 3, 6). The areas directly adjacent to the studied river sites belonged to agricultural lands cultivated mainly as meadows and pastures (Table 1). The bed of the upper section (sites 1–4) of the river was regulated. The lower river course (sites 5–7) has the most unaltered bed. The shores are overgrown by trees (*Salix sp.*, *Alnus sp.*) and the bed is shaded at stations 1 (before the town) and in the lower course (6–7).

Table 1

Characteristics of study sites in the Nida River in 2008–2010

Parameter	Site						
	1	2	3	4	5	6	7
Geographical position	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° 20.56' E 20° 24.78'	N 53° 20.48' E 20° 24.48'	N 53° 20.44' E 20° 24.41'	N 53° 20.21' E 20° 23.62'
River bed width: mean [m]	3.5	3.0	3.5	3.5	4.0	4.0	4.5
Depth: mean [m]	0.3	0.4	0.6	0.6	0.8	0.7	0.8
Velocity [m s ⁻¹]	0.3	0.4	0.2	0.2	0.3	0.3	0.2
Substrate type:							
stones	–	+	–	–	–	–	–
gravel	+	+	+	–	–	+	+
sand	+	+	+	+	+	+	+
clay	–	+	+	+	+	+	+
Character of anthropogenic changes:							
strengthening of the banks – inert fascine	+	+	+	+	+	+	+
piles of stones	–	–	+	+	–	+	–
anthropogenic debris	–	+	–	–	–	–	–
Riparian vegetation:							
trees	+	–	+	–	–	+	+
bushes	+	–	+	+	–	–	–
herbaceous plants	+	+	+	+	+	+	+
Land use category:							
suburban	+	+	–	–	–	–	–
meadow	–	–	+	+	+	+	+
pasture	–	+	–	+	+	+	+
agriculture	–	–	–	–	–	+	+
Others:							
submersed macrophytes	–	+	+	+	+	+	–
woody debris	–	–	–	+	–	–	–
muddy patches	–	+	–	+	–	–	–

The sampling was started before the onset of the renaturalization works in April 2008.

In May 2008, 15 tonnes of rocks and boulders with the maximal diameter of 256 mm and pebbles 64–256 mm in diameter were put into the river. The mineral material was distributed on the sandy bottom of the Wkra, along a 200 – meter-long section, both near the bank under the fascine embankment as heaps of several pebbles and in mid-stream as clusters of several rocks (SKRZYPCZAK et al. 2009). Another 15 tonnes of rocks and boulders were put into the river, approx. 200 m downstream of site 4, where the bottom had been revitalised a year before (SKRZYPCZAK et al. 2010).

Sites 1–5 were selected in the first study year (Figure 1). Site 1 was set up on the river just before it entered the built-up area and site 2 was located at a short distance (30 m) downstream of the town. Sites 3 and 4 were located within the river section where the bottom heterogeneity was increased by the addition of stones between two sampling dates, while site 5 was located downstream of that section. In the second sampling year, two additional sites were established: site 6, located in another river section with the bottom enriched with stones, and site 7, placed downstream of site 6. In 2008, material was collected at four sites (numbers from 2 to 5) in April and at five sites (1–5) in September. In September 2009 and 2010, material was collected at four sites (2, 3, 6, 7 and 1, 2, 3, 6, respectively). New sites had to be set up as the length of the river sections with the revitalized riverbed gradually increased.

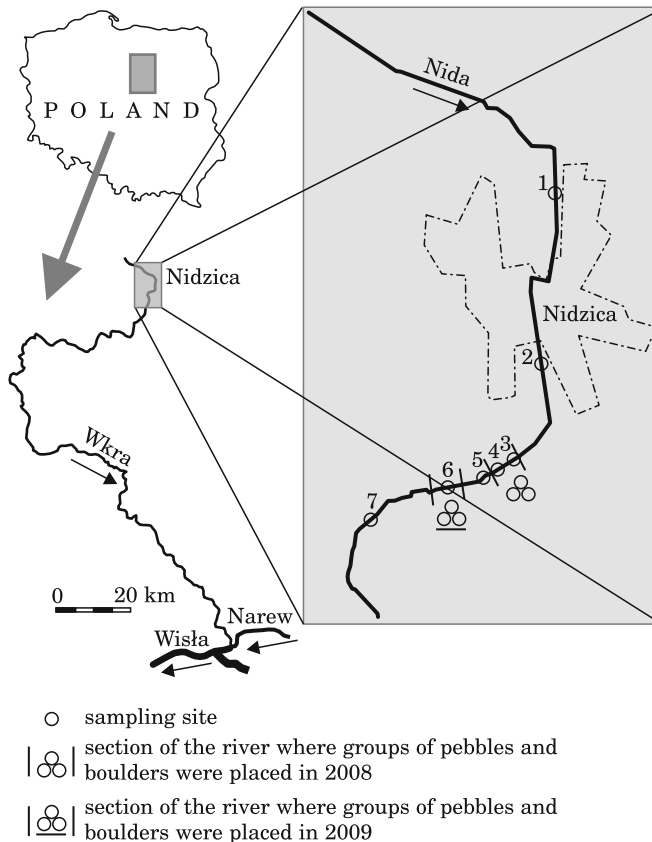


Fig. 1. Location of the sampling sites along the Nida River

A core sampler (steel tube of 29.2 cm² area) was used for collecting bed sediments and invertebrates. Twelve sediment cores were taken across the stream from each station on each sampling occasion, taking into account all kinds of bottom habitats. Samples were washed through a 0.25 mm mesh-size sieve. The material was sorted under a stereoscopic microscope, fixed and preserved in 70% ethanol. Macroinvertebrates were counted and identified to the family level (except the class Oligochaeta).

A Surber net (frame size of 25x25 cm) was used to collect the material during the first year of study (April, September). However, the qualitative samples did not provide any new taxa (families) which should be used in water quality evaluation; they were only helpful for identification of organisms in quantitative determinations.

The BMWP-PL method modified by KOWNACKI et al. (2002) was used for biological evaluation of Nida River water quality. The class of the river water was determined from two calculations. The value of the BMWP-PL index is the sum of scores of individual families present in a sample. Scores of 10–1 were allocated according to their tolerance to pollution. Also, Margalef's diversity index, proposed for biological evaluation of Polish freshwaters, was calculated.

The relationships between the values of biotic indices and environmental variables were tested using Spearman's non-parametric rank coefficient of correlation. Analyses were performed using the results for all the years and sampling sites (BMWP-PL- Margalef's diversity index, $n=17$) or, if a correlation existed between the indices (BMWP-PL, Margalef's diversity index) and environmental variables (dissolved oxygen, pH, total phosphorus, total nitrogen, COD), the data from the research carried out in September in 2008–2010 at site 2 and at the site situated most downstream with respect to the position of the water physicochemical sampling sites in a given year were used (SKRZYPCZAK et al. 2009, 2010, 2011).

The Wilcoxon non-parametric rank sum test was used to compare two groups of samples to determine if changes in the environmental conditions caused by revitalization works affected the BMWP-PL index scores. The groups were created by dividing the samples into the ones taken before and after the addition of stones and fish stocking treatments.

The non-parametric Friedman ANOVA test was applied to test time effects on the values of both of the indices. Kendall's coefficient of concordance was used to measure the degree of association among the three groups of distinguished sites (upstream, modified, downstream) with respect to time.

All statistical analyses were performed using the STATISTICA package, version 9.1 (StatSoft 2010).

Results

From the benthic indicators used for determination of water quality, 24 taxa were found in the studied Nida River. Four families, including Gammaridae, Sphaeridae, Glossiphonidae, Chironomidae and a class of Oligochaeta, were recorded at all of the sampling stations (Table 2).

The highest biological diversity was observed at sites 2 and 3, where as many as 17 taxonomic groups used as water quality indicators were found.

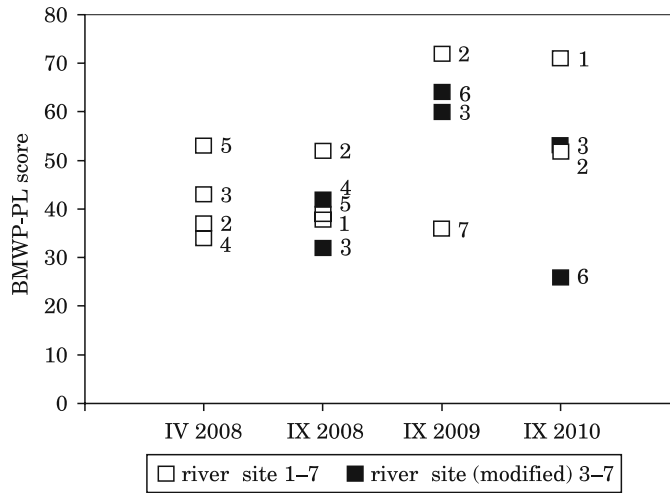


Fig. 2. Changes of the BMWP-PL score at seven sites along the River Nida in 2008–2010

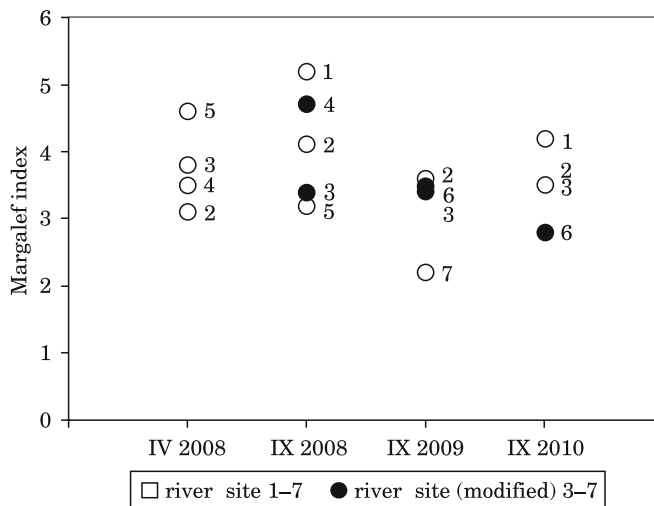


Fig. 3. Changes of the Margalef's score at seven sites along the River Nida in 2008–2010

Table 3
Values of indices – biotic BMWP-PL and Margalef's diversity and classification of water quality in studied sites sampled along the Nida River

Index	Sites																
	1		2				3				4		5		6		7
	09. 2008	09. 2010	04. 2008	09. 2008	09. 2009	09. 2010	04. 2008	09. 2008	09. 2009	09. 2010	04. 2008	09. 2008	04. 2008	09. 2008	09. 2009	09. 2010	09. 2009
BMWp-PL	38	71	37	52	72	52	43	32	60	53	34	42	53	39	64	26	36
	IV	II	IV	III	II	III	III	IV	III	III	IV	III	III	IV	III	IV	IV
Margalef's	5.2	4.2	3.1	4.1	3.6	3.5	3.8	3.4	3.4	3.5	3.5	4.7	4.6	3.2	3.5	2.8	2.2
	II	II	III	II	III	III	III	III	III	III	III	II	II	III	III	III	IV
General classification of water quality	III	II	IV	III	III	III	III	IV	III	III	IV	III	III	IV	III	IV	IV

According to the BMWP-PL scoring system (KOWNACKI et al. 2002), a result indicating 'good water quality' was obtained once – at one site and for one sampling date (Table 3), but in most cases (10) the values of the water quality indices classified the Nida River as water quality class III, corresponding to moderate quality. In the remaining cases (6), the water quality was poor – class IV.

The observed variation in the BMWP-PL index score against sampling dates, illustrated in Figure 2, assumes a sinusoid-like shape. The values of modified Margalef's biodiversity index demonstrated a relatively small range of variation (Figure 3).

Changes in the values of both indices for all sampling sites demonstrated moderate relationships ($R=0.45$), which were not significant.

The values of the BMWP-PL index calculated before and after the riverbed improvement did not show any statistically significant differences. Values of the index changed differently during the experiment and exhibited contradictory trends depending on which river sections were compared.

The values of both indices at the sites where water was sampled for physicochemical analyses showed negative correlation with the environmental variables (pH, total phosphorus, total nitrogen, COD) and positive correlation with the oxygen concentration in water, but the correlations were not statistically significant.

The Friedman Repeated Measures ANOVA test applied in the analysis, based on four data sets (four dates of studies), showed no differences among the three parts of the Nida River ($p = 0.717$), and there were strong differences among the sampling dates (Kendall's Coefficient of Concordance $W=0.11$).

Discussion

The results for water purity obtained using macrozooinvertebrates showed that the river water belonged to the same quality class, a finding confirmed by CZERNIAWSKA-KUSZA (2005), who observed good correlation of the BMWP-PL index with results based on chemical properties.

The results of monitoring observations presented in environmental reports (*Raport...* 2009, *Raport...* 2010) concern the fragment of the Nida River downstream of the sites used in this study. They show the ecological condition in 2008 as moderate and in 2009 as good. The water quality in the Wkra has been gradually improving since the 1990s (PENCZAK et al. 2001); however, considering the requirements set out in the *Water Framework Directive* (2002),

the goal of achieving good water quality, determined based on the aquatic organisms living in it, has not been achieved in the river section where the study was conducted.

The study sites were colonised mainly by taxa representing groups highly tolerant to environmental pollution, such as oligochaetes (VERDONSCHOT 1989), chironomid larvae (WATERHOUSE and FARREL 1985) and members of family Sphaeriidae (small freshwater bivalves) (LOCH et al. 1996), as well as relatively sensitive organisms like gammarids (BLOOR and BANKS 2006). The presence of individuals representing the highest ranked family Goeridae (Trichoptera), as well as the only water quality general classification result indicating good water quality, occurred in the river section situated upstream from the town. However, the results obtained during the investigations indicated the highest values of the BMWP index for the river section most severely exposed to anthropopression – at the site near the outflow from the urban areas. The most probable cause may be the high diversity of the riverbed. Despite locally abundant muddy deposits originating from organic matter transported from the catchment, the bottom was inhabited by the most diverse macroinvertebrate fauna. At this site, 17 taxonomic groups were recorded out of the total of 24 groups found in the river during the investigations. The site was also inhabited by the most taxonomically diversified zooplankton (GOŹDZIEJEWSKA et al. 2010). The direct impact of humans was manifested by the presence of various items found on the riverbed surface, which acted as artificial substrates for algae, macrophytes and macrobenthic fauna. An increase in the taxonomical diversity of benthic fauna after the introduction of artificial substrates was demonstrated by MEIER et al. (1979), and the exposure of artificial substrates was applied in water quality assessment (CZERNIAWSKA-KUSZA 2004). In the future, a similar effect may also be observed in sections of the Nida River where the bottom was enriched with items of natural origin such as pebbles and boulders. So far, the results have not indicated a significant increase in the numbers of indicatory taxa of benthic fauna, in contrast to the observations of many authors reporting a rise in taxonomic diversity after a very short time as an effect of enriching habitats with additional natural elements such as cobbles (HOSE et al. 2007) or large-woody debris (HRODEY et al. 2008). However, diversifying the bottom as an element of restoration does not always give the expected results when the quality of the water in the river is poor (SUNDERMANN et al. 2011). Moreover, MARCHANT et al. (1991) claim that the number of taxa and the rate of their colonization are proportional to the locally-occurring number of taxa and their density. PURCELL et al. (2002) observed recolonization of fauna on restored sites from the upstream sites in the third season of their study. WENN (2008) demonstrated higher BMWP and ASPT scores no sooner than three to four years post-remediation. Having cut off a point source of pollution improved the

chemical water quality, but the macroinvertebrates in the stream called the Spen Beck failed to correspond to the good water quality conditions until the sixth year post-remediation.

One reasons for the worse quality of water in river sections downstream from the town, especially the most distant ones, could be agricultural treatments. The large amount of bivalve shells (mostly of Sphaeriidae) found at site 7 indicates deceleration of the current but could also suggest infiltration of pesticides or fertilizers to the river with the surface runoff from surrounding fields. According to HECKMAN (1981), the feeding behaviour of bivalves makes them particularly sensitive to chemical compounds dissolved in water or deposited in river bed sediments.

Conclusions

The Nida River waters in the investigated section were most often classified as class III and IV (moderate and poor quality status). Based on our analysis of the benthic fauna associations, the introduction of additional mineral substrate on the riverbed surface did not affect the water quality.

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EFFECT OF STOCKING DENSITY IN RELATIONSHIP TO BOTTOM AREAS ON THE GROWTH AND SURVIVAL OF COMMON BARBEL *BARBUS BARBUS* (L.) LARVAE*

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Key words: common barbel, *Barbus barbus*, larviculture, controlled rearing, stocking density, larval development.

Abstract

An experiment was carried out concerning the effect of the initial stocking density per area unit on the growth and survival of the common barbel, *Barbus barbus*, larvae under controlled conditions. During the 21 days of rearing, the effect of fish stocking density between 2500 and 6250 individuals m⁻² was determined. Water temperature was 25°C throughout the experimental period. Fish were fed on live *Artemia* sp. nauplii. During the experiment, the mean total length (TL) of larvae as well as their weight (W) and survival were measured. On the basis of the obtained data, specific growth rate (SGR), Fulton condition coefficient (K) and fish biomass per area unit were calculated. The developmental stage of larvae was also determined. This study proved that initial stocking density of larvae does not affect survival, developmental stage or growth parameters. In all experimental treatments survival rate exceeded 98%. On the last day of rearing, all fish reached juvenile stage and TL of 28.5–30.2 mm. SGR ranged from 14.2 to 15% day⁻¹. The analysis of variance did not reveal statistical differences between the groups ($P>0.05$) for each measured parameter. The results obtained in this study indicate the possibility of a significant increase in the production intensity of fry-stocking material of the common barbel under controlled conditions, which should positively affect the economic effectiveness of rearing.

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WPŁYW ZAGĘSZCZENIA OBSADY NA JEDNOSTKĘ POWIERZCHNI NA WZROST I PRZEŻYWALNOŚĆ LARW BRZANY, *BARBUS BARBUS* (L.)

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Słowa kluczowe: brzana *Barbus barbus* (L.), larwikultura, podchów kontrolowany, zagęszczenie, rozwój larwalny.

Abstrakt

Przeprowadzono eksperyment dotyczący wpływu początkowego zagęszczenia larw w przeliczeniu na jednostkę powierzchni na wzrost i przeżywalność brzany *Barbus barbus* w warunkach kontrolowanych. Badano zagęszczenie ryb od 2500 do 6250 osobn. m⁻². Doświadczenie trwało 21 dni. Podchów prowadzono w temperaturze 25°C. Pokarm ryb stanowiły żywe naupliusy *Artemii* sp. W czasie trwania eksperymentu notowano średnią długość całkowitą larw (TL), masę (W) oraz przeżywalność. Z uzyskanych danych wyliczono specyficzne tempo wzrostu (SGR), współczynnik kondycji Fultona (K) oraz biomasę ryb uzyskaną z jednostki powierzchni. Stopień zaawansowania rozwojowego larw określono na podstawie zgromadzonej dokumentacji. Dowiedziono, że początkowe zagęszczenie larw nie wpływa na przeżywalność, stopień zaawansowania rozwojowego oraz parametry podchowu. We wszystkich wariantach eksperymentalnych przeżywalność przekroczyła 98%. W ostatnim dniu podchowu wszystkie ryby osiągnęły stadium juvenilne dla średniej TL 28.45–30.20 mm. SGR wynosił 14.15–14.99% dzień⁻¹. W analizie wariancji nie wykazano różnic statystycznych między grupami ($P > 0.05$). Uzyskane wyniki wskazują na możliwość znacznego zwiększenia intensywności produkcji materiału zarybieniowego brzany w warunkach kontrolowanych. Powinno to pozytywnie wpłynąć na ekonomiczną efektywność podchowu.

Introduction

In recent years, the level of global fish biodiversity has been characterized by a decreasing trend (ROSS 2008). It is estimated that many natural ecosystems will disappear, resulting in a decrease in the number of existing species. River regulations and pollutions have a direct threat to populations in running waters (KECKEIS et al. 2001). An additional aspect which has had an impact on a decrease stocks of many species is overfishing (POLICAR et al. 2007). This largely concerns rheophilic cyprinids which form the basis of river ichthyofauna (MANN 1996).

A dramatic decrease in the population of many species of fish has become a reason for undertaking actions aimed at restitution or protection of endangered populations of fish. In the active protection of endangered species, sustainable aquaculture plays a very important role (e.g., ROSS 2008, ŻARSKI et

al. 2011b), where the biotechnology of larval rearing under controlled conditions is one of the most effective methods of producing fry-stocking material required for restocking purposes (SHIRI HARZEVILI et al. 2003, HAMACKOVA et al. 2009). Improvement of fry-stocking material production, particularly at the stage of intensive larviculture, can have a significant effect on the quantity and the quality of the fry-stocking material produced, which can determine restocking effectiveness (COWX 1994). On the other hand, production carried out under controlled conditions makes it possible to constantly monitor both the quantity and quality, as well as the growth rate of fish. However, effective procedures of larvae and juveniles rearing of many species of fish are lacking, and data concerning the effect of individual factors on the efficiency of rearing of many fish species are fragmentary.

The common barbel, *Barbus barbus* (L.), is a typical representative of cyprinid rheophilic fish, usually populating the upper and the central course of clean rivers with hard, stony and gravel substrates. An important morphological feature of this species is its adaptation to bottom life (MANN 1996, BRYLIŃSKA 2000). In recent decades, a decrease in the size of population of many species of rheophilic fish, including the common barbel (POLICAR et al. 2007), has been recorded in Europe. In many countries of central Europe, the observed decrease in the common barbel stock has been so serious that this species has become endangered (e.g., PROKES et al. 2006, POLICAR 2007). Consequently, restocking of open waters with the material obtained as a result of controlled reproduction and rearing can significantly support the natural recruitment (COWX 1994, PHILIPPART et al. 1995). In the case of the common barbel, the high price of fry-stocking material, resulting from its lower fecundity and efficiency of artificial reproduction in comparison to other rheophilic cyprinids (KUCHARCZYK et al. 2008, TARGOŃSKA et al. 2011), significantly limits restocking attempts on a large scale. At the same time, production of the common barbel may be one of the most profitable among freshwater aquaculture activities (HAKUĆ-BŁAŻOWSKA et al. 2010). Undertaking all possible actions aiming at increasing rearing intensity can significantly reduce the cost of production and at the same time increase the production. Published data concerning common barbel larviculture concern early ontogenesis (CALTA 1998) and determination of optimum nutritional conditions and the effect of stocking density per volume unit (KUJAWA 2004, WOLNICKI 2005, ŻARSKI et al. 2011a). However, there are no data concerning the effect of larval density per area unit, which in case of bottom-dwelling fish significantly condition the efficiency of rearing (SCHRAM et al. 2006, AKSUNGUR et al. 2007). A negative effect of stocking density on fish growth was reported for many fish species. In many cases, the influence of social interactions, cannibalism and changes in physicochemical parameters of the water affected by application of high

stocking densities indirectly affected fish metabolism and, in consequence, growth rate (e.g. KING et al. 2000, ALVARES-GONZALES et al. 2001, ŻARSKI et al. 2011a,b). However, the mechanism still remains unclear.

The aim of this study was to determine the effect of initial stocking density of barbel larvae per area unit on the growth and survival of fish under controlled conditions.

Materials and Methods

Common barbel larvae originated from controlled reproduction of cultured stock reared and reproduced at the Czarci Jar Fish Farm (North-Eastern Poland). Hormonal stimulation was carried out according to the method described by TARGOŃSKA et al. (2011). The eggs from three females were fertilized using the “dry” method, with a mixture of sperm obtained from five males. Fertilized eggs were incubated in Weiss jars, at a constant temperature of 20°C. Freshly hatched larvae were transferred to a 150 L collective tank, operating in a closed recirculating system. From the moment when all larvae hatched, water temperature was increased by 1°C each day up to 25°C. Larvae were kept under such conditions until the moment they started to swim actively and inflated the posterior chamber of their swim bladder (5 days post hatch [DPH]), after which they were placed in an experimental rearing system.

Larvae were reared in a closed recirculating system in glass tanks (aquaria), with the possibility of water level adjustment (as described by KUJAWA et al. 2000). The surface of each tank was 0.16 m². Depending on the experimental group, the water in aquaria was increased to the volume of: 20, 30, 40 and 50 L. The larval density in each of the experimental treatments was 20 individuals L⁻¹. In this way, a stocking density per area unit amounting to 2500, 3750, 5000 and 6250 fish per m² was obtained. The experiment was carried out in three replications. Water was supplied to the tanks in the form of an upper inflow. At the beginning of the experiment, water flow was adjusted in such a way so that its exchange took place five times an hour in each of the experimental tanks. Water flow was gradually increased to obtain ten-time water exchange at the end of the experiment. Larvae were reared for 21 days at 25°C (±0.1) under a 14h light photoperiod (14L:10D). The fish were fed three times (at 08.00, 12.00, 16.00) a day *ad libitum* with freshly-hatched *Artemia* sp. nauplii in such amounts that the nauplii were present in water from the moment of the first feeding until the end of the light day. Before the first feeding, remains of food, faeces and dead fish (the number of which was recorded) were removed from the rearing tanks. Twice a week,

ammonia and nitrate content were measured in each of the rearing tanks (photometer Slandi, Poland) along with oxygen content in water (HI 9828, Hanna Instruments, Italy).

Fish for total length measurements were sampled on the day when the experiment began, and then on day 7, 14 and 21 of the experiment. During the analysis, the total length of fish was determined (TL, ± 0.1 mm). The mean weight of fish (± 0.1 mg) was determined at the beginning and at the end of the experiment. Measurements were carried out on 30 randomly-selected specimens from each experimental tank (90 specimens per each experimental group were measured). Before taking measurements, the fish were anesthetized in a 2-phenoxyethanol solution (Sigma-Aldrich, Germany) (0.4 mL L^{-1}). After performing measurements, the fish returned to the same tanks from which they were caught.

On the basis of the data obtained, specific growth rate (SGR [$\% \text{ day}^{-1}$]) and Fulton condition coefficient (K) were calculated, according to the following formulas:

$$\text{SGR} = ((\ln W_T - \ln W_t)100)(T - t)^{-1}$$

where:

W_T , W_t – mean body weight at the beginning and at the end of rearing, respectively [mg],

$T - t$ – number of days between measurements.

$$K = 100 (W \text{ TL}^{-3})$$

where:

W – fish body weight [mg],

TL – total length of the fish body [mm].

On the basis of the obtained data, the developmental stage was determined for each experimental group (according to the method described by ŻARSKI et al. 2011a). Additionally, fish biomass obtained from an area unit was calculated (total weight of fish obtained from area unit). Data expressed in percentage before the analysis were subject to arc-sine transformation. An analysis of variance (ANOVA) was applied to establish significant statistical differences between the groups at the level of $\alpha = 0.05$. Linear regression analysis was performed for biomass of fish obtained in individual experimental groups. Statistical analyses were carried out using Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA) and MS Excel for Windows.

Results

During the experiment, the level of oxygen in all rearing tanks did not drop below 85% saturation. Ammonia and nitrates content ranged from 0.05 to 0.1 mg L⁻¹.

At the moment when the experiment started, all larvae were feeding, although some energy originated from incompletely resorbed yolk sacs. Inflation of the anterior chamber of the swim bladder (second in turn) occurred in about 5% of larvae. The fin fold revealed a distinct narrowing in the dorsal part. The dorsal fin and the caudal fin had visible rays. After 7 days of the experiment, the fin fold in the larvae was significantly reduced and its remaining parts were visible near the pelvic areas of the body and in the caudal part. The size of pelvic fins differed in individual fish and in most of them they extended beyond the edge of the fin fold. On day 14 of rearing, in each experimental treatment, the fin fold was visible between the pelvic fins and the anus only in less than 5% of fish. The fin fold completely disappeared on day 21 of rearing, which proves that all fish reached the juvenile stage.

Fish survival, regardless of the experimental group, was very high and amounted to about 99% (Table 1). A high growth rate was recorded for the entire experiment. The analysis of variance did not reveal any statistical differences between the groups ($P > 0.05$). During the study, the highest values of the mean fish body length (30.2 mm) and the mean fish body weight (204.5 mg) were recorded on the last day of rearing in the group where the density amounted to 3750 ind. m⁻². The value of SGR coefficient ranged from 14.15 to 14.99% day⁻¹. In case of K coefficient, the highest value (0.78) was recorded

Table 1
Characteristics of the common barbel (*Barbus barbus*) over 21 days at different stocking densities under controlled conditions (SGR – specific growth rate; K – Fulton's condition coefficient)

	Stocking density [ind. m ⁻²]			
	2500	3750	5000	6250
Total body length [mm] at:				
day 0	12.20 ± 0.41	12.20 ± 0.41	12.20 ± 0.41	12.20 ± 0.41
day 7	17.34 ± 0.50	17.42 ± 0.15	17.36 ± 0.45	17.19 ± 0.37
day 14	23.17 ± 0.10	23.47 ± 0.44	22.56 ± 0.28	23.09 ± 0.76
day 21	28.60 ± 1.69	30.20 ± 0.28	28.45 ± 1.28	29.07 ± 0.52
Wet body weight (mg) at:				
day 0	8.7 ± 0.9	8.7 ± 0.9	8.7 ± 0.9	8.7 ± 0.9
day 21	188.70 ± 16.96	204.55 ± 8.07	178.30 ± 20.85	180.21 ± 13.71
Survival rate [%]	99.00	99.06	98.38	99.43
SGR [% day ⁻¹]	14.57 ± 0.46	14.99 ± 0.17	14.15 ± 0.67	14.38 ± 0.37
K	0.78 ± 0.03	0.74 ± 0.01	0.75 ± 0.01	0.73 ± 0.02

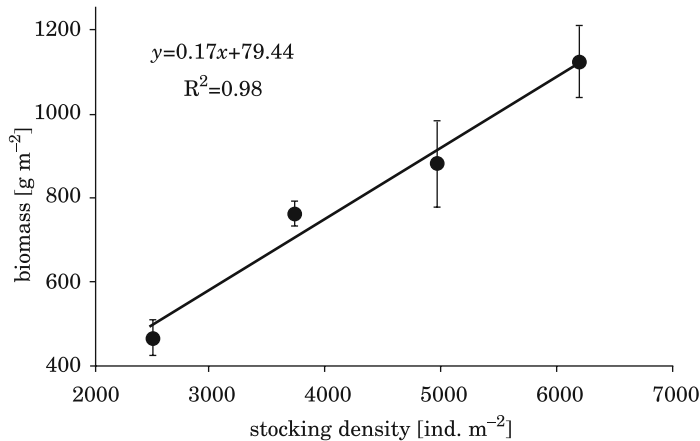


Fig. 1. Correlation between stocking density and mean fish biomass ($n=3$ for each stocking density) after 21 days controlled rearing of common barbel (*Barbus barbus*)

in the group where fish density was 2500 ind. m⁻². A regression analysis revealed a significant linear relation between density and biomass of fish obtained per area unit (Figure 1).

Discussion

The obtained results in this study indicate that the stocking density of barbel larvae per area unit did not have a negative effect on rearing parameters or survival of barbel larvae. WOLNICKI (1997) obtained SGR of 14.82% day⁻¹ during 15-day rearing of barbel larvae at 25°C and density of 30 ind. L⁻¹, while WOLNICKI and GÓRNY (1995) recorded 13.8% day⁻¹ at a density of 40 ind. L⁻¹. POLICAR et al. (2007) obtained an SGR of 14.5% day⁻¹ and a mean LT of 24.3 mm during 21-day rearing of barbel at 21°C. It is worth to mention that POLICAR et al. (2007) started research on 13 DPH. KUJAWA (2004) after 21 days of rearing barbel larvae at 25°C recorded the highest mean total length of 25.6 mm at density of 20 ind. L⁻¹. ŽARSKI et al. (2011a) examined the effect of high density per volume unit reaching up to 200 ind. L⁻¹ obtained similar results (mean final weight of 138.17 mg, mean final TL of 27.11 mm) to this obtained in the present study (mean final weight of 187.94 mg, mean TL of 29.08 mm). In addition, the developmental stage of fish at the end of the experiment was similar in this study with the study of ŽARSKI et al. (2011a). Therefore, the obtained results indicate that the growth rate of barbel larvae observed in this study was the highest growth rate reported so far, which confirms the optimal rearing conditions.

The effectiveness of rearing fish larvae under controlled conditions depends on many factors. They include, first of all, temperature, oxygen content, photoperiod, type and amount of food provided and feeding frequency (WOLNICKI et al. 2003, WOLNICKI 2005, POLICAR et al. 2006, VORLIČKOVÁ et al. 2006). These parameters in our study were identical in all experimental groups. Some authors reported that stocking density is also a very important factor affecting the growth rate and survival of fish (KUJAWA 2004, SZKUDLAREK and ZAKĘŚ 2007, CELADA et al. 2007). KUJAWA (2004) recorded differences in the growth rate between the groups during three-week rearing of barbel larvae at 25°C at the initial stocking density of 25, 50 and 75 ind. L⁻¹. However, ŹARSKI et al. (2011a) did not record any differences between densities reaching 20 and 200 fish L⁻¹ which probably resulted from a much smaller scale of the experiment and consequently, the possibility of maintaining very good experimental conditions of rearing and precise amount of food (number of *Artemia* nauplii per capita instead of *ad libitum* method). The possible effect of rearing conditions on the growth rate of fish in various densities has been already reported (KING et al. 2000). It must be emphasized that the scale of the experiment, water temperature, period of rearing, type and amount (*ad libitum*) of supplied food, as well as larval density used in this experiment were the same as applied by KUJAWA (2004) in the best of experimental treatments. On the other hand, a better growth rate in comparison to results obtained by KUJAWA (2004) could be caused by the 2h longer photoperiod applied in this study, the factor that has been proven to affect the growth rate (WOLNICKI et al. 2003). Additionally, these differences could result from the varied origin of the larvae used in the experiments. KUJAWA (2004) obtained larvae from wild fish, while in this study, the larvae originated from cultured stock. Moreover, it cannot be excluded that the results obtained in this study in various densities (no differences in the growth rate) could depend on the origin of larvae. However, an explanation of those possible variables requires further, more detailed research.

There are also assumptions that the value of density expressed per area unit of the bottom, in case of bottom-dwelling fish, also influences growth parameters (SCHRAM et al. 2006, AKSUNGUR et al. 2007). It was assumed that use of large stocks has a negative impact on the growth rate of the body because of stress which may affect elevated cortisol level in blood plasma (e.g., BOLASINA et al. 2006). However, the results obtained in this study, like the results obtained by ŹARSKI et al. (2011a), show that barbel larvae in the early developmental stages are little susceptible to stress, which eliminates the density factor as a potential stressor. This applies both to the stocking density examined as a number of individuals per volume unit (ŹARSKI et al. 2011a) and per bottom area unit (the present study). Similar dependencies were also

reported for other species of rheophilic cyprinids (KUPREN et al. 2011), as well as for the crucian carp, *Carassius carassius* (ŻARSKI et al. 2011b), where a large stocking density did not affect the growth rate of larvae. Therefore, based on the data published to date, it can be claimed that a negative effect of the stocking density on the final rearing result is most probably related to the size of the fish and its developmental stage (IRWIN et al. 1999, AMBROSIO et al. 2008), where fish in a higher developmental stage are able to more intensely react to stress-inducing stimuli, such as stocking density. This dependency, as a result of stress related to application of large stocks, can have an immediate effect on physiological conditions, and consequently, contribute to a reduction in the growth rate (COSTAS et al. 2008, ŻARSKI et al. 2011a, b). However, this aspect should be more closely studied and in future research the analysis of the cortisol level in the blood plasma should be included because data on the physiological responses (such as excretion of the cortisol) of fish larvae due to the stressful culture conditions is very limited (BOLASINA et al. 2006).

As results from the data presented showed there are no contraindications as to applying large stocking densities in larval rearing and early juvenile stages of the common barbel. No negative effect in applying large stocks on the growth rate and individual development of fish was found, regardless of the method of density examination.

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THE EFFECT OF HIGH PRESSURE ON SELECTED THREE STRAINS OF *LACTOBACILLUS ACIDOPHILUS*

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Key words: *Lactobacillus acidophilus*, pressurization, antibacterial activity, acidifying activity.

Abstract

The experiment was aimed at determining the influence of pressures between 30 and 150 MPa on selected three strains of *Lactobacillus acidophilus* on bacterial count; the antibacterial activity and acidifying activity. Cultures of the following strains: *Lactobacillus acidophilus* LA-5, *Lactobacillus acidophilus* T132/2 and *Lactobacillus acidophilus* T294, in milk and liquid MRS medium were subjected to a pressure treatment of 30, 60, 90, 120 and 150 MPa for 1 minute. Immediately after pressurization and after inoculation, the cultures were determined for survivability, active acidity (pH) and antibacterial activity. The study demonstrated that pressure did not exert any significant effect on the survivability of the strains examined. An increase in the acidifying activity of *Lactobacillus acidophilus* T294 strain was observed after pressurization in a culture run on milk. The antibacterial activity was found to depend on the culture medium. Pressurization had a little effect on the improvement of antibacterial properties of the examined strains of *Lactobacillus acidophilus*.

WPŁYW WYSOKICH CIŚNIEŃ NA WYBRANE TRZY SZCZEPY *LACTOBACILLUS ACIDOPHILUS*

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Słowa kluczowe: *Lactobacillus acidophilus*, presuryzacja, aktywność antybakteryjna.

Abstrakt

Celem doświadczenia było określenie wpływu ciśnień 30–150 MPa na przeżywalność, aktywność kwaszącą i aktywność antybakteryjną szczepów: *Lactobacillus acidophilus* LA-5, *Lactobacillus acidophilus* T132/2 i *Lactobacillus acidophilus* T294. Hodowle szczepów na mleku i na podłożu płynnym MRS poddano działaniu ciśnienia 30, 60, 90, 120 i 150 MPa przez 1 minutę. Bezpośrednio po presuryzacji oraz po przeszczepieniu oznaczano przeżywalność, kwasowość czynną (pH) i aktywność antybakteryjną. Stwierdzono, że ciśnienie nie wpływa znacząco na przeżywalność badanych szczepów. Najniższą aktywność kwaszącą wykazywały bakterie *Lactobacillus acidophilus* T294 w hodowli na mleku. Stwierdzono niewielki wzrost aktywności kwaszącej badanych szczepów po presuryzacji w hodowlach na mleku. W hodowlach na podłożu płynnym MRS aktywność antybakteryjna badanych szczepów była wyższa niż w hodowlach na mleku. Presuryzacja, bez względu na stosowane ciśnienie, wpłynęła nieznacznie na poprawę właściwości antybakteryjnych badanych szczepów *Lactobacillus acidophilus*.

Introduction

Lactobacillus acidophilus is one of the major species of natural microflora colonizing the gastrointestinal tract of humans and animals. In breast-fed infants probiotic bacteria, like *Bifidobacterium* sp., represent 99% of intestinal microflora. Strains of *Lactobacillus acidophilus* are characterized by a number of health-promoting properties, i.e. antibacterial activity against pathogenic microflora, enhancement of the digestion process of lactose, capability to assimilate cholesterol and support the immune system of humans. (JAKUBCZYK and KOSIKOWSKA 1996, OUWEHAND et al. 2002, PARVEZ et al. 2006).

Investigations carried out thus far have indicated that high pressure exerts a variety of effects on microorganisms depending not only on the species but also on the strain. The result of pressurization is affected by both the value of pressure, length of pressure treatment and the course of enzymatic reactions. Thus, pressurization is likely to influence the functioning and metabolism of microorganisms (REPS et al. 1994).

In the reported study, assays were conducted to examine the effect of pressurization at 30–150 MPa on survivability, antibacterial activity and acidifying activity of the following strains: *Lactobacillus acidophilus* LA-5, *Lactobacillus acidophilus* T132/2, *Lactobacillus acidophilus* T294.

Materials and Methods

Materials

The following strains were used in this study: *Lactobacillus acidophilus* LA-5, *Lactobacillus acidophilus* T132/2 and *Lactobacillus acidophilus* T294.

These strains were cultured on sterile reconstituted skim-milk (10% d.m.) and on sterile liquid MRS medium (Merck) at pH 5.4. The inoculum averaged about 10^2 cfu cm^{-3} .

The course of the experiment

In duplicate cultures of *Lactobacillus acidophilus* (passage I) were poured into 10 cm^3 plastic containers and pressurized in a high-pressure generator (Unipress Equipment) using pressures of 30, 60, 90, 120 and 150 MPa for 1 minute at 18°C.

Immediately after pressure treatment, bacterial count, acidifying activity and antibacterial activity, assays were conducted in the non-pressurized and pressurized cultures.

Next, the pressurized and non-pressurized cultures were inoculated at 2% onto reconstituted skim-milk (10% d.m.) and sterile liquid MRS medium (pH 5.4), incubated at 37°C/20 h and then kept at a temperature of 4°C/12 h, thus passage II was obtained. The resultant cultures were subjected to the same assays as those of passage I.

Analytical methods

Bacterial count of *Lactobacillus acidophilus* was determined on MRS-Agar (Merck) medium (pH 5.4) using the plate method. Incubation was run at 37°C/72 h under anaerobic conditions.

The antibacterial activity was determined with a modified well method against 11 test strains: *Escherichia coli* 366, *Escherichia coli* 345, *Escherichia coli* L1, *Enterobacter cloacae*, *Enterobacter cloacae* 10, *Enterobacter cloacae* 11, *Enterobacter cloacae* 17, *Proteus* 6H, *Proteus* dw, *Klebsiella* ssp. 499, *Klebsiella pneumoniae* B. Agar broth medium (1.25%), previously inoculated with a liquid culture of a test strain was poured onto wells so that the number of cells in the medium reached 10^4 – 10^5 cfu cm^{-3} . After setting, wells 10 mm in diameter were cut in the medium. Next, 0.125 cm^3 of the examined material were introduced into each well and the medium was incubated at 37°C/16–24 h under aerobic conditions. Following the incubation, the size of growth inhibition zones of the test strains was measured and expressed in mm.

Active acidity was measured with an HI 221 pH-meter (HANNA instruments).

All the obtained results were subjected to statistical analysis.

Results and Discussion

Effect of pressurization on the survivability of the examined strains of *Lactobacillus acidophilus*

Pressure treatments at 30–150 MPa did not elicit any significant differences in the survivability of *Lactobacillus acidophilus* strains (Student's t-distribution, $\alpha = 1\%$). As compared to the control sample (non-pressurized), the number of bacteria in samples subjected to pressure treatment was lower by less than one logarithmic cycle (Figure 1–3) of the strains examined,

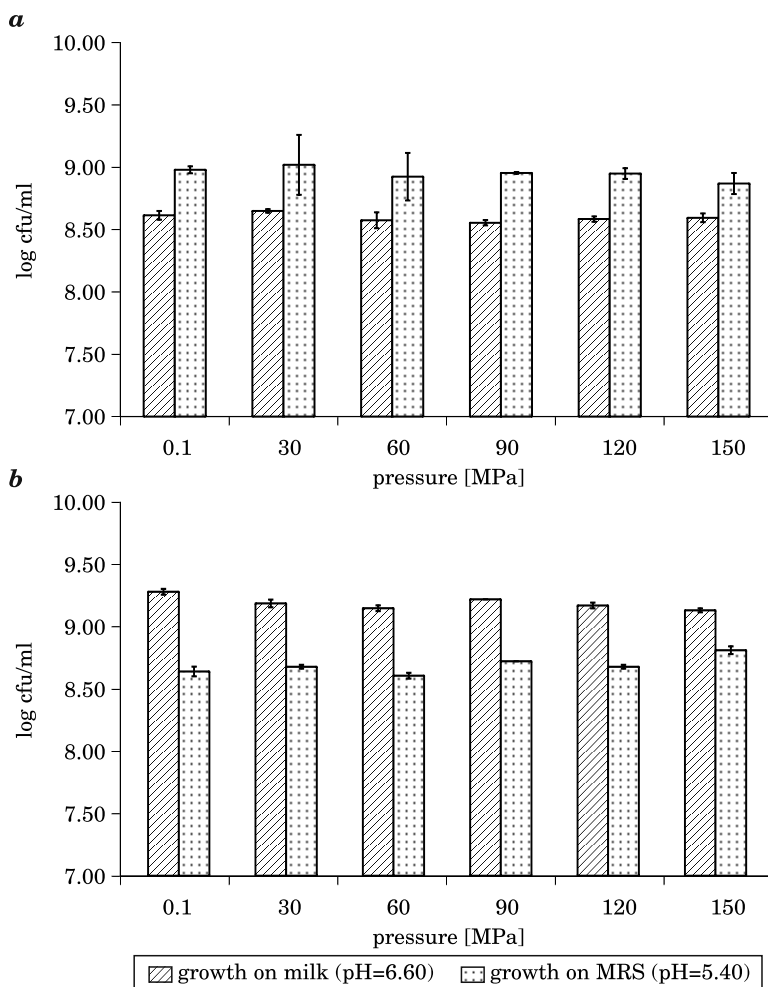


Fig. 1. Survivability of *Lactobacillus acidophilus*: a – LA-5 – I passage, b – LA-5 – II passage

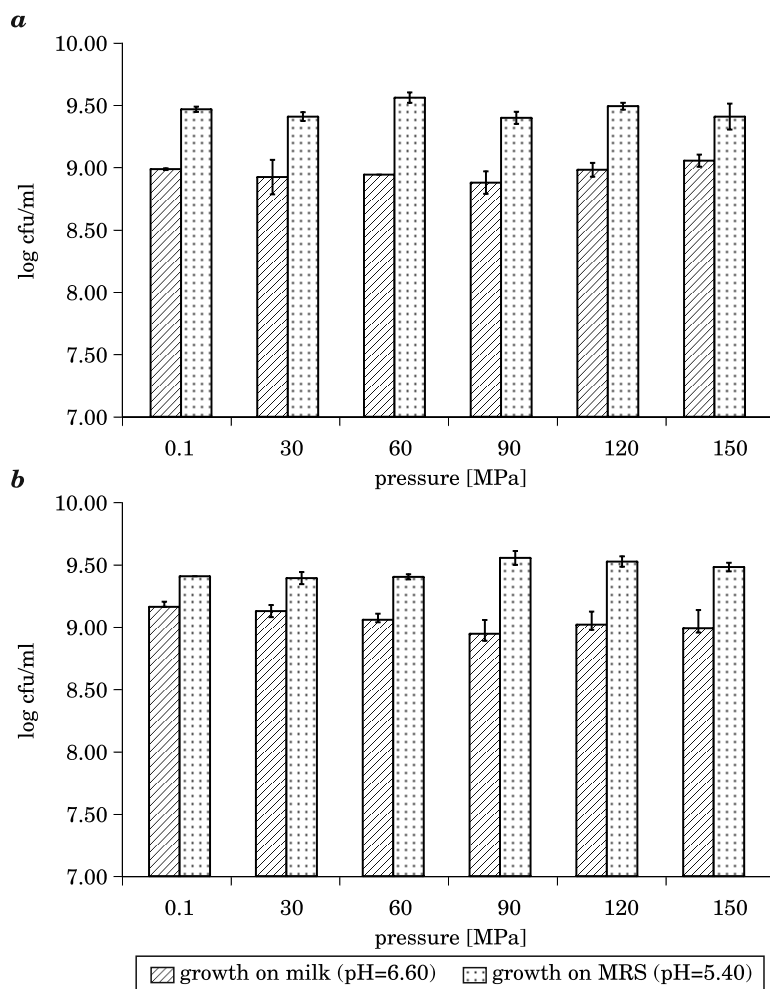


Fig. 2. Survivability of *Lactobacillus acidophilus*: *a* – 132/2 – I passage, *b* – 132/2 – II passage

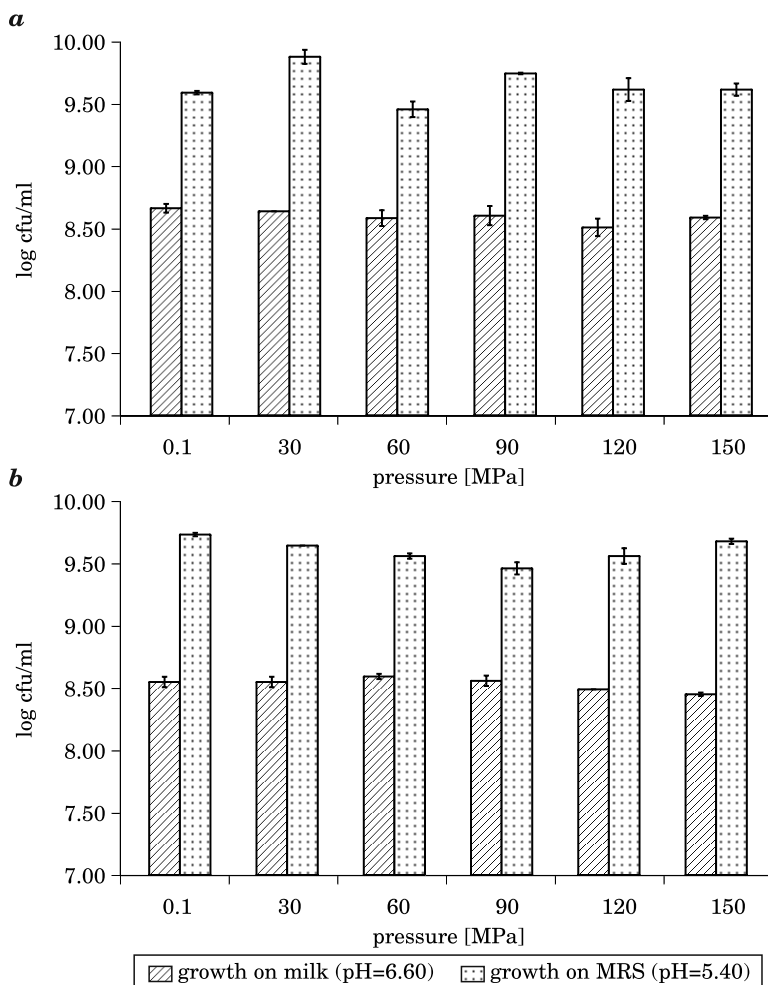


Fig. 3. Survivability of *Lactobacillus acidophilus*: a – 294 – I passage, b – 294 – II passage

the lowest resistance to the action of pressure was observed in the case of *Lactobacillus acidophilus* LA-5 strain.

The survivability of the examined strains of *Lactobacillus acidophilus* pressurized in the culture grown on the MRS medium was higher compared to those strains cultured on milk. No differences were found in the survivability between non-pressurized and pressurized strains in the passage II on milk and MRS medium.

Investigations by WOUTERS et al. (1998) demonstrated that the susceptibility of microorganisms to the action of high pressure depends on the reaction of medium. *Lactobacillus plantarum* grown on a medium at pH 5.0 subjected to

a pressure treatment of 250 MPa was found to be more resistant than in the culture run on a medium at pH 7.0.

LANCIOTTI et al. (2007) investigated the effect of high-pressure homogenization on the survivability of selected bacteria of the genus *Lactobacillus*. They did not observe any significant changes in the number of those bacteria after pressure treatments at 50, 100 and 150 MPa.

In passage II, the strains subjected to pressurization exhibited the same, and in some cases even higher survivability as compared to the non-pressurized strains.

Effect of pressurization on the acidifying activity of the examined strains of *Lactobacillus acidophilus*

The highest acidifying activity was demonstrated for the strain *Lactobacillus acidophilus* LA-5. A slight increase in the acidifying activity of the analyzed strains was found after pressurization in cultures run on milk (passage II) – Figure 4–6.

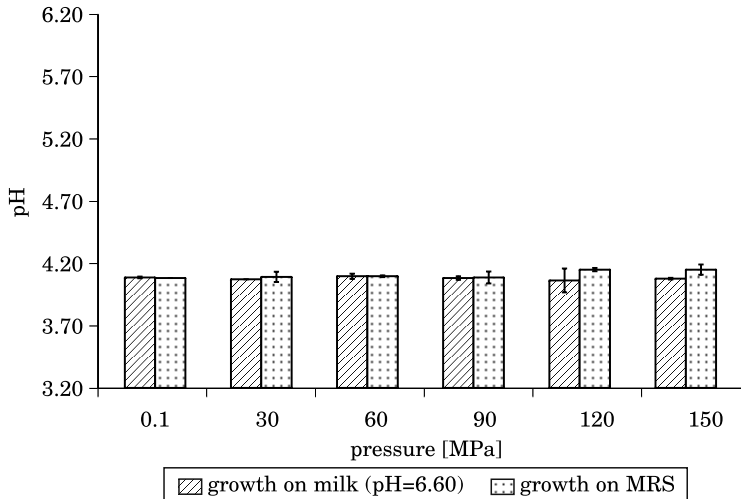


Fig. 4. Acidifying activity of *Lactobacillus acidophilus* LA-5 – II passage

LIKEWISE, LANCIOTTI et al. (2007) observed an increase in the acidifying activity of selected strains of the genus *Lactobacillus* after a pressure treatment at 50 MPa. Additionally their study demonstrated that differences in that activity were strain-dependent.

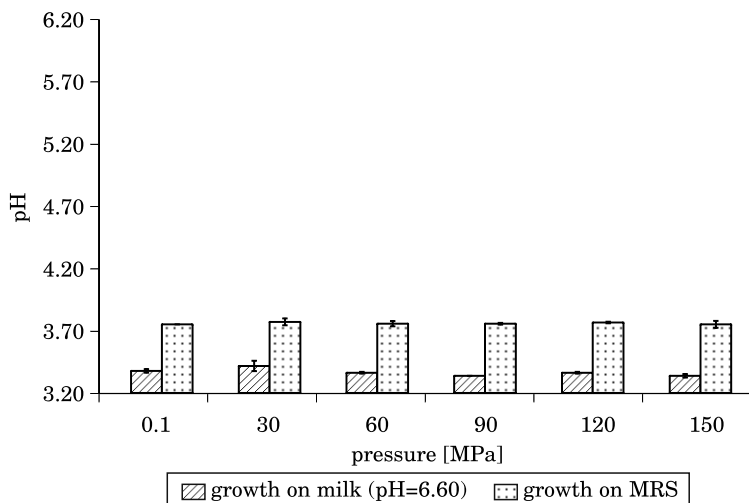


Fig. 5. Acidifying activity of *Lactobacillus acidophilus* 132/2 – II passage

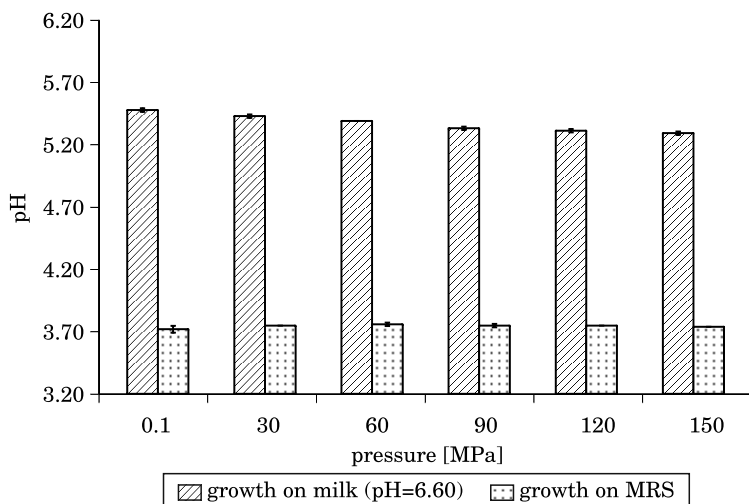


Fig. 6. Acidifying activity of *Lactobacillus acidophilus* 294 – II passage

In turn, a study by KRASOWSKA et al. (2005) demonstrated that the higher the value of the pressure applied, the lower the content of lactic acid in the culture of pressurized bacteria. Pressurization at 300 MPa for 15 min did not cause any significant decrease in the acidifying activity. Only the pressure treatment of 500 MPa/1 min was found to considerably inhibit the acidifying activity of the examined bacteria of *Lactobacillus helveticus*.

Effect of pressurization on the antibacterial activity of the examined strains of *Lactobacillus acidophilus*

The antibacterial activity of the investigated strains was higher in cultures run on MRS medium than in those run on milk. (Table 1 and Table 2).

The highest antibacterial activity was reported for the strain *Lactobacillus acidophilus* T132/2. Pressurization was found not to affect its antibacterial activity, either immediately after pressurization in passage I or after inoculation in passage II, in culture on milk. In contrast, the antibacterial activity was observed to increase in the culture run on the MRS medium immediately after pressurization.

Pressurization did not evoke any changes in the antibacterial activity of the strain *Lactobacillus acidophilus* LA-5 in cultures on milk. In turn, its increase was observed in the culture on the MRS medium both immediately after pressurization in passage I as well as after inoculation in passage II.

The weakest antibacterial activity was exhibited by the strain *Lactobacillus acidophilus* T294. In the culture on milk in passage I it did not inhibit the growth of three, whereas in passage II it inhibited the growth of two out of the 11 test strains. In the culture on the MRS medium, the *Lactobacillus acidophilus* T294 strain inhibited the growth of all test strains. After inoculation in passage II, an increase in the antibacterial activity was observed in the culture run on the MRS medium.

The antibacterial activity is determined, to a significant extent, by the amount of lactic acid produced by bacteria, which is linked to their acidifying activity. A decrease in pH to a value of 4.2–4.5 is generally recognized as sufficient to inhibit the growth of putrefactive bacteria, butyric bacteria and enteropathogens. (WOJTATOWICZ and CHRZANOWSKA, 1998) High-pressure homogenization in a pressure range of 50–150 MPa evokes an increase in the acidifying activity of selected strains of the species *Lactobacillus* sp., which results in an increase in the antibacterial activity against pathogenic microflora. (LANCIOTTI et al. 2007).

Conclusions

- pressurization in a pressure range of 30–150 MPa/ 1 min did not exert any significant effect on the survivability of the examined strains of *Lactobacillus acidophilus*;
- cultures of the examined strains of *Lactobacillus acidophilus* run on milk exhibited lower resistance to a pressure treatment at 30–150 MPa/ 1 min, as compared to the cultures on liquid MRS medium;

Table 1
Antibacterial activity of the *Lactobacillus acidophilus* T132/2 strain – I passage

Pressure [MPa]	Growth medium	The size of growth inhibition zones of the test strains [mm]											
		<i>Escherichia coli</i> 366	<i>Escherichia coli</i> 345	<i>Escherichia coli</i> L1	<i>Enterobacter cloacae</i> 6	<i>Enterobacter cloacae</i> 10	<i>Enterobacter cloacae</i> 11	<i>Enterobacter cloacae</i> 17	<i>Proteus</i> 6H	<i>Proteus</i> du	<i>Klebsiella</i> ssp. 499	<i>Klebsiella pneumoniae</i> B	
0	Passage	I	I	I	I	I	I	I	I	I	I	I	
	milk	12.0 ± 0.0	21.0 ± 1.4	16.5 ± 0.7	17.0 ± 0.0	13.0 ± 0.0	24.5 ± 0.7	13.0 ± 0.0	23.0 ± 0.0	16.5 ± 0.7	16.0 ± 0.0	14.0 ± 0.0	
	MRS	22.5 ± 0.7	35.5 ± 0.7	28.5 ± 0.7	29.0 ± 1.4	24.5 ± 0.7	33.5 ± 0.7	30.5 ± 0.7	50.0 ± 0.0	45.0 ± 0.0	44.5 ± 2.1	31.0 ± 0.0	
	milk	13.0 ± 0.0 ^s	21.0 ± 1.4 ⁿ	16.5 ± 0.7 ⁿ	17.0 ± 0.0 ⁿ	13.5 ± 0.7 ⁿ	24.5 ± 0.7 ⁿ	13.0 ± 0.0 ⁿ	22.5 ± 0.7 ⁿ	16.0 ± 0.0 ⁿ	15.0 ± 0.0 ^s	14.5 ± 0.7 ⁿ	
30	MRS	23.0 ± 1.4 ⁿ	37.0 ± 1.4 ⁿ	28.5 ± 2.1 ⁿ	29.5 ± 2.1 ⁿ	22.0 ± 0.0 ⁿ	33.5 ± 0.7 ⁿ	31.0 ± 1.4 ⁿ	50.0 ± 0.0 ⁿ	44.0 ± 1.4 ⁿ	48.0 ± 0.0 ^s	34.5 ± 0.7 ^s	
	milk	13.0 ± 0.0 ^s	20.5 ± 0.7 ⁿ	16.5 ± 0.7 ⁿ	17.5 ± 0.7 ⁿ	14.5 ± 0.7 ⁿ	24.5 ± 0.7 ⁿ	13.0 ± 0.0 ⁿ	23.5 ± 0.7 ⁿ	16.5 ± 0.7 ⁿ	15.0 ± 0.0 ^s	15.0 ± 0.0 ^s	
	MRS	26.0 ± 1.4 ^s	37.5 ± 0.7 ⁿ	30.5 ± 2.1 ⁿ	32.5 ± 2.1 ⁿ	22.5 ± 0.7 ⁿ	32.0 ± 0.0 ⁿ	31.5 ± 0.7 ⁿ	50.0 ± 0.0 ⁿ	44.5 ± 0.7 ⁿ	49.0 ± 1.4 ^s	36.0 ± 0.0 ^s	
	milk	13.5 ± 0.7 ^s	20.0 ± 0.0 ⁿ	17.0 ± 0.0 ⁿ	17.0 ± 0.0 ⁿ	14.5 ± 0.7 ⁿ	26.0 ± 0.0 ⁿ	14.0 ± 0.0 ⁿ	24.0 ± 1.4 ⁿ	15.5 ± 0.7 ⁿ	15.5 ± 0.7 ⁿ	15.0 ± 0.7 ^s	
90	MRS	21.5 ± 0.7 ⁿ	38.0 ± 1.4 ⁿ	30.0 ± 0.0 ⁿ	28.0 ± 0.0 ⁿ	24.5 ± 0.7 ⁿ	31.5 ± 2.1 ⁿ	30.0 ± 0.0 ⁿ	50.0 ± 0.0 ⁿ	43.0 ± 0.0 ^s	45.0 ± 0.0 ⁿ	35.0 ± 0.0 ^s	
	milk	13.0 ± 0.0 ^s	20.5 ± 0.7 ⁿ	16.5 ± 0.7 ⁿ	16.5 ± 0.7 ^s	14.0 ± 0.0 ⁿ	24.0 ± 0.0 ⁿ	13.0 ± 0.0 ⁿ	23.5 ± 0.7 ⁿ	15.0 ± 0.0 ^s	15.0 ± 0.0 ^s	14.0 ± 0.0 ⁿ	
	MRS	23.0 ± 1.4 ⁿ	39.0 ± 1.4 ^s	30.0 ± 0.0 ⁿ	29.5 ± 2.1 ⁿ	24.5 ± 2.1 ⁿ	31.0 ± 1.4 ⁿ	32.0 ± 1.4 ⁿ	50.0 ± 0.0 ⁿ	42.0 ± 0.0 ^s	46.0 ± 1.4 ⁿ	36.0 ± 1.4 ^s	
	milk	13.0 ± 0.0 ^s	21.0 ± 1.4 ⁿ	17.5 ± 0.7 ⁿ	16.0 ± 0.0 ^s	14.0 ± 0.0 ⁿ	26.5 ± 0.7 ⁿ	14.0 ± 0.0 ⁿ	22.5 ± 0.7 ⁿ	15.5 ± 0.7 ⁿ	15.5 ± 0.7 ⁿ	14.0 ± 0.0 ⁿ	
150	milk	13.0 ± 0.0 ^s	21.0 ± 1.4 ⁿ	17.5 ± 0.7 ⁿ	16.0 ± 0.0 ^s	14.0 ± 0.0 ⁿ	26.5 ± 0.7 ⁿ	14.0 ± 0.0 ⁿ	22.5 ± 0.7 ⁿ	15.5 ± 0.7 ⁿ	15.5 ± 0.7 ⁿ	14.0 ± 0.0 ⁿ	
	MRS	24.5 ± 0.7 ⁿ	37.5 ± 0.7 ⁿ	32.0 ± 0.0 ^s	31.0 ± 1.4 ⁿ	24.0 ± 1.4 ⁿ	33.5 ± 0.7 ⁿ	30.5 ± 0.7 ⁿ	50.0 ± 0.0 ⁿ	42.5 ± 0.7 ^s	50.0 ± 0.0 ^s	37.5 ± 2.1 ^s	

n – not significant; *s* – significant, *P* ≤ 0.05

Table 2
Antibacterial activity of the *Lactobacillus acidophilus* T132/2 strain – II passage

Pressure [MPa]	Growth medium	The size of growth inhibition zones of the test strains [mm]											
		<i>Escherichia coli</i> 366	<i>Escherichia coli</i> 345	<i>Escherichia coli</i> L1	<i>Enterobacter cloacae</i> 6	<i>Enterobacter cloacae</i> 10	<i>Enterobacter cloacae</i> 11	<i>Enterobacter cloacae</i> 17	<i>Proteus</i> 6H	<i>Proteus</i> du	<i>Klebsiella</i> ssp. 499	<i>Klebsiella pneumoniae</i> B	
0	Passage	II	II	II	II	II	II	II	II	II	II	II	
	milk	17.0 ± 0.0	23.0 ± 0.0	22.5 ± 0.7	21.0 ± 1.4	16.0 ± 0.0	25.0 ± 0.0	19.5 ± 0.7	36.5 ± 0.7	32.5 ± 0.7	30.5 ± 2.1	30.0 ± 0.0	
	MRS	28.0 ± 0.0	39.0 ± 1.4	34.5 ± 2.1	47.5 ± 0.7	26.0 ± 1.4	32.5 ± 0.7	31.5 ± 2.1	53.5 ± 2.1	54.0 ± 2.8	55.0 ± 1.4	29.5 ± 0.7	
	milk	17.5 ± 0.7 ⁿ	23.0 ± 0.0 ⁿ	21.0 ± 0.0 ⁿ	20.0 ± 0.0 ⁿ	17.0 ± 0.0 ^s	23.0 ± 0.0 ^s	19.0 ± 0.0 ⁿ	35.5 ± 0.7 ⁿ	32.0 ± 0.0 ⁿ	31.5 ± 0.7 ⁿ	30.0 ± 0.0 ⁿ	
30	MRS	29.5 ± 2.1 ⁿ	39.0 ± 1.4 ⁿ	35.5 ± 2.1 ⁿ	48.5 ± 0.7 ⁿ	24.5 ± 2.1 ⁿ	31.5 ± 0.7 ⁿ	31.0 ± 1.4 ⁿ	54.5 ± 0.7 ⁿ	49.0 ± 1.4 ^s	52.5 ± 3.5 ⁿ	32.0 ± 1.4 ^s	
	milk	17.0 ± 0.0 ⁿ	25.0 ± 0.0 ^s	21.0 ± 0.0 ⁿ	21.0 ± 0.0 ⁿ	16.0 ± 0.0 ⁿ	24.0 ± 1.4 ⁿ	19.0 ± 0.0 ⁿ	36.0 ± 0.0 ⁿ	32.5 ± 0.7 ⁿ	28.5 ± 0.7 ⁿ	29.0 ± 1.4 ⁿ	
	MRS	28.0 ± 0.0 ⁿ	37.0 ± 1.4 ⁿ	33.0 ± 1.4 ⁿ	40.0 ± 0.0 ^s	23.0 ± 1.4 ^s	28.0 ± 0.0 ^s	31.5 ± 2.1 ⁿ	54.5 ± 0.7 ⁿ	50.0 ± 0.0 ⁿ	55.0 ± 1.4 ⁿ	28.0 ± 0.0 ⁿ	
	milk	19.0 ± 0.0 ^s	24.0 ± 0.0 ^s	22.0 ± 0.0 ⁿ	20.0 ± 1.4 ⁿ	16.0 ± 0.0 ⁿ	24.0 ± 0.0 ⁿ	19.5 ± 0.7 ⁿ	36.5 ± 0.7 ⁿ	33.5 ± 0.7 ⁿ	30.0 ± 0.0 ⁿ	32.0 ± 0.0 ^s	
90	MRS	26.5 ± 0.7 ⁿ	36.0 ± 0.0 ^s	31.5 ± 0.7 ⁿ	45.5 ± 3.5 ⁿ	26.5 ± 0.7 ⁿ	28.0 ± 2.8 ⁿ	31.0 ± 1.4 ⁿ	49.5 ± 2.1 ^s	49.0 ± 0.0 ^s	55.0 ± 0.0 ⁿ	29.0 ± 0.0 ⁿ	
	milk	18.0 ± 0.0 ^s	23.5 ± 0.7 ⁿ	21.0 ± 1.4 ⁿ	20.5 ± 0.7 ⁿ	17.5 ± 0.7 ^s	23.5 ± 0.7 ⁿ	20.0 ± 0.0 ⁿ	37.5 ± 0.7 ⁿ	36.5 ± 0.7 ^s	30.0 ± 0.0 ⁿ	30.0 ± 0.0 ⁿ	
	MRS	25.0 ± 1.4 ^s	36.0 ± 0.0 ^s	32.5 ± 0.7 ⁿ	40.5 ± 0.7 ^s	27.0 ± 1.4 ⁿ	30.5 ± 0.7 ⁿ	27.0 ± 1.4 ^s	51.0 ± 1.4 ⁿ	49.0 ± 1.4 ^s	50.0 ± 0.0 ^s	30.0 ± 0.0 ⁿ	
	milk	19.0 ± 0.0 ^s	25.0 ± 0.0 ^s	21.0 ± 0.0 ⁿ	21.5 ± 0.7 ⁿ	18.0 ± 0.0 ^s	24.5 ± 0.7 ⁿ	20.0 ± 0.0 ⁿ	38.0 ± 0.0 ^s	37.0 ± 0.0 ^s	30.0 ± 0.0 ⁿ	32.0 ± 0.0 ^s	
150	MRS	28.0 ± 0.0 ⁿ	37.0 ± 0.0 ⁿ	33.0 ± 1.4 ⁿ	49.0 ± 1.4 ^s	26.5 ± 0.7 ⁿ	32.0 ± 2.8 ⁿ	31.0 ± 1.4 ⁿ	49.0 ± 1.4 ^s	54.0 ± 2.8 ⁿ	56.0 ± 0.0 ⁿ	32.5 ± 0.7 ^s	

ⁿ – not significant; ^s – significant, $P \leq 0.05$

- acidifying activity of *Lactobacillus acidophilus* strains of the culture run on milk was higher than that of the culture established on the liquid MRS medium;
- an increase was observed in the acidifying activity of the strain *Lactobacillus acidophilus* T294 after pressurization of the culture run on milk;
- antibacterial activity of *Lactobacillus acidophilus* in the culture established in the MRS medium was higher than that of the culture run on milk;
- pressurization was observed to evoke a slight increase in antibacterial activities of the examined strains of *Lactobacillus acidophilus*.

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ANATOMY OF THE COMMON CALCANEAL TENDON IN RAT (*RATTUS NORWEGICUS*)

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Key words: tendon, rat, common calcaneal tendon, rotation, fascicles.

Abstract

The aim of the study was to describe anatomy of the common calcaneal tendon in rat (*Rattus norvegicus*) and to correlate individual parts of the tendon with the muscles that act with the aid of them.

Ten pelvic extremities of adult rats were fixed in 10% of formaldehyde and were dissected layer-by-layer method with microsurgical instruments under the operating microscope (4 and 10 fold magnification). The fascicles of the soleus muscle and the lateral head of the gastrocnemius muscle compose the deepest layer of the tendon. The fascicle of the medial head of the gastrocnemius muscle is located more superficially. The strong tendon of the plantaris muscle covers the fascicles listed above. The common calcaneal tendon in rat is composed of twisted fascicles attaching on the tuber calcanei. Fascicles of the following muscles: gastrocnemius and soleus are twisted along the long axis of the common calcaneal tendon, which is additionally strengthened from behind by the plantaris tendon.

A detailed knowledge of anatomy of the common calcaneal tendon in rats provides a better understanding of pathology of the tendon. Conclusions are often extrapolated to the human calcaneal tendon.

ANATOMIA ŚCIĘGNA PIĘTOWEGO WSPÓLNEGO U SZCZURA (*RATTUS NORWEGICUS*)

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Słowa kluczowe: ścięgno, szczur, ścięgno piętowe wspólne, rotacja, pęczki.

Abstrakt

Celem pracy było zbadanie budowy ścięgna piętowego wspólnego u szczura (*Rattus norvegicus*) oraz powiązanie części ścięgna z odpowiednimi mięśniami.

Metodą warstwową z użyciem narzędzi mikrochirurgicznych pod mikroskopem operacyjnym (powiększenie 4x i 10x) preparowano dziesięć kończyn miednicznych dorosłych szczurów utrwalonych w 10% formalinie. Najgłębszą warstwę ścięgna tworzą pęczki ścięgna mięśni: płaszczkowatego oraz głowy bocznej mięśnia brzuchatego łydki. Bardziej powierzchownie znajduje się pęczek od głowy przysrodkowej mięśnia brzuchatego łydki. Wymienione pęczki pokryte są przez silne ścięgno mięśnia podeszwowego.

Ścięgno piętowe wspólne szczura składa się ze zrotowanych pęczków przyczepiających się na guzie piętowym. Pęczki ścięgna mięśnia brzuchatego łydki i mięśnia płaszczkowatego ulegają rotacji wokół osi długiej ścięgna piętowego wspólnego, dodatkowo od tyłu wzmocnione są przez ścięgno mięśnia podeszwowego.

Dokładna znajomość budowy ścięgna piętowego wspólnego u szczura pomoże lepiej zrozumieć procesy związane z jego patologią. Wnioski z nich często ekstrapolowane są bezpośrednio na człowieka.

Introduction

Rats belong to the semiplantigrade animals (SIKORSKA-PIWOWSKA 1984) and in scientific research are animal model for tendon related researches. Studies concerning injection of the corticosteroids to the tendon or the impact of stimuli on the tendon (ALMEIDA et al. 2009) as well as the effect of drugs (e.g. ciprofloxacin) on the tendon structure were conducted. There are no detailed data on the structure of the common calcaneal tendon of rat in the available literature. Neither are there further details regarding a comparison of the rat tendon with its human analogue, Achilles tendon. According to PARSONS (1894) the anatomy of the beavers' common calcaneal tendon is similar to that seen in other rodents. The common calcaneal tendon in wood rat (*Phloeomys cumingi*), which is tree-living (aboreal) and ground-living (nonaboreal) animal, is composed of the fused tendons of the gastrocnemius muscle, soleus muscle and plantaris muscle (BRAZIER 1926). There is an inaccuracy regarding the nomenclature of the muscles in rat. Some authors distinguish in rats the flexor digitorum superficialis muscle (ETTEMA et al. 2006, THOMAS et al. 2009) while other authors identify this muscle as the plantaris muscle (DEGENS et al. 2009, ISHIHARA et al. 1998). Such differences may result from the type of locomotion (SIKORSKA-PIWOWSKA 1984). The plantaris muscle is present in plantigrade animals (e.g. bear), while in digitigrades animals the flexor digitorum superficialis muscle is distinguished (eg. dog). Rats are digitigrade and plantigrade animals. In our study we used name "plantaris muscle" since this term is most commonly used in the literature referring to this tendon.

The aim of the study

The aim of the study was to describe internal anatomy of the common calcaneal tendon in rat. In our research the individual parts of the tendon were correlated with muscles that act with the aid of them.

Material and Method

Specimens of adult rats, fixed in 10% formaldehyde solution, came from the collection of the Department of Descriptive and Clinical Anatomy at Medical University of Warsaw.

Ten pelvic limbs (5 right and 5 left) of rats were dissected under surgical microscope (4 and 10 fold magnification was used) with layer-by-layer method. Initially the skin was removed, then the fascia was dissected and eventually the individual parts of the common calcaneal tendon were analyzed with their correlation with the muscles that act with the aid of them. Finally, the tendons fibres (tendon fascicles), which are a direct continuation of the tendon, were dissected. Photographic documentation was made at each stage of the study.

Results

The triceps surae muscle in rat is composed of the gastrocnemius muscle and the soleus muscle (Figure 1, Figure 2). The common calcaneal tendon is covered by the plantaris tendon at the level of the tuber calcanei (Figure 3). Tendinous fibres of the plantaris tendon are located superficially and they cover the lower part of the common calcaneal tendon which is mostly composed of the fascicles from the gastrocnemius muscle. At the level of the tuber calcanei, the plantaris tendon covers it, becomes a little wider and partially attaches to it (Figure 2).

In the superior part of rats' leg the biceps femoris muscle covers the triceps surae muscle. In the inferior part it is located superficially (Figure 1).

During the dissection of the common calcaneal tendon we observed that the tendon fibres are loosely connected one to another and their separation during a gentle dissection was relatively easy. The fusion between the individual parts of the common calcaneal tendon that occurred just above the tuber calcanei was distinctly stronger.

The most superficial fibres in the common calcaneal tendon were those coming from the plantaris tendon (Figure 3). Tendinous fibres from the gastrocnemius are located deeper than the fibres coming from the plantaris



Fig. 1. The common calcaneal tendon in rat (left side), posterolateral aspect: 1 – the medial head of the gastrocnemius muscle; 1a – the fascicle from the medial head of the gastrocnemius muscle; 2 – the lateral head of the gastrocnemius muscle (* – aponeurosis); 2a – the fascicle from the lateral head of the gastrocnemius muscle; 3 – the plantaris muscle; 4a – the plantaris tendon; 5 – the tuber calcanei

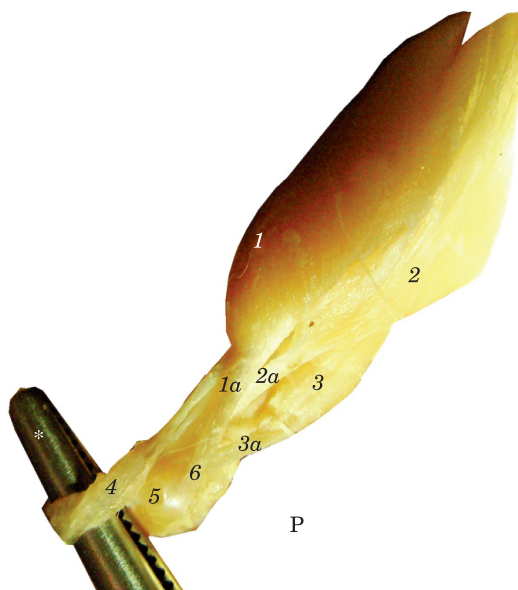


Fig. 2. The right common calcaneal tendon in rat, posterior aspect: 1 – the medial head of the gastrocnemius muscle; 1a – the fascicle from the medial head of the gastrocnemius muscle; 2 – the lateral head of the gastrocnemius muscle; 2a – the fascicle from the lateral head of the gastrocnemius muscle; 3 – the soleus muscle; 4 – the plantaris tendon; 5 – the tuber calcanei; 6 – the level of the connection between the fascicles of the triceps surae muscle, the common calcaneal tendon

tendon (Figure 4). Considering the two heads of the gastrocnemius, the medial one sends more superficial fibres lying beneath the fibres from the plantaris tendon. The deepest layer of the common calcaneal tendon is formed by tendineous fibres from the soleus muscle and from the lateral head of the gastrocnemius muscle (Figure 5).

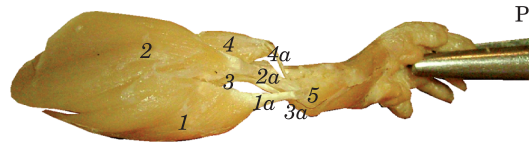


Fig. 3. The right common calcaneal tendon in rat. Posterior aspect: 1 – the medial head of the gastrocnemius muscle; 1a – the fascicle from the medial head of the gastrocnemius muscle; 2 – the lateral head of the gastrocnemius muscle; 2a – the fascicle from the lateral head of the gastrocnemius muscle; 3 – the plantaris muscle; 3a – the fascicle from the plantaris muscle; 4 – the soleus muscle; 4a – the fascicle from the soleus muscle

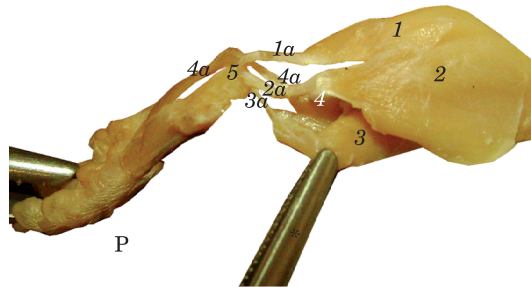


Fig. 4. The common calcaneal tendon in rat (right side). Posterolateral aspect: 1 – the medial head of the gastrocnemius muscle; 1a – the fascicle from the medial head of the gastrocnemius muscle; 2 – the lateral head of the gastrocnemius muscle; 2a – the fascicle from the lateral head of the gastrocnemius muscle; 3 – the soleus muscle; 3a – the fascicle from the soleus muscle; 4 – the plantaris muscle; 4a – the fascicle from the plantaris muscle; 5 – the tuber calcanei

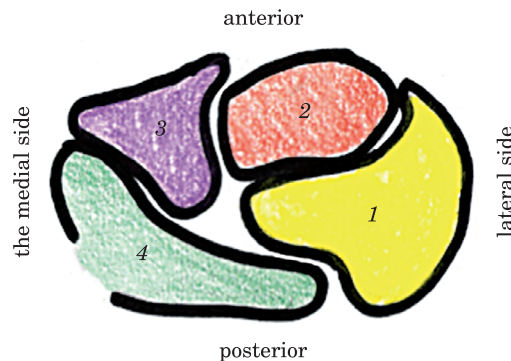


Fig. 5. The transverse cross section of the common calcaneal tendon in rat: 1 – the fascicle from the medial head of the gastrocnemius muscle; 2 – the fascicle from the lateral head of the gastrocnemius muscle; 3 – the fascicle from the soleus muscle; 4 – the plantaris tendon

Discussion

The tendons that we examined in the study came from the adult rats in which the ultimate structure of the tendon, as well as its attachment, was developed (MURATA et al. 2000, RUFAI et al. 1992). In our material tendons fibres of the common calcaneal tendon come from the gastrocnemius muscle, the soleus muscle and the plantaris muscle, which is in accordance with observation made by other authors (BRAZIER 1926). This conception is also in accordance with the generally accepted scheme or pattern of the anatomy of the common calcaneal tendon in animals (KRYSIK 1981, KRYSIK et al. 2001, *Nomina Anatomica Veterinaria* 2005). As it was mentioned in the introduction, most of the research studies done on the common calcaneal tendon in rats are related to experimentally induced tendinitis or peritendinitis, rupture of the tendon, testing different therapeutic methods (eg. influence of drugs) and investigations of the tendon healing. The results of such researches (in which rats are used) are quite arbitrarily extrapolated to the human calcaneal tendon (SHAKIBAEI and STAHLMANN 2001). Differences in the internal structure of tendons and type of locomotion are not taken under consideration. The findings from the literature review confirm a lack of data on the internal structure of the common calcaneal tendon in rats and its comparison to the human analogue. There is information, given by BRAZIER (1926) who studied the anatomy of the wood rat that the fascicle of the medial head of the gastrocnemius muscle runs downwards and laterally. Nevertheless, as far as the lateral head of the gastrocnemius muscle is concerned similar report is not given which makes his description incomplete.

In our study we revealed that the common calcaneal tendon in rat is composed of twisted fascicles, which refers to the anatomy of the human calcaneal tendon (SZARO et al. 2009). However, there is a difference in number of tendons' components in both species. In humans the plantaris tendon is not a part of the Achilles tendon. In rats the plantaris tendon is relatively bigger and it contributes to the formation of the posterior part of the common calcaneal tendon. The fascicles of rats' common calcaneal tendon are loosely connected, as it was already mentioned, while tight junctions connect the fascicles of the human Achilles tendon. Comparing the common calcaneal tendon in rat and horse (SZARO et al. 2011) we can see that there is a tendency to multiply the fascicles in digitigrade animals such as horse (SIKORSKA-PIWOWSKA 1984). It helps to stabilize the autopodium in the digitigrade animals in the upright position. In the human Achilles tendon there are three fascicles participating in the formation of the tendon. They come from the medial and lateral heads of the gastrocnemius and from the soleus muscle. The common calcaneal tendon in the horse is composed of the five fascicles coming

from the two heads of the gastrocnemius muscle, the soleus muscle and from two calcaneal stripes derived from the muscles of the thigh (SZARO et al. 2011). As far as the number of the fascicles of the tendon is concerned, the common calcaneal tendon in the rat is more similar to the Achilles tendon in humans (representing the plantigrade locomotion) than to the analogue of this tendon in horses (which are digitigrade animals).

As it was mentioned in the results section the distinct connections between the fascicles of the common calcaneal tendon occur just above the tuber calcanei and this is where the tendon becomes “common” indeed, since it practically unifies the fascicles at this level and not above. A term “the calcaneal cord” which is used by some authors seems to be a very suitable and an alternative name for the common calcaneal tendon. This name emphasizes the fact that individual parts (or the fascicles) of the common calcaneal tendon form an integral structure. It has been proved in the current study but it was also previously reported in our study concerning the structure of the common calcaneal tendon in the horse (SZARO et al. 2011). Some authors believe that such observations (the fusion of the fascicles of the gastrocnemius, soleus and plantaris) can be the starting point for distinguishing the muscle complex called “guadriceps surae muscle” (BRAZIER 1926). However, such a term does not exist in the modern veterinary nomenclature (MILART 2002, *Nomina Anatomica Veterinaria* 2005).

Conclusions

The common calcaneal tendon in rat is not a homogeneous structure but it is composed of the mutually rotated fascicles. Tendon fascicles connect the muscle bellies (which act with the aid of the tendon) with the tuber calcanei. Complexity of the internal structure of the common calcaneal tendon in rats is probably related to a type of locomotion.

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