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INFLUENCE OF NITROGEN FERTILISATION ON THE TECHNOLOGICAL VALUE OF SEMI-DWARF GRAIN WINTER TRITICALE VARIETIES ALEKTO AND GNIEWKO

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K e y w o r d s: semi-dwarf winter triticale, varieties, nitrogen fertilisation, quality features, test baking.

A b s t r a c t

This paper examines the milling value of grain and baking quality of flour from two semi-dwarf varieties of winter triticale (Alekto and Gniewko) grown at different nitrogen fertilisation doses (60, 90 (60+30), 120 (90+30), 150 (90+60) kg N·ha^{-1}. Grain harvested in two seasons: 2008/2009 and 2010/2011, was subjected to evaluation. The nitrogen fertilisation was significant influence on test weight, flour extract, protein content, sedimentation value, dough development and dough stability. Tested varieties were differ in flour extract, sedimentation value and flour colour. It has been demonstrated that the grain obtained from semi-dwarf triticale cultivars cultivated at different levels of nitrogen supply did not meet the requirements regarding the baking value set for grains used to bake good quality bread. The main reasons were a low falling number and low content as well as poor quality of wet gluten in grain, which precluded the production of dough with good farinographic properties or achievement of sufficiently high bread yield and bread volume. The correlation analysis showed significant positive relationships between the nitrogen fertilisation versus the content of protein or wet gluten in winter triticale grain as well as the development and stability of dough.

WPŁYW NAWOŻENIA AZOTEM NA JAKOŚĆ TECHNOLOGICZNĄ ZIARNA PÓŁKARŁOWEGO PSZENŻYTA OZIMEGO ODMIAN ALEKTO I GNIEWKO

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S ł o w a k l u c z o w e: półkarłowe pszenżyto ozime, odmiany, nawożenie azotem, cechy jakościowe, próbny wypiek.

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A b s t r a k t

W pracy oceniono wartość przemiałową i wypiekową ziarna i mąki dwóch odmian półkarłowych pszenżyta ozimego (Alekto i Gniewko) uprawianego w warunkach zróżnicowanych dawek azotu (60, 90 (60+30), 120 (90+30), 150 (90+60) kg N·ha^{-1}). Ocenie poddano surowiec zebrany w sezonie wegetacyjnym 2008/2009 i 2010/2011. Nawożenie azotem istotnie wpłynęło na gęstość ziarna, wyciąg mąki, zawartość białka, test sedymentacji, rozwój i stałość ciasta. Porównywane odmiany wykazały istotne zróżnicowanie w wyciągu mąki, teście sedymentacji i kolorze mąki. Wykazano, że otrzymany w doświadczeniu surowiec pochodzący z odmian półkarłowych pszenżyta ozimego uprawianego w warunkach różnych dawek azotu nie spełniał wymagań co do wartości wypiekowej jakie stawia się dla ziarna przeznaczonego do wyrobu pieczywa o dobrej jakości. Decydowały o tym głównie niska liczba opadania (wysoka aktywność α-amylazy) oraz mała zawartość i niska jakość glutenu mokrego w ziarnie, które nie pozwalały na wytworzenie ciasta o dobrych cechach farinograficznych, a także odpowiednio wysokiej wydajności i objętości chleba. Przeprowadzona analiza korelacji wykazała istotny, dodatni związek pomiędzy nawożeniem azotem a zawartością białka i glutenu mokrego w ziarnie pszenżyta ozimego oraz rozwojem i stałością ciasta.

Introduction

Owing to its high yielding potential and good nutritional values (JONNALA et al. 2010, HANSEN 2012), triticale has received much interest as foodstuff for the food industry (DOXASTAKIS et al. 2002, NAEEM et al. 2002, RAKHA et al. 2011, NAKURTE et al. 2012, PATTISON and TRETHOWAN 2013). The most promising future use of triticale as food depends on its identified suitability for baking (MCKEVITH 2004, TOHVER et al. 2005, RAKHA et al. 2011, NAUKURTE et al. 2012). Unfortunately, the technological quality of triticale grain is limited by the high activity of α-amylase, high ash content and low content and quality of. Although triticale can be a useful component in human diet, it now occupies a minor position on the food market (AGUIRRE et al. 2011).

Possible improvement of triticale grain quality traits can be achieved through progress in the breeding of new cultivars, which encodes the chemical composition, mainly proteins and carbohydrates (CEGLIŃSKA et al. 2006) and in the elaboration of adequate cultivation technologies. Nitrogen fertilisation is the agronomic factor that most distinctly modifies the quality traits of grain (MUT et al. 2005). An appropriate dose and application schedule can facilitate the production of a good quality plant product and are very important for effective use of nitrogen. The current triticale breeding programme aims at producing cultivars useful in the baking industry, able to retain the yielding potential of wheat and adaptability of rye from almost all geographical locations (MCGOVERIN et al. 2011).

The breeding of winter triticale has developed two forms: traditional and semi-dwarf. Semi-dwarf forms have their own, characteristic agronomic requirements. They are more resistant to lodging (shorter stem), which makes them particularly suitable for intensive cultivation, including better utilization

of higher nitrogen doses. Recognition of their response to nitrogen fertilisation levels in the context of modelling the quality of grain and baking flour would open up a new vista for further expansion of this crop. Such experiments on semi-dwarf triticale have not been conducted until now, which has encouraged us to undertake this research with the aim of analysing the milling and baking value of grain harvested from semi-dwarf winter triticale varieties grown at different nitrogen fertilisation doses.

The working hypothesis assumes that, when fertilised with a certain nitrogen dose, the selected winter triticale cultivars (Gniewko and Alekto) would meet the qualitative requirements for grain intended for bread baking and would thus determine a new direction of triticale flour utilisation for baking. The aim of the study was determining the suitability of the new winter triticale grain in the baking industry, including evaluation of grain quality traits selected depending on the applied nitrogen fertilisation on the value of the milling and baking.

Materials and Methods

The analyses were based on results obtained from a two-year controlled field experiment conducted at the Experimental Station in Bałcyny, Poland (N=53°35'49''; E=19°51'20.3''). The experiment was set up on grey-brown podozolic soil developed on light clay, according to the split-plot method with four replications. Two semi-dwarf winter triticale cultivars (Alekto and Gniewko) were grown on plots supplied with different doses of nitrogen, in the following scheme: A – 60 kg·ha^{-1} with a single application of the whole dose, B - 90 kg·ha^{-1} split into 60+30, C – 120 kg·ha^{-1} split into 90+30 and D – 150 kg·ha^{-1} split into 90+60. The whole dose of 60 kg·ha^{-1} (variant A) or the first part of a higher dose (variants B, C and D) were applied in spring, at the resumed plant growth (29 stage on the BBCH scale), while the second part was supplied at the 38 BBCH plant growth stage. Phosphorus and potassium fertilisation was carried out in a single application before sowing, in doses of 30 kg P·ha^{-1} and 75 kg K·ha^{-1}. Winter triticale was sown in the last ten days of September, using 400 seeds per 1 m^{-2}. The seeds were treated with the seed dressing preparation Baytan Universal 094 FS (active ingredient *triadimenol* + *imazalil* + *fuberidazole*). The antifungal protection consisted of a spraying treatment with the preparation Input 460 EC in the amount of 1 l·ha^{-1} (*spiroxamine* + *prothiocanazole*) during the first node phase (31 BBCH) and the application of the fungicide Prosaro 250 EC in the dose of 0.6 1 l·ha^{-1}) (*tebuconazole* + *prothiocanazole*) during the full heading phase (58 BBCH). Weed control was composed of a single spraying treatment with a mixture of

herbicides (Boxer 800 EC 2 l·ha^{-1} – a.i. *prosulfocarb* , Glean 75 WG 5 g·ha^{-1} – a.i. *chlorosulfuron*, Legato 500 SC 0.5 l·ha^{-1} – a.i. d*iflufenican*) carried out in autumn, during the BBCH 29 growth stage.

Alekto is semi dwarf variety (one of the shortest among triticale varieties registered in Poland), distinguished by a high yield potential in different soil and climatic. Alecto has very good resistance against most fungal diseases, variety with good winter hardiness. The advantage of this variety is high in protein. Gniewko is semi dwarf variety also but with a relatively small frost resistance. High resistance to *Blumeria graminis* and *Puccinia recondita* and average resistance to *Septoria tritici* and *nodorum*, low resistance to *Fusarium* spp. and stem base diseases. The plants are characterized by rather low resistance to sprouting of grain in the ear and small falling number. The scientific literature to date has not presented research results with the above-mentioned winter triticale varieties. There is a new opportunity to improve the value of this cereal grain baking. In 2006, entered into the National Register semi-dwarf, Gniewko variety of high protein content, which stated by PCR method increased participation of macromolecular gluten proteins.

Samples of grains were analysed, including the following determinations: bulk density of grain using a densitometer, protein content with Kjeldahl's method (N x 6.25) according to the ICC-105/2 standard modified by Tecator, sedimentation rate in a solution of SDS (sodium dodecyl sulphate) (AXFORD et al. 1979), falling number according to ICC standard 107/1 Falling Number 1400 (ICCH), wet gluten content according to ICC standard 155 (a Glutomatic 2200 system, ICCH), flour yield on a Quadrumat Senior Brabender. Farinographic analyses determined: hygroscopicity of flour, dough development, stability and weakening. These determinations were made according to ICC standard 115/1 on a Brabender farinograph (ICCH). Samples of grain were ground in an MLU 202 laboratory mill (manufactured by Buhler) according to Sitkowski's method. Whiteness of the flour was determined in a Karl Zeiss Jena leucometer. The bread baking test made in a laboratory electric oven provided data for determinations of the bread yield and volume.

For the statistical analysis have been selected average samples from nitrogen fertilisation objects. The results were submitted as two factorial Anova for the model of the completely randomized design (CRD) with Statistica®10. Tukey's test at a significance of 0.05 was applied to verify the significance of differences. Pearson correlation coefficients were computed.

The two years when the field experiments were conducted were varied in the weather conditions, especially in the distribution of rainfalls during the growing season (fig. 1). This had a direct impact on the growth and development of winter triticale. In the analyzed seasons, the early autumn plant

growth was accompanied by rainfall shortages and temperatures higher than multi-annual average. In the growing seasons of 2008 April was a very dry and warm month, while May and June were wet. In the growing season 2010 wet conditions were similar to multi-annual average. Air temperatures higher than the average occurred in July, which was favorable for the plants as it helped the grain and straw to dry properly. Triticale harvest was particularly troublesome in 2011, when the rainfall in July was as much as 211% higher than the multi-year mean.

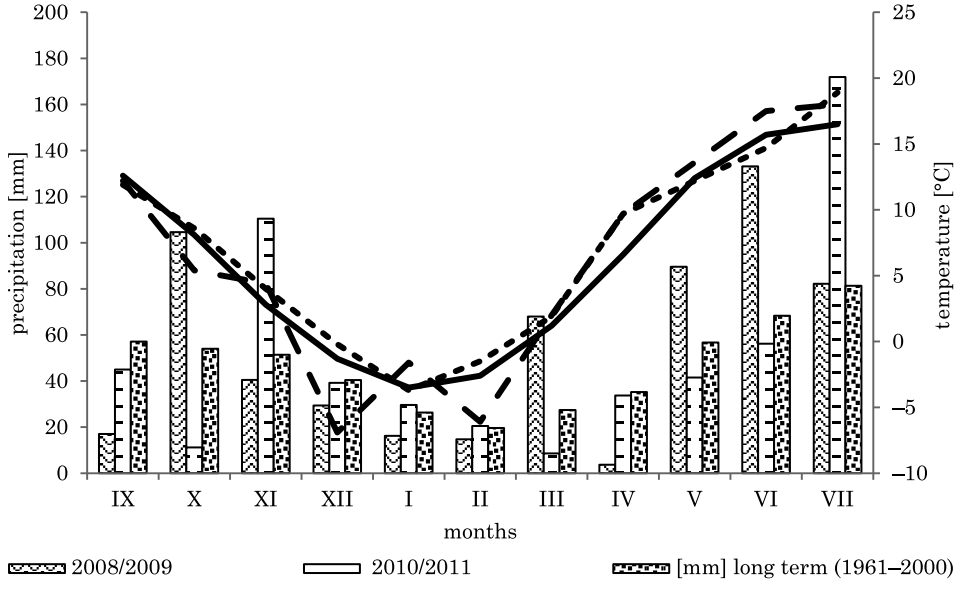

Fig. 1. Rainfall and temperature distribution during the growing seasons

Results and Discussion

Grain density is a volumetric measure of grain mass in kilos per hectolitre. High grain density confirms good grain filling, whereas low density suggests that kernels are wrinkled and the endosperm is poorly filled. In our analyses, triticale grain demonstrated a volumetric mass in the range of 68.5–71.4 kg·hl^{-1}, significantly differentiated by the nitrogen doses (Table 1). Similar grain density values for this cereal species (65.4–71.5 kg·hl^{-1}) were experimentally assessed by KALNIN et al. (2013). In our experiment, the highest triticale grain density was achieved from the plot supplied 90 kg N·ha^{-1}. The cultivar x nitrogen fertilisation interactions demonstrated that the highest grain volumetric mass was produced by cv. Alekto in response to the nitrogen doses of 60 and 90 kg N·ha^{-1}, and by cv. Gniewko fertilised supplied 90 and 120 kg N·ha^{-1}.

Table 1

Milling and baking features of winter triticale grain (mean value)

Parameter	Variety	Fertilisation N (kg·ha^{-1})				Mean
		60	90	120	150	
Test weight (kg·hl^{-1})	Gniewko	70.7	71.4	69.7	68.5	70.0
	Alekto	68.9	70.1	70.3	69.8	69.7
	mean	69.8	70.7	70.0	69.1	–
$LSD_{(0.05)}$ nitrogen fertilisation – 0.89, interaction – nitrogen fertilisation x varieties – 1.26, other – n.s.t						
Flour extract (%)	Gniewko	66.3	65.6	64.8	65.0	65.4
	Alekto	67.6	67.8	67.4	66.7	67.4
	mean	67.0	66.7	66.1	65.9	–
$LSD_{(0.05)}$ nitrogen fertilisation – 0.63, varieties – 0.44, other – n.s.						
Protein content (% s.m.)	Gniewko	9.6	10.4	11.5	11.7	10.8
	Alekto	10.8	11.0	11.5	11.8	11.3
	mean	10.2	10.7	11.5	11.7	–
$LSD_{(0.05)}$ nitrogen fertilisation – 0.82, other – n.s.						
Gluten content (%)	Gniewko	10.4	13.0	17.5	17.8	14.7
	Alekto	13.8	17.3	18.7	17.7	16.9
	mean	12.1	15.1	18.1	17.8	–
$LSD_{(0.05)}$ not significant difference						
SDS sediment value (ml)	Gniewko	35.6	38.4	41.3	41.3	39.1
	Alekto	45.5	46.5	45.5	48.5	46.5
	mean	40.5	42.4	43.4	44.9	–
$LSD_{(0.05)}$ nitrogen fertilisation – 1.58, varieties – 1.12, interaction nitrogen fertilization x varieties – 2.25						
Falling number (s)	Gniewko	118	125	145	136	131
	Alekto	176	128	139	164	152
	mean	147	127	142	150	–
$LSD_{(0.05)}$ not significant difference						

The flour extract from grain yielded by the two analysed triticale cultivars ranged from 64.8 to 67.8%, which is a rather low percentage range, as verified by the results of other authors (SOBCZYK et al. 2009). In our research, a significantly higher flour yield was obtained from cv. Alekto grain (Table 1). The most favourable flour extract was achieved from the plots fertilised with 60 or 90 kg N·ha^{-1}, while any further increase in nitrogen nourishment significantly depreciated this parameter. Similarly, in a study by CEGLIŃSKA et al. (2005), elevated nitrogen fertilisation depressed the yield of flour from triticale grain. However, other researchers (SOBCZYK et al. 2009) observed a small decline in flour yield at a nitrogen dose of 40 kg N·ha^{-1}, whereas higher doses (80 kg and 120 kg N·ha^{-1}) resulted in a relatively high increase of this parameter.

The grain baking value depends on the protein content. PELTONEN-SAINIO et al. (2012) claim that triticale is an interesting and promising cereal species, able to ensure a satisfactory protein yield per field area unit. The grain for

bread baking should contain around 11.5% of protein. In our study, it ranged from 9.6 to 11.8%, and was significantly dependent on the nitrogen fertilisation level (Table 1). The highest percentage of protein in grain was about 8.6% recorded by JANUŠAUSKAITĖ (2013), 10.5–13.3% determined by DENNETT and TRETHOWAN (2013) and 17% reported by EREKUL and KÖHN (2006). In our investigations, higher doses of nitrogen under triticale caused an increase in the protein content in grain, with significant increments noted up to the dose of 120 kg $N \cdot ha^{-1}$ (Table 1). The protein content expected from bread baking grain was obtained only when triticale had received nitrogen doses of 120 or 150 kg $N \cdot ha^{-1}$. The positive relationship between nitrogen fertilisation and protein content in triticale grain was confirmed by the correlation analysis ($r = 0.77$) (Table 3). Likewise, in studies by CEGLIŃSKA et al. (2005), KARA and UYSAL (2009), GULMEZOGLU and AYTAC (2010), a higher dose of nitrogen supplied under triticale caused an increase in the total protein content in all tested cultivars. Similar observations were made by CIMRIN et al. (2004). According to ZHENG et al. (2009), the protein content in grain depends on the availability of nitrogen in soil. Its deficit depressed yields and the grain content of protein. ALARU et al. (2003) claimed that the protein concentration in triticale grain is more strongly dependent on the cultivar (i.e. genetic predispositions) than the environmental conditions. However, BUREŠOVÁ et al. (2010) would disagree, having observed a significant difference in the protein content in triticale grain depending on years of the research, while failing to detect such differences between the tested varieties.

For the baking industry, the content and composition of gluten proteins in flour are of utmost importance (BRANLARD et al. 2001, KOEHLERA et al. 2010). Grain which will be ground to flour should contain at least 25% of wet gluten, as this component plays an important part in technological processes involved in dough making and bread baking (UTHAYAKUMARAN et al. 1999). In our investigation, the content of wet gluten in grain was low, ranging from 10.4 to 18.7%, which is less than the required minimum (Table 1). Its content in grain was not significantly differentiated by the cultivar or nitrogen fertilisation level. There was just a tendency for a higher wet gluten content in grain from cv. Alekto and in response to the nitrogen doses raised to 120 kg $N \cdot ha^{-1}$. The analysis of correlations showed a significant, albeit weaker than for the protein content, relationship between the nitrogen fertilisation and wet gluten content ($r = 0.59$) (Table 3). TOHVER et al. (2005) concluded that triticale gluten behaves analogously to gluten in rye grain, but in terms of quality it is worse than gluten in bread baked from wheat flour.

A fundamental measure in the quality assessment of gluten proteins is the sedimentation rate. In our study, this property ranged from 35.6 ml to 48.5 ml, indicating poor quality of gluten. Its value depended on the cultivar and

nitrogen fertilisation level (Table 1). A significantly higher sedimentation rate was obtained for the grain produced by cv. Alekto. Higher doses of nitrogen had a positive effect on the protein composition measured by the SDS test, with the improved results achieved for cv. Gniewko up to the level of 120 kg $N \cdot ha^{-1}$, and for cv. Alekto – up to 150 kg $N \cdot ha^{-1}$. The beneficial influence of increasing nitrogen doses on the value of sedimentation rate has also been implicated by others (CEGLIŃSKA et al. 2005).

The baking quality assessment devotes much attention to the enzymatic properties of grain, in particular the activity of α-amylase, which is measured by the falling number. It determines the usefulness of grain for baking and is strictly associated with the resistance of grain to sprouting. Grain with a moderate amylolytic activity, whose falling number is within 200–300 seconds, is best for flour production. In our experiment, the amylolytic activity of triticale grain was high (low falling numbers within 118–164 seconds) and rather weakly differentiated by the cultivars or nitrogen doses. Also, EREKUL and KÖHN (2006) determined a lower falling number for triticale than for wheat or rye. The literature contains frequent mentions of the falling number around 62–70 seconds (MARTINEK et al. 2008, KALNINA et al. 2013), 120 seconds (depending on the weather) (ALARU et al. 2009), up to 180 seconds, but the mean value is below 100 seconds (YASEEN et al. 2007). A low falling number is generally connected with kernels sprouting on triticale plants even before harvest, but other causes can be involved (MARES and MARVA 2008).

The colour of the flour was differentiated only by the cultivar (Table 2). Significantly whiter flour was obtained from grain of cv. Alekto. The influence of a cultivar and nitrogen dose on the water absorption by flour was statistically insignificant (Table 2). There was only a trend for a slightly better hygroscopicity of flour from the cv. Gniewko grain. CEGLIŃSKA et al. (2005) also did not demonstrate any significant differences in the water absorption by flour caused by cultivars or nitrogen fertilisation.

The dough development time and its stability were significantly varied depending on the nitrogen fertilisation levels (Table 2). Higher doses of nitrogen supplied to triticale fields resulted in the dough taking longer to develop, with significant differences observed between the doses of 60 and 150 kg $N \cdot ha^{-1}$. The best dough stability was achieved for flour from the grain of triticale fertilised with the nitrogen dose equal 120 kg $N \cdot ha^{-1}$. The correlation analysis showed a positive relationship between nitrogen nourishment and the time of dough development as well as dough stability ($r = 0.63$ and $r = 0.68$, respectively) (Table 3). Dough produced from flour obtained from cv. Gniewko grain, compared to cv. Alekto, revealed just a slight tendency for possessing an improved stability.

Table 2

Flour, dough and bread properties depending on nitrogen fertilisation (mean value)

Parameter	Variety	Fertilisation N (kg·ha^{-1})				Mean
		60	90	120	150	
Flour colour (% pattern)	Gniewko	80.9	81.3	81.1	81.5	81.2
	Alekto	84.8	84.6	84.2	84.3	84.5
	mean	82.8	82.9	82.6	82.9	–
LSD $_{(0.05)}$ varieties – 1.89, other – n.s.						
Flour water absorption (%)	Gniewko	50.6	50.9	51.9	51.8	51.3
	Alekto	46.9	48.2	48.1	49.5	48.1
	mean	50.6	50.9	51.9	51.8	–
LSD $_{(0.05)}$ not significant difference						
Dough development (min.)	Gniewko	0.9	1.3	1.1	1.8	1.2
	Alekto	1.0	1.2	1.4	1.2	1.2
	mean	0.9	1.2	1.3	1.5	–
LSD $_{(0.05)}$ nitrogen fertilisation – 0.36, other – n.s.						
Dough stability (min.)	Gniewko	1.0	1.9	2.7	2.3	2.0
	Alekto	0.6	1.0	2.3	1.8	1.4
	mean	0.8	1.5	2.5	2.0	–
LSD $_{(0.05)}$ nitrogen fertilisation – 0.80, other – n.s.						
Dough softening (uB)	Gniewko	181	170	168	171	172
	Alekto	146	180	152	153	158
	mean	163	175	160	162	–
LSD $_{(0.05)}$ not significant difference						
Yield of bread (%)	Gniewko	140	140	139	141	140
	Alekto	134	134	133	133	133
	mean	137	137	136	137	–
LSD $_{(0.05)}$ not significant difference						
Volume of bread (cm^3)	Gniewko	548	528	546	528	537
	Alekto	507	540	540	547	533
	mean	527	534	543	537	–
LSD $_{(0.05)}$ not significant difference						

Table 3

Coefficient correlation between nitrogen fertilisation of winter triticale and grain quality, flour, dough and bread traits

Test characteristic	Significance levels (p)	Correlation coefficient (r)
Test weight (kg·hl^{-1})	p=0.23	-0.32
Flour extract (%)	p=0.15	-0.38
Protein content (% s.m.)	p=0.00	0.77*
Wet gluten content (%)	p=0.02	0.59*
SDS sediment value (ml)	p=0.15	0.37
Falling number (s)	p=0.65	0.12
Flour colour (% pattern)	p=0.98	-0.01
Flour water absorption (%)	p=0.49	0.18
Dough development (min.)	p=0.01	0.63*
Dough stability (min.)	p=0.00	0.68*
Dough softening (uB)	p=0.71	-0.10
Yield of bread (%)	p=0.97	-0.01
Volume of bread (cm^3)	p=0.70	0.10

* coefficients statistically significant

The effect of a cultivar or nitrogen fertilisation on dough weakening was not significant (Table 2). A slightly higher value of dough weakening (worse quality dough) was determined for cv. Gniewko grain and for plots fertilised with a dose of 90 kg $N \cdot ha^{-1}$.

Baking bread on a laboratory scale is a direct method to assess the baking quality of flour. In our study, the bread baked in a laboratory was characterized by poor volume yield, ranging from 507 to 548 cm^3. No significant effect of the cultivars or nitrogen fertilisation levels was demonstrated on this property of bread (Table 2), which is confirmed by the results reported by CEGLIŃSKA et al. (2005). In our study, the cultivar or nitrogen fertilisation factors also did not significantly modify the bread yield (Table 2) as was also reported by CEGLIŃSKA et al. (2005).

Conclusions

1. The nitrogen fertilisation had significant influence on the test weight, flour extract, protein content, sedimentation value, dough development and dough stability. Analised triticale varieties were differ in flour extract, sedimentation value and flour colour.

2. The grain harvested from semi-dwarf, winter triticale cultivars Alekto and Gniewko, grown at different nitrogen fertilisation levels, did not meet the requirements in terms of baking quality set for grains used to bake good quality bread.

3. The main reasons were a low falling number (high activity h-amylase) and a low content and poor quality of wet gluten in grain, which precluded making dough with good farinographic properties, or production of bread with sufficiently good yield and volume.

4. The correlation analysis showed a significant, positive relationship between the nitrogen fertilisation and the content of protein and wet gluten in winter triticale grain as well as the dough development and stability.

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YIELDS OF WINTER TRITICALE UNDER THE INFLUENCE OF NITROGEN FERTILISATION AND FUNGICIDE APPLICATION

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K e y w o r d s: triticosecale, grain yield, yield components, nitrogen doses, antifungal plant protection.

A b s t r a c t

This research encompassed the results of a three-year (2009–2011) field experiment conducted at the Research Station in Bałcyny near Ostróda. The first order factor was nitrogen fertilisation (kg N·ha^{-1}): 30, 60, 90, 120 and 150. The second order factor was the level of protection against fungal diseases: seed dressing, seed dressing + one application of fungicides, seed dressing + two applications of fungicides. The aim the researches was analyzing production output, expressed by grain yield and its components, as affected by different levels of nitrogen fertilisation (the yield forming factor) and protection against pathogens (the yield protecting factor). Statistical analysis of the results showed that the grain yield was significantly affected by the year of the trial, nitrogen fertilisation, fungicidal treatment and interaction of the first and second order factors. The yield structure were significatly influenced by the year of the experiment (1.000 grains weight, ears number m^2), nitrogen fertilisation (1.000 grains weight, grain number per ear), fungicial treatment (1.000 grains weight, grain weight per ear). Calculated regression equation between the dose of nitrogen and grain yield shows that the maximum yield of grain in a field experiment with the variety 'Gniewko' can be achieved at 145 kg N·ha^{-1}. The highest yield obtained (in all research years) from objects with full protection against pathogens.

PLONOWANIE PSZENŻYTA OZIMEGO POD WPŁYWEM NAWOŻENIA AZOTEM I OCHRONY FUNGICYDOWEJ

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S ł o w a k l u c z o w e: pszenżyto, plon ziarna, komponenty plonu, dawki azotu, przeciwgrzybowa ochrona roślin.

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Abstrakt

Materiał badawczy stanowiły wyniki trzyletniego (2009–2011) doświadczenia polowego zlokalizowanego w Zakładzie Produkcyjno-Doświadczalnym w Bałcynach k. Ostródy. Czynnikiem pierwszego rzędu było nawożenie azotem (kg $N \cdot ha^{-1}$): 30, 60, 90, 120, 150. Czynnik drugiego rzędu stanowił poziom ochrony przed chorobami grzybowymi: zaprawa nasienna, zaprawa nasienna + jeden zabieg ochronny, zaprawa nasienna + dwa zabiegi ochronne. Celem badań było poznanie efektów produkcyjnych – wyrażonych plonem ziarna i jego strukturą – pod wpływem zróżnicowanego poziomu nawożenia azotem (czynnik plonotwórczy) i ochrony przed patogenami (czynnik plonochronny). Analiza statystyczna wyników wykazała, iż na plon ziarna istotnie oddziaływały lata badań, nawożenie azotem, ochrona przeciwgrzybowa oraz interakcja czynników pierwszego i drugiego rzędu. Na elementy struktury plonu istotny wpływ miały lata badań (masa 1000 ziarn, liczba kłosów na m^2), nawożenie azotem (masa 1000 ziarn, liczba ziarn w kłosie), ochrona przeciwgrzybowa (masa 1000 ziarn, masa ziarna z kłosa). Z zastosowanego w doświadczeniu rachunku regresji wynika, iż optymalna dawka azotu dla pszenżyta odmiany „Gniewko" kształtuje się na poziomie 145 kg $N \cdot ha^{-1}$. We wszystkich latach badań najwyższy plon otrzymano z obiektów z pełną ochroną przeciwgrzybową.

Introduction

Triticale fields are a distinguishing feature of the Polish agriculture, a large producer of cereal grains in the EU. The total acreage cropped with triticale in Poland corresponds to about 27% of the farmland under this crop in the whole world. European agriculture also appreciated this strain of cereal. Over the last ten years, the triticale total acreage has increased by 26.8%, which clearly shows that the species is gaining importance.

Triticale was designed in order to obtain a species which combines wheat;s good quality of grain yield with the tolerance to abiotic and biotic stresses, i.e. a plant suitable for cultivation in unfavorable conditions, where the yielding of typical cereals is somewhat limited (OETTLER 2005, EREKUL and KÖHN 2006, UGARTE et al. 2007, ESTRADA-CAMPUZANO et al. 2008, VILLEGAS et al. 2010, ESTRADA-CAMPUZANO et al. 2012).

Triticale possesses a broad spectrum of use. Although it is primarily grown as animal fodder (MCGOVERIN et al. 2011), triticale is also a useful feedstock for bioethanol production, and a promising resource for making biogas (GOWDA et al. 2011). According to PELTONEN-SAINO et al. (2009), in the near future, winter cereals will be grown in northern countries on a larger acreage than today.

One of the major agrotechnical factors which affect grain yield and enable farmers to take advantage of the high production potential of cereals is mineral fertilisation, especially nitrogen nutrition (GIUNTA and MOTZO 2004, GIBSON et al. 2007, LESTINGI et al. 2010, ZEČEVIC et al. 2010). JAŚKIEWICZ (2011) claims that yields close to the maximum yielding capacity of a cultivar are achievable only when the agrotechnical requirements of a given cultivar are satisfied, hence the need to determine the response of cultvars to basic agrotechnical conditions.

Bearing in mind the above considerations, the present investigations were undertaken, in which the winter triticale cultivar 'Gniewko' was examined, with the aim of analyzing production output, expressed by grain yield and its components, as affected by different levels of nitrogen fertilisation (the yield forming factor) and protection against pathogens (the yield protecting factor).

Material and Methods

Results of a controlled field experiment, carried out at the Research Station in Bałcyny, Poland (N=53°35'49''; E=19°51'20,3'') were analyzed. The experiment was set up in a random, split-plot design, with four replications. The first order factor was nitrogen fertilisation (kg N·ha^{-1}): A-30, B-60, C-90 (60+30), D-120 (90+30), E-150 (90+60). Doses of nitrogen equal 30 and 60 kg N:ha^{-1} were applied in early spring (BBCH 27). Higher doses (90, 120, 150 kg·ha^{-1}) were applied on two dates: in the resumed plant growth (BBCH 27 and BBCH 38). Phosphorus and potassium fertilisation was applied before sowing in the total amounts of 30 kg P·ha^{-1} and 75 kg K·ha^{-1}. The second order factor was the level of plant protection against fungal diseases: a-seed dressing, b-seed dressing + one application of fungicide, c-seed dressing and two applications of fungicide. The seed dressing preparation was Baytan Universal 094 FS (*active ingredient triadimenol + imazalil + fuberidazole)*. The first fungicidal treatment comprised the spraying of plants with the preparation Input 460 EC in a dose of 1 l·ha^{-1} *(spiroxamine + prothioconazole)* during the first node phase (BBCH 31). The second application of a fungicide took place in the full phase of earing (BBCH 58), when the preparation Prosaro 250 EC *(tebuconazole + prothioconazole)* in a dose of 0.6 l·ha^{-1} was applied. Weeds were controlled by a single autumn spray of a mixture of herbicides (Boxer 800 EC 2 l·ha^{-1} – a.i. *prosulfocarb*, Glean 75 WG 5 g·ha^{-1} – a.i. *chlorosulfuron*, Legato 500 SC 0.5 l·ha^{-1} – a.i. *diflufenican*). The soil was classified as representing the soil valuation class III a, complex 2 (good wheat complex) (Table 1). Phosphorus content was determined by colorimetric method according to PN-R-04023:1996. Potassium content was determined by flame photometry according to PN-R-04022:1996. Magnesium content was determined by ASA according to PN-R-04020:1996. Total nitrogen was determined by colorimetric method according to PB 29 ed. 2. During the three-year research period, winter triticale was preceded by winter oilseed rape. Dressed seed material was sown at the density of 450 germinating kernels per 1 m^2. The results were submitted to analysis of variance in a Statistica®10 software package. Tukey's test at the significance level of 0.05 was run to evaluate the significance of differences and Pearson correlation coefficients were computed.

Table 1

Soil properties

Specification	Vegetation period		
	2008/2009	2009/2010	2010/2011
Soil type	lessives typical		
Soils pecies	medium clay		
Soil pH (1 M KCl)	6.5	6.4	6.5
Soil valuation class	R – IIIa		
Soil suitability complex	good wheat		
Content of nutrients (mg/kg of soil)			
– P	77.4	69.8	73.8
– K	176.7	164.9	157.2
– Mg	92.0	97.0	95.0
– N_{min} (0-90 cm)	19.4	18.5	17.2

Results and Discussion

The three years when the field experiments were conducted were highly varied in the weather conditions, especially in the distribution of rainfalls during the growing season (Figure 1). This had a direct impact on the growth and development of winter triticale. In the analyzed seasons, the early autumn plant growth was accompanied by rainfall shortages and temperatures higher

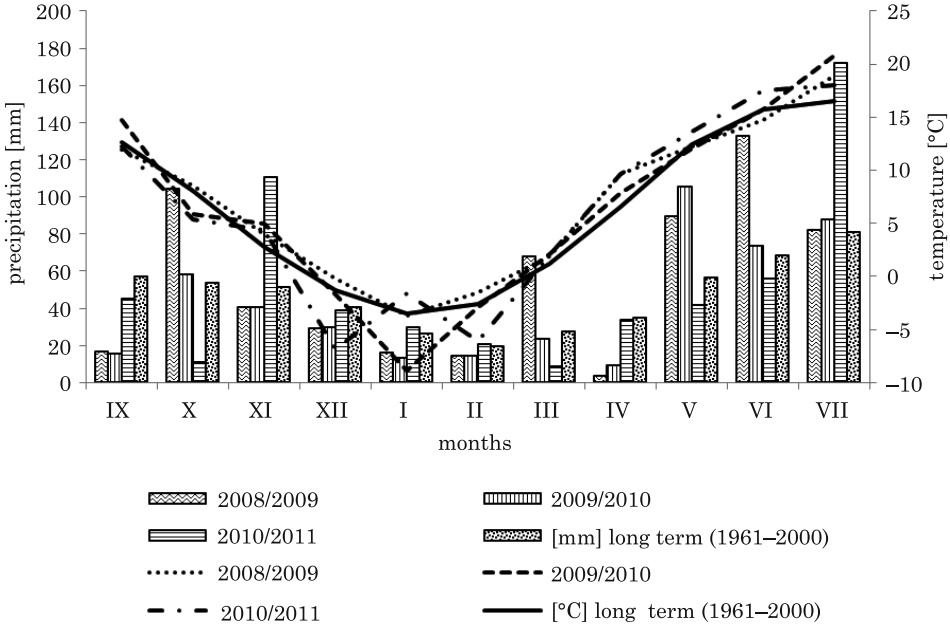

Fig. 1. Rainfall and temperature distribution during the growing seasons

than multi-annual average. In the growing seasons of 2009 and 2010, April was a very dry and warm month, while May was wet. Air temperatures higher than the average occurred in July, which was favorable for the plants as it helped the grain and straw to dry properly. Triticale harvest was particularly troublesome in 2011, when the rainfall in July was as much as 211% higher than the multi-year mean.

The number of ears per 1 m^2 of a field depended on the growing season (Table 2). The highest value of this trait was recorded in 2010. Significantly fewer ears per land unit were determined in 2011, by 9%. Nitrogen fertilisation as well as fungicidal treatments did not have any influence on the value of this yield component. A tendency was noticed for a higher number of ears per square meter on a field fertilized with growing nitrogen doses. The way triticale was protected against pathogens was not significant relative to the ears density. The number of ears per 1 m^2 tended to be higher on the treatments where seed dressing was accompanied by one or two applications of fungicides; it was the lowest on the treatments with seed dressing alone. In another experiment, JAŚKIEWICZ (2011) reported that the intensity of triticale chemical protection against pathogens did not differentiate significantly the number of ears per 1 m^2. In turn, Wróbel and Jabłoński (2004) as well as ADAMIAK et al. (2008) claimed that a triticale protection technology had a significant effect on the number of ears per 1 m^2, and triticale on a treatment with a double application of fungicides grew the most dense field cover in terms of the number of ears.

The grain number in ear was significantly conditioned by the nitrogen fertilisation level. As the amount of applied nitrogen increased, the above yield trait achieved a higher value. However, it was not until the application of 90 kg $N \cdot ha^{-1}$ that the number of grains per ear in winter triticale increased significantly. The highest dose of 150 kg $N \cdot ha^{-1}$ had a positive effect on the number of grains per ear, but the difference was not significant. The current results confirm the reports by DOPKA (2006). In our experiment, the grain number in ear was higher on treatments with the highest level of protection against diseases but without significant difference. ADAMIAK et al. (2008) verified experimentally that the application of fungicides improves the quality parameters of ear. In contrast, this yield structure component was not modified significantly by various protection technologies in the research conducted by WRÓBEL and JABŁOŃSKI (2004) or JAŚKIEWICZ (2011).

Nitrogen fertilisation did not play any larger role in the formation of grain weight per ear. Similar results were reported by DOPKA (2006). Statistical analysis showed that the second experimental factor, such as the way triticale was protected against pathogens, had a significant effect on that trait. The grain weight per ear tended to increase as the intensity of fungicidal protection

Table 2

Grain yield and yield components of winter triticale as affected by year, N fertilisation and protection method

Treatment	Yield (t·ha^{-1})				1000 grain weight (g)				Grain number in ear				Grain weight per ear	Ear number m^2			
	2009	2010	2011	mean	2009	2010	2011	mean	2009	2010	2011	mean	2009–2011 mean	2009	2010	2011	mean
N rate (kg·ha^{-1})																	
30	8.46	8.36	7.06	7.96	36.9	38.9	43.0	39.6	37.4	37.1	36.6	37.0	1.35	608	643	508	586
60	8.84	8.68	8.00	8.51	34.9	38.2	39.9	37.7	38.6	36.1	38.2	37.6	1.36	612	651	589	617
90	9.03	9.07	8.57	8.89	36.2	39.5	43.2	39.6	39.2	38.9	39.8	39.3	1.41	650	653	597	633
120	9.11	9.26	8.97	9.11	34.6	36.7	39.0	36.7	42.3	39.9	40.6	40.9	1.44	648	660	605	638
150	9.25	9.39	9.23	9.29	34.0	36.2	40.6	36.9	40.2	41.6	43.0	41.6	1.40	661	678	662	667
Protection method																	
a	8.49	8.21	8.13	8.28	33.5	35.7	39.4	36.2	39.4	38.6	38.7	38.9	1.34	639	613	609	620
b	8.94	8.92	8.33	8.73	35.4	38.0	41.4	38.3	38.7	39.6	39.3	39.2	1.38	645	655	602	634
c	9.39	9.72	8.64	9.25	37.0	40.0	42.7	39.9	40.4	38.0	40.9	39.8	1.47	623	707	570	633
mean	8.94	8.96	8.37	–	35.3	37.9	41.2	–	39.5	38.7	39.6	–	1.39	636	657	592	–
HSD (0.05)																	
Year (Y)	0.18				1.8				n.s.				n.s.	21.3			
Nitrogen (N)	0.22				2.3				1.5				n.s.	n.s.			
Protect. method (P)	0.18				1.7				n.s.				0.07	n.s.			
YxN	0.40				n.s.				n.s.				n.s.	n.s.			
YxP	0.31				n.s.				n.s.				n.s.	n.s.			
NxP	n.s.				n.s.				n.s.				n.s.	n.s.			
YxNxP	n.s.				n.s.				n.s.				n.s.	n.s.			

dose. In our studies, the weight of grain was from 1.34 g in the treatment with seed dressing alone up to 1.47 g in the treatment with complete protection. A significant increase in the weight of grains from a single ear was observed between these two types of treatments. This finding is confirmed by the experiment of ADAMIAK et al. (2008). On the other hand, JAŚKIEWICZ (2011) did not verify any statistical significance of different levels of triticale protection versus the weight of grains per ear.

The statistical analysis proved that the 1.000 grains weight was significantly affected by the year of experiment, nitrogen fertilisation and fungicidal protection. The lowest 1.000 grains weight was noted in the first year of the research (35.3 g), while the highest one was achieved in the third year (41.2). The references indicate that the 1.000 grains weight of triticale ranges from 35 to 55 g (EREKUL and KÖHN 2006, KOZAK et al. 2007). Nitrogen nutrition affected this trait adversely, but this effect was not unidirectional. In general, the 1,000 grains weight was decreasing as the doses of nitrogen fertiliser increased. The most robust grain was harvested from the treatment which had received the lowest nitrogen fertilisation. Significantly lower values of the 1,000 grains weight were recorded on the treatments given 120 kg $N \cdot ha^{-1}$ than from the ones supplied 30 kg $N \cdot ha^{-1}$. However, the highest dose of nitrogen resulted slightly higher increase of the 1.000 grains weight. SAMBORSKI et al. (2008) also showed that the highest dose of nitrogen had a negative effect on the 1.000 grains weight. In the study by ALARU et al. (2004), a dose of nitrogen above 60 $kg \cdot ha^{-1}$ did not have any significant influence on the 1.000 grains weight. MUT et al. (2005) observed a significant increase of the 1.000 grains weight under the influence of nitrogen fertilisation. In our research, the robustness of grains depended significantly also on the protection of plants from diseases. The highest 1.000 grains weight was recorded under complete protection. Lower weight of 1.000 grains was determined on treatments with a single application of fungicide. Significantly less robust grains were harvested from treatment where seed dressing was the only form of protection against pathogens. The results reported by WRÓBEL and JABŁOŃSKI (2004) coincide with the above information. Contrary results are presented by ADAMIAK et al. (2008), who did not record any significant effect of disease prevention methods on the mass of 1.000 grains.

Our statistical analysis of the results demonstrated a significant effect of the year of the experiments on the yields of winter triticale. The year 2011 was the least favorable for the winter triticale grain yield. Triticale produced significantly higher yields in 2009 and 2010 (Table 2). Among the most important factors affecting grain yields, BIBERDŽIĆ et al. (2013) mentioned the climate. The difference in grain yields between the best and the worst year was 6.6%. In the growing seasons of 2008/2009 and 2009/2010, in response to the

subsequently higher nitrogen doses up to 90 kg N·ha^{-1}, the grain yield increased significantly. The response of triticale to nitrogen was slightly different in the final year of the experiment. A significant increase in grain yield was noticed following the application of 120 kg N·ha^{-1}. MUT et al. (2005) obtained the highest grain yield of triticale supplied with a dose of nitrogen equal 180 kg·ha^{-1}. A dose of nitrogen above 60 kg·ha^{-1} in the study by ALARU et al. (2004), and a dose above 66 kg·ha^{-1} in the experiment by GIBSON et al. (2007) did not affect significantly the volume of grain yield. In our trials, a the highest increase in grain yields (by 0.55 t·ha^{-1}) was observed after an application of just 60 kg N·ha^{-1}. Raising the nitrogen fertilisation level to 90 kg·ha^{-1} caused significantly higher grain yields. The highest yield was harvested from treatments fertilised with 150 kg N·ha^{-1}, the difference was significant versus the 90 kg·ha^{-1} dose. MAŁECKA et al. (2004) reported that the grain yield from winter triticale increased significantly as the nitrogen fertilisation was gradually elevated to 120 kg·ha^{-1}. Its further increase did not cause a significant difference in the volume of grain yields. In the trial run by DOPKA (2006), the effects of 100 and 150 kg N·ha^{-1} were similar. SAMBORSKI et al. (2008) demonstrated a yield-stimulating effect of nitrogen at a dose rising up to 80 kg N·ha^{-1}, with an average yield increase of 21%, and following the application of 170 kg N·ha^{-1}, when a 9.2% yield increase occurred. The technology used to protect triticale from fungal diseases was another factor which strongly differentiated the yield. The weakest yield stimulating effect was produced by seed dressing. Triticale from the treatments with seed dressing and a single application of fungicide yielded significantly better. The highest yield was harvested from the treatments with full protection against fungi. On average, triticale protected by seed dressing alone yielded by 0.45 t·ha^{-1} (5.5%) worse than the same crop given seed dressing and a single spraying of fungicide. Two applications of fungicide in a growing season significantly increased the yield compared with treatments with a single treatment (0.52 t·ha^{-1}, i.e. 6% higher yield).

The influence of the antifungal protection on the yield of winter triticale differed between the years, which was confirmed by the interactions of years and levels of fungicide application. This effect was most distinct in the second year of the experiment, when an increase in grain yields from treatments with complete protection versus the ones with the lowest plant protection input was 1.51 t·ha^{-1}. WRÓBEL and JABŁOŃSKI (2004) recorded the highest yield from treatments with the complete fungicide application. When the protection against fungal diseases had been completely abandoned, the yield decreased by 15%. In the study by ADAMIAK et al. (2008), the yield was 7–16% higher, depending on a cultivar, following a double application of chemicals against fungal pathogens. In our study, an interaction between the years and level of

antifungal protection was determined. ALARU et al. (2009) concluded that the strongest influence on yield and yield structure was produced by the year of an experiment, followed by a cultivar and a division of the nitrogen fertilisation dosage.

Grain yields of winter triticale varieties 'Gniewko' were very high. Yield variability was fairly narrow range. The reason could be the cultivation of triticale after a very good forecrop and high content of mineral nitrogen in the soil. From the calculated regression equation between the dose of nitrogen and grain yield, which is a second-degree curve, it follows that the maximum yield of grain in a field experiment with the variety 'Gniewko' can be achieved at 145 kg N·ha^{-1} (Figure 2).

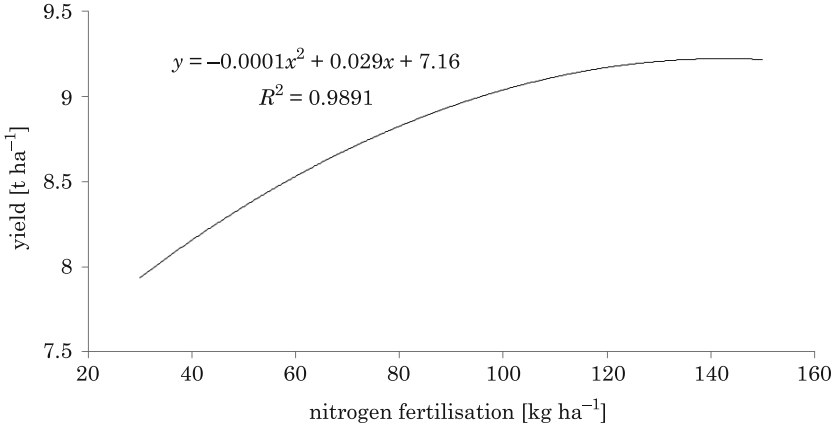

Fig. 2. Regression curve of grain yield (y) of winter triticale cv. „Gniewko" depending on nitrogen fertilisation dose (x)

Table 3 shows the correlation coefficients between the grain yield and its components. Significant positive correlations were observed between yield and grain number in ear, 1000 grain weight and grain weight per ear, grain number in ear and grain weight per ear weight. Table 4 presents the correlation coefficients between the examined traits in the years of study. In the first research year by testing correlation coefficients between yield and 1000-grain weight (r=0.31*), grain weight per ear (r=0.32*), ear number m^{-2} (r=0,27*) positive and significant correlations were found. However, grain number in ear was significantly and positively correlated with grain yield only in the third year (r=0.47*). Table 4 shows the correlation coefficients between the analyzed traits, depending on studied fertilization and protection method treatments. Significant negative correlations were observed between yield and 1000-grain weight in the treatment 30 kg·ha^{-1} N (r=-0.47*), 'a' treatment

(r=-0.26) and 'c' treatment (r=-0.37*). Positive and strong correlations were observed between yield and grain number in ear in the treatment 'b' (r=0.33). The present results confirm the opinion of many authors that the traits analyzed and their correlations are genetically determined, but are strongly modified by the nutrient status of the environment and weather conditions (MÁRTON 2008, BOLTON 2009).

Table 3

Correlation coefficients between grain yield and its components (significant level (p)/ correlation coefficient (r))

Traits	Yield	1000 grain weight	Grain number in ear	Grain weight per ear	Ears number 1 m^2
Yield	1.00	p=0.09/-0.13	p=0.00/0.23*	p=0.17/0.10	p=0.25/0.09
1000 grain weight		1.00	p=0.59/0.04	p=0.01/0.20*	p=0.19/0.09
Grain number in ear			1.00	p=0.00/0.45*	p=0.83/-0.02
Grain weight per ear				1.00	p=0.29/0.08

* significant at 0.05

Table 4

Correlation coefficients between the yield components and year, nitrogen fertilisation and protection method

Specyfication	1000 grain weight	Grain number in ear	Grain weight per ear	Ears number m^2
Correlations between the examined traits				
2008/09	0.31*	0.16	0.32*	0.27*
2009/10	0.42*	0.15	0.10	0.21
2010/11	-0.20	0.47*	0.08	-0.09
Correlations between the traits analyzed in the N fertilisation				
30	-0.47*	0.13	-0.14	0.06
60	0.08	-0.00	0.17	-0.21
90	-0.07	-0.15	-0.02	0.27
120	-0.02	0.19	0.31	-0.03
150	0.32	-0.07	-0.31	0.09
Correlations between the traits analyzed in the protection method				
a	-0.26*	0.18	-0.15	-0.07
b	-0.25	0.33*	0.03	0.04
c	-0.37*	0.16	-0.02	0.22

* significant at 0.05

Conclusions

1. Effect of nitrogen fertilisation had significant influence on grain yield, 1000-grain weight and grain number in ear. Effect of antifungal protection method treatments on grain yield, 1000-grain weight and grain weight per ear.

The weather conditions in the research years were statistically significantly influence on yields, 1000 grain weight and ears number per m^2. Also, the effect of interaction of the year x fertilisation and year x protection method on the yield was significant.

2. Regression equation between the dose of nitrogen and grain yield shows that the maximum yield of grain in a field experiment with the variety „Gniewko" can be achieved at 145 kg $N \cdot ha^{-1}$. The highest yield obtained from objects with full antifungal protection.

3. The triticale grain yield was significantly positively correlated with grain number in ear (0.47). Significant positive correlations of 1000 grain weight, grain weight per ear and ears number m^2 in the first year and positive correlations of 1000-grain weight and grain number in ear in the second year and third year were determined.

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EFFECT OF CURING AND THERMAL TREATMENT OF THE QUALITY OF MEAT PRODUCTS FROM TURKEYS FED DIETS ENRICHED PROTEIN--XANTHOPHYLL EXTRACT OF ALFALFA

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K e y w o r d s: alfalfa, feed supplementation, turkey, meat product, yield of product, chemical composition, sensory evaluation.

A b s t r a c t

The aim of the study was to evaluate the effect of the method of curing and thermal processing on yield of product, chemical composition and sensory quality of meat products from turkeys fed diets enriched in protein-xanthophyll extract of alfalfa (PX). The experiment involved 120 turkeys allotted to groups: 1 – control without any supplementation; 2 and 3 with 1.5% and 3% supplementation of PX, respectively. After slaughter and carcasses division breast and thigh muscles of each group were divided into two parts. One part of the mixture were cured and dried and after that smoked and cooked. To the other sample salt was added then roasted. Water and salt content, pH, the sensory evaluation, instrumental color were determined after production. The result showed that the feedstuff supplementation with 1.5% use of PX in turkeys diet does not significantly *(P*<0.05) affect on tested quality factors of the meat products. Feedstuff with addition the 3% PX caused a significant deterioration in products flavor.

WPŁYW SPOSOBU PEKLOWANIA I OBRÓBKI TERMICZNEJ NA JAKOŚĆ PRODUKTÓW Z MIĘSA INDYKÓW ŻYWIONYCH PASZĄ WZBOGACONĄ W EKSTRAKT Z LUCERNY

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S ł o w a k l u c z o w e: lucerna, suplementacja paszy, indyki, wyrób mięsny, wydajność produktu, skład chemiczny, ocena sensoryczna.

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Abstrakt

Celem pracy była ocena wpływu sposobu peklowania i obróbki termicznej na wydajność technologiczną, skład chemiczny i jakość sensoryczną pieczonych i wędzonych wyrobów z mięsa indyków żywionych paszą wzbogaconą w ekstrakt białkowo-ksantofilowy preparatu z lucerny (PX). W doświadczeniu zwierzęta podzielono na 3 grupy: grupę 1 – kontrolną – żywiono niesuplementowaną paszą, grupie 2 dodawano do paszy 1,5% PX, zaś grupie 3 – 3% PX. Po uboju i dysekcji tuszki mięśnie piersi i ud każdej z grup dzielono na dwie części. Jedną część peklowano, wędzono i parzono, drugą solono i pieczono. W otrzymanych wyrobach oznaczano zawartość wody i soli, kwasowość, barwę (L*,a*,b*) oraz przeprowadzono ocenę sensoryczną. Stwierdzono, że 1,5-procentowy dodatek PX do paszy indyków nie wpływa istotnie (P<0,05) na badane wyróżniki jakości wędzonych i pieczonych wyrobów z mięsa tych zwierząt. Dwukrotne zwiększenie suplementacji tego preparatu w paszy spowodowało znaczne pogorszenie smaku wyrobów.

Introduction

Sensory quality is one of the most important features in food, including meat products, too. There are many knowing factors affecting the meat quality and thus the consumers desire and its acceptance. Among all factors: feeding habits, feedstuff type and its composition can be mentioned. Feed components influence the nutritional and physico-chemical properties of meat and its sensory characteristics, which in turn are reflected in the quality of meat products.

In the recent years there a lot of plant ingredients (fitobiotics) as potential feed ingredients for poultry have been researched. One of such ingredient is an alfalfa (Medicago sativa L.) and various alfalfa extracts and preparations. Alfalfa is a valuable source of nutrients including essential amino acids, vitamins (A, B, C, D, E, K), and minerals (Ca, Cu, Fe, Mg, Mn, P, Zn, Si) (BEN AZIZ et al. 2006, GAWEŁ 2012) and it contains many biologically active substances. Many alfalfa products contain toxic alkaloid canavanine and coumestrol. Extraction process removes canavanine and coumestrol from alfalfa lives providing potent form of saponins that has been shown to have antiatherosclerotic activity (KHALEEL et al. 2005), antibacterial activity (AVATO et al. 2006) and antifungal activity (POLACHECK et al. 1991). Plant sterols (β-sitosterol, campesterol, cycloartenol, α-spinasterol and stigmasterol) and polyphenols having estrogenic activity and isoflavones (biochanin A, daidzein, genistein and formonoetyna) are also the source of phytoestrogens (*e.g.* coumestrol) which reduce neurovegetative symptoms during menopause, the incidence of ischemic heart disease and hormone-dependent breast and prostate cancer with bone osteoporosis process (LAMSAL et al. 2007). Animal studies by MEHRENJANI et al. (2007) have also shown alfalfa antidiabetic effect and studies by DONG et al. (2007) the immunostimulatory effect.

Alfalfa has long been used in animal nutrition (CARRILHO et al. 2009, RIPOLL et al. 2012). It was found that preparations of alfalfa added to feed, improve feed efficiency increasing the body weight gain and reducing feed consumption as well as increased the fatty acid content in muscle and improve its profile (JIANG et al. 2012). The quality of feed components has an impact on animal health and meat quality (PETTIGREW and ESNAOLA 2001). Several nutrients and additives are transferred from feed to muscles and adipose tissue when fed to monogastric animals. The extract of alfalfa is free from mycotoxins and pesticide contamination (GAWEŁ 2012). Many authors suggest that the addition of alfalfa concentrate to turkey diets did not cause the loss of meat quality (KARWOWSKA et al. 2007, KARWOWSKA 2008, BLANCO et al. 2010, DEL CAMPO et al. 2010, KARWOWSKA et al. 2010, RESCONI et al. 2010, HUTCHISON et al. 2012, PORDOMINGO et al. 2012). However, not much is known about the effects of alfalfa feeding or feed containing preparations of alfalfa on sensory characteristics of products produced from meat of these animals.

The objective was to evaluate the effects of dietary protein-xanthophylls concentrate (PX) of alfalfa to turkey diets (at 1.5 and 3.0%) on the yield of product, chemical composition and sensory quality of smoked and roasted breast and thigh muscles.

Material and Methods

Animals and diet. One hundred and twenty 42-day turkey poults (Big-6 type) were selected at random and divided into three groups of 40. A control group (group 1) was fed according to the Poultry Feeding Standards (NRC 1994). The other groups were fed with 1.5% (group 2) and 3.0% (group 3) protein-xanthophylls (PX) concentrate of alfalfa, instead of extracted soybean extracted meal. The ingredients of the diets are presented in Table 1. Alfalfa concentrate was prepared by condensing the juice of alfalfa leaves. The method of obtaining a protein concentrate from the leaves of alfalfa was developed as a result of thermocoagulation of protein at the temperature of 85–90°C prior to conventional dehydration. (CAILLOT 2008, Commission Decision 2009/826/EC). Feed and water was provided *ad libitum*. After 11 weeks, the turkeys with an average live weight of 16.9 ± 0.5 kg were slaughtered under commercial conditions (Council Regulation (EC) No 1099/2009). The breast and thigh muscles were stored at 4°C for 24 h.

Manufacturing of products. Breast (without bones and skin) and thigh (with a bone and skin) of each group were divided into two parts. The cured dry mixture (99.4–99.5% sodium chloride, 0.5–0.6% sodium nitrite) at a ratio 2.5% of the meat was added to the first part. The same rate of salt was added to

Table 1

Composition of basal diets (%)

Item	Feeding period (weeks)		
	7–9	10–12	13–18
Maize	10.0	10.0	10.0
Wheat	46.63	51.32	57.69
Soybean extracted meal (47% CP)	36.00	31.00	24.50
Soya oil	2.50	3.00	3.60
Calcium phosphate	1.85	1.75	1.60
Ronozyme P 5000	0.015	0.015	0.0
Ronozyme WX	0.02	0.02	0.0
Limestone	1.30	1.27	1.10
NaCl	0.23	0.23	0.22
Sour sodium carbonate ($NaHCO_3$)	0.11	0.10	0.11
Betafin S1 (Betaine)	0.10	0.10	0.0
Choline chloride (60%)	0.10	0.10	0.0
Lysine (98%)	0.35	0.325	0.0
Methionine DL (99%)	0.25	0.25	0.0
Threonine L (98%)	0.02	–	0.03
Vitamin-mineral premix	0.53	0.52	1.15

another sample. Then the meat was stored at 4°C for 48 h. Next, the cured samples were smoked (thick smoke, 50°C, 30 min.) and cooked (75°C, until a final temperature of 70°C was reached in center of product) in the laboratory smoking-cooking chamber (Jugema, Poland). The salted samples were roasted in the roaster (XF135, Unox S.p.A., Italy) at 180°C until the final temperature of 70°C was reached in center of product. Subsequently, the samples were cooled to 20–25°C for 1 h and stored in a refrigerator (4°C) for 24 h. The experimental variants of turkey meat products showed are in Table 2.

Table 2

The experimental variants of turkey meat products

Part of turkey carcasses	Type of processing	Group of turkeys		
		Control (group 1) (n=40)	1.5% PX (group 2) (n=40)	3.0% PX (group 3) (n=40)
Thigh	Smoked and cooked (n=3) Roasted (n=3)	TS1 TR1	TS2 TR2	TS3 TR3
Breast	Smoked and cooked (n=3) Roasted (n=3)	BS1 BR1	BS2 BR2	BS3 BR3

Yield of the final product. The yield of the final product was calculated from the weight of the product (after heat treatment and 24 h cooling) as a percentage of the weight of the raw meat (uncured, unsalted) (U.S. Department of Agriculture, 2012).

Measurement of pH (ISO 2917:2001). Ten grams of minced meat product was homogenized with 100 ml of potassium chloride solution (0,1 mol/L) for 1 min using the homogenizer (IKA ULTRA-TURRAX T25 Basic, Germany). The homogenate was filtered through filter paper and the pH of the filtrate was measured with the digital pH-meter CPC-501 (Elmetron, Poland) equipped with the pH electrode (ERH-111, Elmetron, Poland).

The moisture and total chlorides were quantified according to the ISO recommended standards (ISO 1442:1997) and to the argentometric Volhard method (ISO 1841-1:1996), respectively. The total chloride calculated on salt.

Instrumental color measurements were taken after production (day 1). Color parameters (CIE L*a*b*) were measured on the freshly cut surface of a product in 10 measuring points using 8200 Series reflection spectrocolorimeter (X-Rite Inc., USA) with a D65 illuminant and a 10° standard observer. Color coordinates were determined using the CIE Lab system. The results were expressed as lightness (L*, 100 = white and 0 = black), redness /greenness (a*, positive = red) and yellowness /blueness (b*, positive = yellow). Prior to use, the spectrocolorimeter was calibrated against white and black standard tiles.

Sensory evaluation. To determine the sensory quality, 5-point hedonic scale was used where 1 indicated extremely undesirable property, and 5 – very desirable (ISO 4121: 2003, ISO 5492: 2008). Sensory attributes of products from turkey breast: color and structure of section, consistency, odor and overall flavor were measured. The assessment was made by the 8-person panel consisting of employees of the Department of Meat Technology and Food Quality, University of Life Sciences in Lublin. The panel had years of experience in sensory evaluation practice (ISO 8586: 2014). They were trained theoretically and practically for the methods applied. Meat product samples were sliced into approximately equal size and weight (around 10 g) and placed in plastic odorless, disposable, covered with lids boxes (volume, 125 mL). All samples were separately coded with three digits and were randomly served to avoid carry-over effects. The test samples were kept in boxes at room temperature (22 ± 1°C) for 30 min before analysis. They were presented to panelists in plastic boxes with covers on a white background with assessments cards. Water and unsalted crackers were provided to cleanse the palate between samples.

Statistical analysis

The experiment was repeated three times. Physicochemical analyses were performed in six replicates in each experiment. Analysis of variance (ANOVA) was performed on all variables using the General Linear Model process of the SAS statistical software. The significance of the differences between mean

values was calculated at a significance level of $P<0.05$ using the *T-Tukey's* range test.

Results and Discussion

A weight loss, primarily water during thermal treatment is an important feature affecting the sensory evaluation of meat products. Their size determines the degree of tenderness, juiciness and other sensory attributes experienced during the test. The earlier study revealed that the chemical composition of raw breast and thigh muscle of turkeys fed with diets supplemented with protein-xanthophylls concentrate of alfalfa was not affected by the diet (KARWOWSKA *et al*. 2010). However, in current study chemical composition was influenced by dietary treatment showing significantly higher yield of smoked and cooked breast (BS) and thighs (TS) in comparison to roasted breast (BR) and thighs (TR) (Table 3). The BS is characterized by an increase of about 10 to 17 percentage points productivity compared to BR. This is mainly due to the method of heat treatment of the samples roasted i.e. using higher temperatures and forced air. The yield of control sample of smoked (TS1) were statistically significantly ($P<0.05$) higher than yield of the thighs of turkeys fed diet with alfalfa preparation (TS2 and TS3). Yield of roasted breast (BR) was at the level 66.30% of the sample BR2 to 70.87% in the control sample (BR1). Difference in the yield of these samples was statistically significant and will certainly have an impact on their chemical composition.

Table 3
Yield of product, moisture and salt content, pH values of meat products stored 24 h at 4°C

Sample	Yield of product [%]	Moisture [%]	NaCl [%]	pH
TR1	66.16 ± 1.45	60.95 ± 0.01	3.81 ± 0.03^{ab}	5.87 ± 0.03
TR2	64.90 ± 2.23	62.27 ± 0.03	1.46 ± 0.05^{b}	5.69 ± 0.01
TR3	64.71 ± 2.86	62.06 ± 0.00	1.75 ± 0.02^{a}	5.81 ± 0.02
TS1	92.05 ± 4.68^{ab}	61.10 ± 0.00^{ab}	1.75 ± 0.04	5.81 ± 0.02
TS2	86.81 ± 4.63^{b}	70.18 ± 0.00^{b}	2.07 ± 0.03	5.79 ± 0.01
TS3	86.14 ± 3.58^{a}	68.67 ± 0.18^{a}	1.90 ± 0.02	5.8 ± 0.01
BR1	70.87 ± 1.56^{a}	45.69 ± 0.06^{ab}	2.63 ± 0.08^{a}	5.89 ± 0.02
BR2	66.30 ± 3.45^{a}	35.59 ± 0.05^{b}	2.97 ± 0.06	5.83 ± 0.03
BR3	69.03 ± 2.12	33.28 ± 0.02^{a}	3.07 ± 0.10^{a}	5.88 ± 0.01
BS1	83.98 ± 5.69	60.92 ± 0.08^{a}	3.22 ± 0.12^{ab}	5.76 ± 0.02
BS2	83.30 ± 4.87	37.29 ± 0.04^{a}	2.34 ± 0.03^{b}	5.61 ± 0.02
BS3	79.91 ± 3.25	56.27 ± 0.18^{a}	2.66 ± 0.08^{a}	5.75 ± 0.04

Values are given as mean ± SD (standard deviation), n = 18
Values, in columns within the same groups (part of turkey carcasses, treatment, parameter), marked with the same characters are significantly different ($P<0.05$)

The salt content in meat products influences the degree of hydration of the proteins and changes color after heat treatment. Furthermore, it forms their durability by lowering water activity. Moreover, from sensory evaluation of meat products point of view, the flavor is primarily determined by salt. In the production process, the same percentage of salt addition or cured dry mixture (2.5%) is used in the samples. However, due to different heat treatment with different yield of finished product, salt content in the finished products differ significantly (Table 3). Salt content was lower in the roasted or smoked thigh samples than in the breast samples ($P<0.05$).

The pH of a meat product is important because it affects many quality factors, including color, texture and flavor (LEE et al. 2010). In the earlier study by KARWOWSKA et al. (2010), the chemical characterization of the raw meat of turkeys fed with protein-xanthophylls concentrate of alfalfa supplemented diets showed that the supplementation had no significant effect on pH of the samples during 120 h of storage ($P<0.05$). The pH values of breast muscles were lower than those of the thigh muscles. Storage time did not affect the pH values. Similar relationships were observed in this study. The pH values between samples did not differ significantly ($P<0.05$).

In consumers opinion color appears to be very important quality characteristic of meat products. The results obtained in this experiment and statistical analysis of color parameters showed no significant effect of the addition of the preparation of the alfalfa fed to turkeys in the value of the parameter L*, a* and b* of meat products (Table 4). Similar relationship was observed in the raw lamb meat (RIPOLL et al. 2012), rabbit meat (CARRILHO et al. 2009) and pork meat, as well as smoked and cooked ham from pigs fed diets supplemented with the alfalfa extract (KARWOWSKA et al. 2007, KARWOWSKA 2008).

Table 4

Color values in CIE L* a* b* of turkey meat products stored 24 h at 4°C

Sample	L*	a*	b*
TR1	62.23 ± 4.20	3.72 ± 0.81	14.36 ± 1.84
TR2	68.19 ± 2.36	3.71 ± 0.13	14.64 ± 0.64
TR3	65.45 ± 1.41	3.82 ± 0.35	15.19 ± 0.45
TS1	66.14 ± 1.09	9.62 ± 1.41	9.98 ± 1.86
TS2	66.44 ± 4.39	10.22 ± 0.95	11.11 ± 1.13
TS3	62.96 ± 4.15	11.63 ± 1.13	11.25 ± 1.55
BR1	77.92 ± 3.68	2.25 ± 1.89	12.65 ± 1.54
BR2	76.04 ± 1.74	1.41 ± 0.34	13.80 ± 0.82
BR3	79.12 ± 1.73	1.46 ± 0.43	13.81 ± 1.18
BS1	74.12 ± 5.51	5.80 ± 1.09	8.36 ± 1.20
BS2	80.78 ± 0.60	5.15 ± 0.18	9.50 ± 0.14
BS3	77.48 ± 3.86	5.38 ± 0.38	9.65 ± 0.70

Values are given as mean ± SD (standard deviation), $n = 30$

Statistical analysis showed a significant effect of dietary addition of alfalfa extract on color, texture and overall flavor of tested meat products (Table 5). The lowest color score was observed in cross-roast breast from turkeys fed with the 3% protein-xanthophylls (PX) concentrate of alfalfa. Moreover, the flavor of sample from this experimental group was rated as the lowest, regardless of the heat treatment. The evaluator's team has sensed it as non-specific, off-taste and off-odors. This result does not agree with earlier study by KARWOWSKA et al. (2007) where sensory of smoked and cured ham from pigs fed with the same preparation with protein-alfalfa xanthophylls was evaluated.

Table 5

Results of sensory evaluation [points] of products from turkey breast stored 24 h at 4°C

Sample	Structure of section	Color of section	Consistency	Odor	Overall flavor
BR1	4.3 ± 0.21	3.6 ± 0.08^{a}	4.4 ± 0.09^{a}	4.6 ± 0.12	4.6 ± 0.21^{a}
BR2	4.1 ± 0.19^{a}	4.0 ± 0.21^{ab}	3.8 ± 0.14^{ab}	4.6 ± 0.11	4.3 ± 0.22^{b}
BR3	4.7 ± 0.11^{a}	3.4 ± 0.12^{b}	4.4 ± 0.23^{b}	4.8 ± 0.09	3.9 ± 0.14^{ab}
BS1	4.3 ± 0.12	3.9 ± 0.13^{ab}	3.9 ± 0.24^{a}	4.6 ± 0.13	4.5 ± 0.31^{a}
BS2	4.5 ± 0.43	4.6 ± 0.22^{b}	4.3 ± 0.38^{a}	4.7 ± 0.10	4.4 ± 0.19^{b}
BS3	4.4 ± 0.33	4.8 ± 0.29^{a}	4.8 ± 0.31^{a}	4.6 ± 0.12	4.0 ± 0.08^{ab}

Values are given as mean ± SD (standard deviation), n = 24
Values, in columns within the same groups (part of turkey carcasses, treatment, parameter), marked with the same characters are significantly different $P<0.05$)

Conclusions

Meat products from turkeys fed diet supplemented with protein-xanthophyll concentrate of alfalfa yield significantly lower and were lower in water content in comparison to the products from birds fed diet without the supplement. The content of protein-xanthophyll concentrate of alfalfa in turkey's diet did not affect the color parameters of tested products. Protein-xanthophyll extract of alfalfa included at 3% of the diets has caused a significant deterioration in flavor of meat products.

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THE EFFECT OF GROWTH RATE AND LEAN MEAT CONTENT IN POLISH LARGE WHITE BOARS ON THEIR SEMEN CHARACTERISTICS

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K e y w o r d s: boars, growth rate, lean meat, ejaculates.

A b s t r a c t

The aim of the study was to determine the effect of growth rate and lean meat content in Polish Large White boars on their semen characteristics. On the basis of the results of performance testing carried out on the 180th day of life, the boars analysed were divided into three groups according to their daily weight gain (I – 751–800 g; II – 801–850 g; and III – 851–900), and additionally into three groups according to lean meat content (I – 58–59%; II – 60–61%; and III – 62–63%). Ejaculates were evaluated for the following characteristics: volume, sperm concentration, percentage of sperm with progressive movement, number of sperm with progressive movement per ejaculate and per insemination dose, and number of insemination doses obtained per ejaculate. High daily weight gain in the Polish Large White boars during the rearing period had no negative effect on their semen. The lean meat content of the boars significantly affected the characteristics of their ejaculates. Boars whose lean meat content ranged from 62% to 63% had significantly less favourable ejaculate parameters than those with lower meat content.

WPŁYW INTENSYWNOŚCI WZROSTU I MIĘSNOŚCI KNURÓW RASY WIELKIEJ BIAŁEJ POLSKIEJ NA CECHY ICH NASIENIA

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S ł o w a k l u c z o w e: knury, przyrosty dzienne, mięsność, ejakulaty.

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Abstrakt

Celem badań było określenie wpływu intensywności wzrostu i mięsności knurów rasy wielkiej białej polskiej na cechy ich nasienia. Na podstawie wyników z oceny przyżyciowej, przeprowadzonej w 180 dniu życia, analizowane knury podzielono według kryterium przyrostów dobowych na trzy grupy: I – 751–800 g; II – 801–850 g; III – 851–900 g oraz według mięsności, również na trzy grupy: I – 58–59%; II – 60–61%; III – 62–63%. Ocenę ejakulatów przeprowadzono na podstawie następujących cech: objętość, koncentrację plemników, procent plemników o ruchu postępowym, liczbę plemników o ruchu postępowym w ejakulacie i w dawce inseminacyjnej oraz liczbę dawek inseminacyjnych uzyskanych z jednego ejakulatu. Wysokie przyrosty dobowe knurów rasy wielkiej białej polskiej w okresie odchowu nie miały ujemnego wpłynęły na cechy ich nasienia. Mięsność knurów miała istotny wpływa na kształtowanie się cech ejakulatów. Knury, których mięsność mieściła się w granicy 62–63% charakteryzowały się istotnie gorszymi parametrami ejakulatów w stosunku do knurów o niższej mięsności.

Introduction

In recent years work on improvement of pigs has focused in part on increasing their growth rate and lean meat content. Intensive selection for these traits, apart from achieving its objective, may also have negative consequences, such as poorer development of the reproductive, digestive and musculoskeletal systems, an increase in circulatory system failure, and lower resistance to stress (RYDHMER 1993, KAWĘCKA 2002). The role of the boar and insemination is vital in obtaining rapid and significant genetic gain. Therefore in addition to normal development of the reproductive organs, high libido and high-quality semen, males should also have very good feedlot performance and carcass traits. Such individuals are very difficult to breed because some of these characteristics are negatively correlated (RYDHMER 1993, MILEWSKA 2007). ŁYCZYŃSKI (1991), in a study on the correlations between performance test results and suitability for breeding, found that the semen of boars with substantial weight gain was characterized by lower ejaculate volume and a lower total number of sperm per ejaculate. FALKENBERG et al. (1989) found that boars with better musculature produced semen with sperm of greater motility, while those with greater fat cover produced semen of inferior quality. KAWĘCKA et al. (2000) state that an increase in muscularity can cause a slight deterioration in semen characteristics in boars. The authors found low but favourable correlations between daily weight gain up to the 180th day of life and certain semen characteristics in boars. Many authors, however, express the view that the correlations between growth rate and muscularity in boars and their semen characteristics are generally low and insignificant.

The indefinite results published by different authors, both in Poland and abroad, may result from genetic differences in the animals tested and in the scale of the factors analysed.

The aim of the present study was to analyse the effect of growth rate and lean meat content in Polish Large White boars on their semen characteristics.

Material and Methods

The material for the study consisted of 24 boars of the breed Polish Large White used at the Sow Insemination Station in Białka in 2010–2014. On the basis of the results of performance testing carried out on the boars' 180th day of life, taken from breeding documentation, the boars were divided into three groups according to daily weight gain (I – 751–800 g; II – 801–850 g; and III – 851–900) and additionally into three groups according to lean meat content (I – 58–59%; II – 60–61%; and III – 62–63%).

During the growth period the boars were kept in groups and fed complete mixed rations according to *Swine Feeding Standards* (1993). From the start of their exploitation for breeding the boars were kept in identical environmental conditions, in individual pens with litter.

Semen was collected from the boars every other day by the manual method, using a phantom. A detailed quantitative and qualitative evaluation of the ejaculates was performed using common methods. Evaluation of ejaculates was based on the following characteristics: volume, sperm concentration, percentage of sperm with progressive movement, number of sperm with progressive movement per ejaculate and per insemination dose, and number of insemination doses obtained per ejaculate.

The material was analysed statistically using one-way analysis of variance. The significance of differences between groups was determined using DuncanYs test.

Results and Discussion

Sow Insemination Stations purchase boars with very high performance testing results, so genetic gain in terms of feedlot and meat characteristics can be rapidly achieved and expanded. The insemination boars included in the study had very good daily weight gain, ranging from 751 to 900 grams (Table 1), and in this respect their results surpassed those of boars of this breed evaluated in Poland in 2013 as well as those presented by ECKERT and SZYNDLER-NĘDZA (2014). Analysis of the traits characterizing the ejaculates of boars with different growth rates revealed that boars with faster growth rates up to their 180th day of life produced ejaculates with more favourable parameters. The highest average ejaculate volume (281.3 ml) was noted in the

Table 1

Traits of ejaculates depending on their intensity of growth

Traits of semen	Daily gain to 180 day of life						Significance of differences
	I 751-800 (g)		II 801-850 (g)		III 851-900 (g)		
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	
Ejaculate volume (ml)	229.826	5.238	269.616	3.085	281.261	5.882	1–2***; 3***
Concentration of spermatozoa (thous./mm^3)	240.478	4.450	260.944	2.921	256.811	5.115	1–2***; 3*
Percentage of progressive spermatozoa (%)	75.217	0.546	76.418	0.293	79.910	0.090	1–2*; 3***; 2–3***
Number of live spermatozoa (bln)	42.083	1.456	55.039	1.083	59.636	2.175	1–2***; 3***; 2–3*
Number of spermatozoa per insemination dose (bln)	2.589	0.015	2.570	0.007	2.536	0.011	1–3**; 2–3*
Number of insemination doses	16.315	0.581	21.325	0.425	23.550	0.868	1–2***; 3***; 2–3**

*** P<0,001 ** P<0,01 * P<0,05

Table 2

Traits of ejaculates depending on their lean meat content

Traits of semen	Lean meat (%)						Significance of differences
	I 58-59		II 60-61		III 62-63		
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	
Ejaculate volume (ml)	272.500	8.042	280.562	3.249	251.010	5.338	2–3***
Concentration of spermatozoa (thous./mm^3)	272.150	9.371	263.943	3.037	246.899	4.647	1–3*; 2–3**
Percentage of progressive spermatozoa (%)	80.000	–	78.185	0.230	75.859	0.498	1–3***; 2–3***
Number of live spermatozoa (bln)	59.800	3.240	59.137	1.187	48.716	1.749	1–3*; 2–3***
Number of spermatozoa per insemination dose (bln)	2.505	0.028	2.558	0.006	2.579	0.013	1–2*, 3**
Number of insemination doses	23.900	1.401	23.206	0.473	18.485	0.653	1–3**; 2–3***

*** P<0,001 ** P<0,01 * P<0,05

boars of group III, whose daily weight gain ranged from 851 to 900 g. Significantly lower ($P<0.001$) average volume was observed in the ejaculates of boars from group I (229.8 ml), whose daily weight gain was 751–800 g, and group II (269.6 ml), with daily weight gain of 801–850 g.

The highest average sperm concentration (260 thousand/mm^3) in the ejaculates was noted in the boars with daily weight gain of 801–850 g (group II), differing highly significantly from the boars of group I ($P<0.001$) and significantly from the boars of group III ($P<0.05$).

The ejaculates of the group III boars also had the highest percentage of sperm with progressive movement. The differences in the mean values for this trait between the males of this group and the other groups were highly significant ($P<0.001$). The values for these traits in the ejaculates of boars with different growth rates during the rearing period affected the number of insemination doses obtained per ejaculate. The most insemination doses were obtained from the ejaculates of boars from group III (23.5) and the fewest from the ejaculates of the boars in group I (16.3). The differences between groups were highly significant.

The results obtained in this study for the quantitative and qualitative characteristics of semen depending on the growth rate of boars during rearing partially correspond to the results obtained by KAWĘCKA et al. (2000), who found that young boars with somewhat faster growth rates have better semen characteristics. In a study by FALKENBERG et al. (1989), boars with faster weight gain exhibited greater sexual activity during semen collection. Sexual activity in males has a positive effect on semen characteristics (OBERLENDER et al. 2012, KONDRACKI et al. 2013). The literature contains studies indicating that the rate of daily weight gain in boars is correlated with the size and weight of their testes, which according to many authors is closely linked to semen production (SCHINCKEL et al. 1983, TOELLE et al. 1984, YOUNG et al. 1986, KAWĘCKA et al. 1997).

Faster weight gain in animals undoubtedly accelerates their sexual maturity, which may positively influence the process of spermatogenesis. ŁYCZYŃSKI (1991), in a study on the relationship between performance testing results in boars and their suitability for breeding, found that an increase in daily weight gain shortens the period of exploitation. The author claims that the semen of boars with rapid weight gain was characterized by lower volume, higher sperm concentration and a lower total sperm number per ejaculate. In a study by MILEWSKA (2007), boars with rapid weight gain produced ejaculates of significantly lower volume than boars with lower weight gain. However, these ejaculates had significantly better parameters in terms of the percentage and number of sperm with progressive movement, which is consistent with the results of our study. In contrast, a study by FIAŁKOWSKA et al. (2000) suggests

a lack of influence of growth rate in boars during rearing on the characteristics of their semen.

Table 2 presents the average values for the characteristics of the semen of the boars depending on their lean meat content on the 180th day of life. The greatest ejaculate volume (280.6 ml) was noted in the boars whose lean meat content ranged from 60% to 61%. Somewhat lower ejaculate volume (272.5 ml) was observed in the boars with meat content of 58–59%, and the lowest ejaculate volume (251.0 ml) was found in the boars with meat content of 62–63%. The differences in ejaculate volume between the boars from the second and third groups were highly significant ($P<0.001$).

Sperm concentration was highest in the ejaculates of boars with the lowest lean meat content (group I) and differed significantly ($P<0.05$) from the sperm concentration in the ejaculates of boars from group III. The percentage of sperm cells with progressive movement was also significantly higher in the ejaculates of the boars with the lowest meat content (group I). The lowest percentage of sperm cells with progressive movement was noted in the ejaculates of boars with the highest meat content (75.9%) – group III. Owing to the more favourable parameters obtained in the ejaculates of the boars with the lowest meat content (58–59%), such as ejaculate volume, sperm concentration in the ejaculates and the percentage of sperm cells with progressive movement, the highest mean number of insemination doses per ejaculate (23.9) was obtained from them. The number of insemination doses per ejaculate is very important and directly influences economic effects from semen production. According to KONDRACKI et al. (2012), age-related changes in the ejaculate characteristics of Polish Large White boars have a beneficial effect on the performance of boars of this breed measured as the number of insemination doses per ejaculate, which increases from about 22 portions obtained in the initial period of exploitation to 30 portions at the age of 19–21 months.

A similar number of insemination doses per ejaculate (23.2) was obtained from the boars with lean meat content of 60–61% (group II). The differences between groups I and III and between groups II and III were highly significant.

The results of our study on the effect of lean meat content of boars on semen quality indicate that excessive (over 61%) muscularity in boars leads to a quantitative and qualitative reduction in their semen characteristics, which is consistent with the results of other researchers (KAWĘCKA et al. 2000, 2003, MILEWSKA 2007). Excessive muscle growth in young boars during the rearing period may lead to inhibition of the development of the testes, which in turn leads to a delay in sexual maturity and decreased serum concentration of sex hormones, and in consequence to lower semen quality (DZIADEK 1999, KAWĘCKA et al. 2003).

Conclusions

High daily weight gain in Polish Large White boars during the rearing period had no negative effect on the characteristics of their semen. Boars with daily weight gain of 851–900 g up to the 180th day of life had the most favourable ejaculate parameters.

The lean meat content in the Polish Large White boars had a significant effect on their ejaculate characteristics. Boars whose meat content measured on the 180th day of life ranged from 62–63% had significantly less favourable ejaculate parameters than boars with lower meat content.

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FIELD AND FOREST WATER PONDS AS LANDSCAPE ELEMENTS AFFECTING THE BIODIVERSITY OF CARABID BEETLES (COL.; CARABIDAE)

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K e y w o r d s: ground beetles, landscape, small water ponds, assemblages, diversity.

A b s t r a c t

The study was carried out in 2006, in Tomaszkowo near Olsztyn, north-eastern Poland. It comprised two small water ponds: A – in a forest, and B – in a field. Carabid beetles were caught into Barber's traps from May to October 2006. In total, 1408 individuals belonging to Carabidae were captured: 629 individuals representing 47 species around the forest pond and 779 individuals representing 56 species around the field pond. It has been concluded that small water bodies, which improve water relations in the landscape, can considerably influence the increased diversity of Carabidae, as well as stimulate the presence of rare and valuable stenobiotic species.

ŚRÓDPOLNE I ŚRÓDLEŚNE ZBIORNIKI WODNE JAKO ELEMENTY KRAJOBRAZU WPŁYWAJĄCE NA BIORÓŻNORODNOŚĆ BIEGACZOWATYCH (COL.; CARABIDAE)

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A b s t r a c t

Badania prowadzono w roku 2006 w Tomaszkowie koło Olsztyna w północno-wschodniej części Polski. Dotyczyły one dwóch małych zbiorników wodnych: A – oczko leśne i B – śródpolne. Chrząszcze z rodziny biegaczowatych odławiano do zmodyfikowanych pułapek glebowych typu Barbera od maja do końca października 2006 roku. W czasie badań odłowiono łącznie 1408 osobników Carabidae,

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629 osobników należących do 47 gatunków wokół zbiornika śródleśnego i 779 osobników należących do 56 gatunków z okolic zbiornika śródpolnego. Wyniki analizy ekologicznej ich zgrupowań wskazują, że pozostawianie i ochrona drobnych zbiorników wodnych w krajobrazie rolniczym lub leśnym może mieć korzystny wpływ na zasiedlające je zgrupowania epigeicznych biegaczowatych, wyrażający się przede wszystkim zwiększeniem różnorodności gatunkowej Carabidae oraz pojawieniem się gatunków rzadkich i o wąskim zakresie tolerancji względem wilgotności siedliska.

Introduction

In the natural environment, so often re-shaped by people, the fragmentation of habitats is a frequent development. For biodiversity, this is definitely a positive consequence. In spatially diversified habitats, there are many half-empty niches, which facilitate easy adaptation and help to sustain greater diversity and species richness among animals (TEWS et al. 2004, TWARDOWSKI et al. 2009). The preservation of various water bodies or wetlands with their plant cover in the spatial landscape structure means the conservation of valuable natural habitats owing to the contained flora and fauna. An important group of animals representing epigeic fauna are the beetles of the family Carabidae, because most of them are predators able to withhold gradations of phytophags, both forest dwelling ones and those which inhabit agricultural biocenoses. They are also a popular zoological bioindicators (THIELE 1977, RAINIO and NIEMELA 2003, KOTZE et al. 2011).

The purpose of this study has been to describe communities of Carabidae dwelling near shores of small water ponds lying in fields and forests. It seemed important to pay more attention to such apparently inconspicuous objects as small ponds situated in various habitats, and their role in the formation of assemblages of ground beetles living around these water bodies.

The following research hypotheses were made for studied sites:

1. The areas adjacent to forest water bodies differ in species abundance of Carabidae from areas with midfield ponds.

2. The presence of small water reservoirs in the landscape fosters the appearance of stenobiontic species both in the forests and in open areas.

Methods and research area

The observations were carried out in 2006, in a village called Tomaszkowo, situated near Olsztyn (UTM DE 65), in north-eastern Poland. The study comprised two small water bodies: A – a pond in a forest, and B – a pond in a field. The former covered an area of 0.4 ha and was surrounded by about 70-year-old mixed fresh forest with the dominant pine (*Pinus sylvestris* L.).

The pond shores were overgrown with alder (*Alnus glutinosa* Gaertn.), rowans (*Sorbus aucuparia* L.) and oaks (*Quercus robur* L.); the shrubs were mostly composed of willow (*Salix alba* L.). The understory was found to comprise raspberries (*Rubus idaeus* L.), nettles (*Urtica dioica* L.) and impatiens (*Impatiens noli-tangere* L.). The second object (B) was a field pond with the surface area of 0.3 ha, supplied by rainfalls and largely overgrown with reeds (*Phragmites australis* (Cav.) Trin. ex Steud.). To the north, it bordered with a tree assemblage growing on 0.2 ha of a field and composed of such species as alder (*Alnus glutinosa* Gaertn.), birch (*Betula pendula* Roth.), poplar (*Populus alba* L.), ash (*Fraxinus excelsior* L.). The lower level consisted of willow (*Salix alba* L.), European black elder (*Sambucus nigra* L.), plum trees (*Prunus spinosa* L.), raspberries and blackberries (*Rubus idaeus* L., *Rubus plicatus* Weihe Nees), and dog rose (*Rosa canina* L.). The herbal plants included bulrush (*Scirpus sylvaticus* L.), sedge (*Carex hirta* L.), tussock-grass (*Poa pratensis* L.), cocksfoot (*Dactylis glomerata* L.). The investigated pond was surrounded by arable fields cropped with rye, wheat and barley.

The research material was captured into the Barber's traps from May to October. The traps were emptied every 10 days. Five traps were placed along the shores of each pond, about 2–5 m from the water table. In small water bodies located in forest (A), trap A1 was set at the edge of the forest, about 30 m from a forest meadow. The subsequent traps (A2 to A5) were set every 20-30 m along the shores of the pond. In study area located among fields (B), traps B1, B2 and B3 were set between the pond and fields, trap B4 was placed at the edge of the tree community and B5 was inside that groups of trees, on the edge of the water pond (Figure 1).

Fig. 1. Localization of study sites and the layout of Barber's traps (A1-A5 – traps around the pond in a forest, B1-B5 – traps around the pond in a field)

The material was species classified according to the keys by PAWŁOWSKI (1974), WATAŁA (1995) and HŮRKA (1996), using the terminology developed by ALEKSANDROWICZ (2004). The captured specimens of Carabidae were analyzed in terms of species composition and dominance structure. The following dominance classes were distinguished: dominants (<5%), subdominants (3>5%), recedents (1>3%), subrecedents (>1%). The ecological classification of the caught carabid beetles was made according to their preferences with respect to feeding, habitat, humidity and type of development. When describing the results, the Shannon's diversity index (H'), the Pielou index of evenness (J') and dominance index by Simpson (D) were applied. The significance of differences between the mean values of these indices and the abundance and number of species was assessed with a one-factorial analysis of variance ANOVA. The dependence between structure of Carabidae assemblies and type of habitat was determined by redundancy analysis (RDA) (TER BRAAK 1986). The RDA method had been selected based on the analysis of data distribution (DCA). The RDA method was applied to arrange the data and demonstrate relationships between the caught Carabidae species and habitat-specific features (distance to the forest – Forest; distance to the fields – Fields and presence of woodlots – Woodlots). The statistical significance of canonical axes was verified by the Monte Carlo test. All statistical calculations and their graphic interpretation were performed using the software packages Statistica 10 PL and Canoco 4.56 (TER BRAAK and MILAUER 1998).

Results

In total, 1408 Carabidae individuals were captured, including 629 individuals from 47 species around the forest pond and 779 individuals from 56 species around the field pond (Table 1). No statistically significant differences in the number of individuals and species, as well as in the average values of the examined indicators of the diversity between the studied habitats were observed. High values of the Shannon (H') (A – 2.95; B – 3.08) and Pielou (J') (A – 0.77; B – 0.77) indices prove quite large species diversity and evenness of the examined habitats. The Simpson index (D), had rather low values in the analyzed environments (A – 0.08; B – 0.07), possibly implying large stability of the observed communities of Carabidae. The same conclusion can be drawn from the structure of dominance (tab. 2). Although the share of dominants in both environments was high (54 and 64%), it is divided between several species, none of which obtaining dominance values high enough to distort the structure of a whole assemblage. Forest zoophages beetles prevailed in the group of carabid beetles found around the field water pond. The species

attaining the highest share was *Pterostichus oblongopunctatus* (19.87%), a forest, medium-size zoophages beetle. The high moisture of studied sites favours frequent presence of hygrophilous species, like *Agonum fuliginosum* and *Oxypselaphus obscurus*. In the vicinity of the forest pond, the dominant species were mostly open area Carabidae. The highest shares were achieved by the aphid-eating, medium-size zoophages beetle *Anchomenus dorsalis* (12.07%).

Table 1

Species composition and number of individuals of Carabidae caught in the studied area

Species	Abbreviation	Pond in a forest (A)		Pond in a field (B)	
		n	D*(%)	N	D (%)
1	2	3	4	5	6
Agonum ericeti (Panzer,1809)	Ag_eri	0	0.00	1	0.13
A. fuliginosum (Panzer,1809)	A_ful	67	10.65	25	3.21
A. sexpunctatum (Linnaeus,1758)	Ag_sex	1	0.16	0	0.00
Amara aenea (Degeer,1774)	Am_aen	3	0.48	6	0.77
A. aulica (Panzer,1797)	Am_aul	0	0.00	1	0.13
A. bifrons (Gyllenhal,1810)	Am_bif	0	0.00	1	0.13
A. communis (Panzer,1797)	Am_com	12	1.91	2	0.26
A. convexior Stephens,1828	Am_conv	3	0.48	11	1.41
A. littorea Thomson,1857	Am_litt	1	0.16	0	0.00
A. lunicollis Schiodte,1837	Am_lun	15	2.38	8	1.03
A. plebeja (Gyllenhal,1810)	Am_ple	12	1.91	2	0.26
A. similata (Gyllenhal,1810)	Am_sim	0	0.00	2	0.26
Anchomenus dorsalis (Pontoppidan,1763)	Anch_dor	1	0.16	94	12.07
Anisodactylus binotatus (Fabricius,1787)	Ani_bin	1	0.16	0	0.00
Asaphidion flavipes (Linnaeus,1761)	Asa_fla	0	0.00	6	0.77
Badister lacertosus (Sturm,1815)	Bad_lac	3	0.48	2	0.26
Bembidion femoratum (Sturm,1825)	Bem_fem	0	0.00	1	0.13
B. lampros (Herbst,1784)	Bem_lam	3	0.48	1	0.13
B. properans (Stephens,1828)	Bem_prop	1	0.16	2	0.26
Calathus ambiguus (Paykull,1790)	Cal_amb	1	0.16	0	0.00
C. fuscipes (Goeze,1777)	Cal_fus	11	1.75	22	2.82
C. melanocephalus (Linnaeus,1758)	Cal_mel	1	0.16	0	0.00
Carabus arcensis (Herbst,1784)	Car_arc	4	0.64	1	0.13
C. cancellatus (Illiger,1798)	Car_can	1	0.16	2	0.26
C. granulatus (Linnaeus,1758)	Car_gra	53	8.43	64	8.22
C. hortensis (Linnaeus,1758)	Car_hor	38	6.04	37	4.75
C. nemoralis (O.F.Muller,1764)	Car_nem	2	0.32	25	3.21
C. violaceus (Linnaeus,1758)	Car_vio	11	1.75	1	0.13
Clivina fossor (Linnaeus,1758)	Cli_fos	0	0.00	1	0.13
Cychrus caraboides (Linnaeus,1758)	Cych_car	13	2.07	11	1.41
Dicheirotrichus placidus (Gyllenhal,1827)	Dich_pla	0	0.00	1	0.13
Dolichus halensis (Schaller,1783)	Dol_hal	1	0.16	1	0.13
Harpalus affinis (Schrank,1781)	H_aff	0	0.00	2	0.26
H. griseus (Duftschmid,1812)	H_gri	1	0.16	0	0.00
H. laevipes (Dejean,1829)	H_lea	2	0.32	8	1.03
H. latus (Linnaeus,1758)	H_lat	0	0.00	4	0.51
H. rubripes (Duftschmid,1812)	H_rub	0	0.00	8	1.03
H. rufipalpis (Sturm,1818)	H_rufip	0	0.00	1	0.13

cont. Table 1

1	2	3	4	5	6
H. rufipes (Degeer,1774)	H_ruf	24	3.82	13	1.67
H. tardus (Panzer,1797)	H_tar	0	0.00	5	0.64
Leistus terminatus (Hellwig,1793)	Lei_term	0	0.00	2	0.26
Limodromus assimilis (Paykull,1790)	Lim_as	29	4.61	63	8.09
Loricera pilicornis (Fabricius,1775)	Lo_pil	5	0.79	4	0.51
Nebria brevicollis (Fabricius,1792)	Ne_brevi	2	0.32	4	0.51
Notiophilus palustris (Duftschmid,1812)	N_pal	2	0.32	3	0.39
Oodes helopioides (Fabricius,1792)	Oo_hel	1	0.16	4	0.51
Oxypselaphus obscurus (Herbst,1784)	Oxy_obs	31	4.93	10	1.28
Panagaeus bipustulatus (Fabricius,1775)	Pan_bipu	0	0.00	1	0.13
Patrobus atrorufus (Strom,1768)	Pat_atr	2	0.32	0	0.00
Poecilus cupreus (Linnaeus,1758)	Po_cupr	3	0.48	59	7.57
P. lepidus (Leske,1785)	Po_lepi	9	1.43	1	0.13
P. versicolor (Sturm,1824)	Po_ver	11	1.75	51	6.55
Pterostichus aethiops (Panzer,1797)	Pt_aeth	0	0.00	1	0.13
P. anthracinus (Illiger,1798)	Pt_anth	2	0.32	6	0.77
P. diligens (Sturm,1824)	Pt_dil	1	0.16	3	0.39
P. melanarius (Illiger,1798)	Pt_mela	59	9.38	88	11.3
P. minor (Gyllenhal,1827)	Pt_min	12	1.91	4	0.51
P. niger (Schaller,1783)	Pt_nig	31	4.93	11	1.41
P. nigrita (Paykull,1790)	Pt_nigr	2	0.32	7	0.9
P. oblongopunctatus (Fabricius,1787)	Pt_oblo	125	19.89	79	10.14
P. quadrifoveolatus (Letzner,1852)	Pt_quadr	1	0.16	0	0.00
P. rhaeticus (Heer,1838)	Pt_rhae	0	0.00	2	0.26
P. strenuus (Panzer,1797)	Pt_stre	14	2.23	2	0.26
Synuchus vivalis (Illiger,1798)	Syn_viv	0	0.00	2	0.26
Trechus quadristriatus (Schrank,1781)	Tre_qua	1	0.16	0	0.00
Number of individuals		629		779	
Number of species		47		56	
Shannon' diversity (H') (Log Base 2,718)		2.95		3.08	
Evenness Pielou J'		0.77		0.77	
Simpson's Diversity (D)		0.08		0.07	

*D [%] – dominance coefficient

While searching for dependences between habitat conditions and the occurrence of Carabidae species, the redundancy analysis (RDA) was carried out. By analyzing the presence of particular Carabidae species in the context of varied habitat conditions such as the presence of fields, forests or groups of trees, groups of species characteristic for the specific habitats were clearly distinguished.

The presence of trees and shrubs as well as such common species as *Pterostichus melanarius, Harpalus rufipes* and *Limodromus assimilis* was positively correlated with the first ordination axis, describing almost 46% of the variation (fig. 2). In addition, the distribution of species in the diagram RDA, especially correlation of higrophilic species such as *Pterostichus nigrita, P. rhaeticus, P. aethiops* and *P. diligens* with the I ordination axis may indicate that habitat humidity is a factor strongly influencing the occurrence of ground beetles assemblages and stenotopic species.

Table 2

Share of carabid beetles in the studied areas according to the dominance classes

Dominance class	Pond in a forest (A)		Pond in a field (B)	
	Species	D*[%]	Species	D*[%]
Dominant species (<5%)	*Pterostichus oblongopunctatus*	19.87	*Anchomenus dorsalis*	12.07
	Agonum fuliginosum	10.65	*Pterostichus melanarius*	11.30
	Pterostichus melanarius	9.38	*Pterostichus oblongopunctatus*	10.14
	Carabus granulatus	8.43	*Carabus granulatus*	8.22
	Carabus hortensis	6.04	*Limodromus assimilis*	8.09
			Poecilus cupreus	7.57
			Poecilus versicolor	6.55
		54.37		63.93
Sub-dominant species (3>5%)	*Oxypselaphus obscurus*	4.93	*Carabus hortensis*	4.75
	Pterostichus niger	4.93	*Carabus nemoralis*	3.21
	Limodromus assimilis	4.61	*Agonum fuliginosum*	3.21
	Harpalus rufipes	3.82		
		18.28		11.17
Recedent species (1>3%)	10 gatunków	19.08	9 gatunków	13.09
Sub-recedent species (>1%)	28 gatunków	8.27	37 gatunków	11.81

*D [%] – dominance coefficient

The second ordination axis, describing over 30% of the variation, was found to correlate positively with the vicinity of fields. This variable was associated with a whole series of carabid beetles demonstrating highly varied habitat requirements.

Based on the results of the RDA, an attempt was made to verify whether there is any relationship between habitat conditions and the presence of specific ecological groups of Carabidae (Figure 3).

It was shown that trap B5, located on the edge of the water body, in a group of trees adjacent to the observed field pond, was most strongly correlated with the first ordination axis. Probably the tree patch gave shelter to many specimens representing various ecological groups of Carabidae. There were large and medium zoophages, eurytopic species and species dwelling on peat bogs among the captured specimens.

The second canonical axis corresponded to the presence of open area carabids, which in some sense was connected with trap B3, situated between the pond and an arable field. Negative correlation was observed between the second ordination axis and the forest pond. To the north-east, this pond was adjacent to a small xerothermic meadow, which was manifested by the presence of small zoophages beetles, typical of field and meadow habitats, in trap A1, which was the closest to the said meadow.

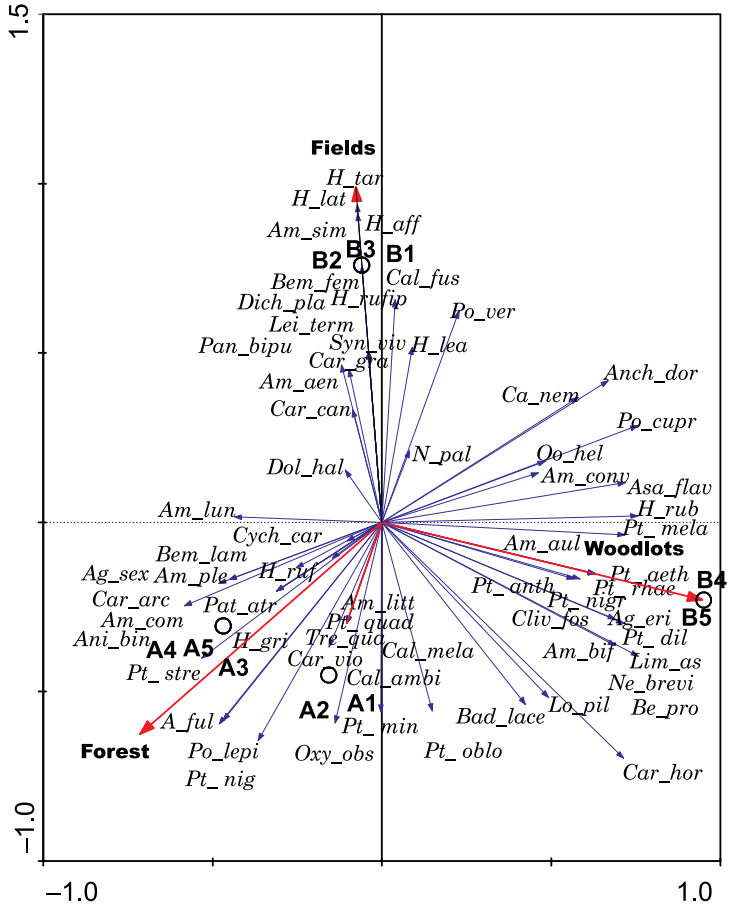

Fig. 2. Diagram of RDA analysis for the examined assemblages of Carabidae and selected environmental variables. Explanation and abbreviations of species are given in Table 1 and methods

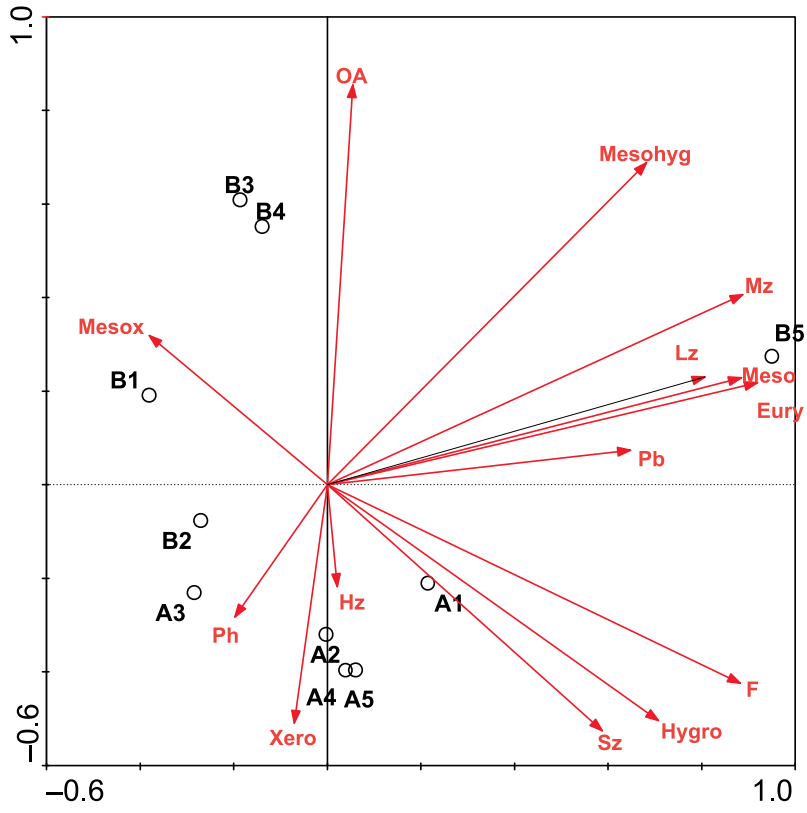

Fig. 3. Diagram of the redundancy analysis of RDA representing dependences between the analyzed habitats and ecological groups of Carabidae (Lz – Large zoophages, Mz – Medium zoophages, Sz – Small zoophages, Hz – Hemizoophages, Ph – Phytophages, F – Forest species, OA – Open area species, Pb – Peatbog species, Eury – Eurytopic species, Xero – Xerophilous species, Mesox – Mesoxerophilous species, Meso – Mesophilous species, Mezohyg – Mesohygrophilous species, Hygro – Hygrophilous species)

Discussion

In this study the hypothesis was not confirmed that the areas adjacent to forest water bodies differ in species richness of Carabidae from areas with midfield ponds. Despite the fact that near the midfield ponds more species of beetles than in the forest were recorded, these differences were not statistically significant. However high values of Shannon species diversity index should be noted in both the examined sites. In other studies on Carabidae of fields and woodlots from Tomaszkowo (KOSEWSKA at al. 2006, 2007, 2008, 2009) significantly lower values of this index were observed, in the fields, as well as wooded areas. This may prove that this type of habitat is very valuable for biodiversity conservation.

Small water bodies located in different habitats may appear to be rather inostensible landscape components, but more thorough examination will reveal their substantial influence, e.g. on the occurrence of Carabidae. Nowadays, when agriculture grows dynamically, swamps, peat bogs and other wetland habitats – due to drainage works and water retention engineering – are among disappearing habitats, which leads to a complete loss of peat-dwelling fauna and its transformation into a less valuable variant of carabid assemblages associated with fields (ALEKSANDROWICZ 2002). Even small water bodies, by improving water relations in the natural environment, contribute to a more numerous appearance of moisture favouring ground beetles which was confirmed in the studies. JĘDRYCZKOWSKI and KUPRYJANOWICZ (2005) argue that as the relative humidity of a habitat increases, so does the number of beetle species. Also, WOJAS (2008) reveals the richness of carabid fauna on the shores of stagnant water bodies and all types of wet and muddy environments. NIETUPSKI et al. (2007) observed carabid communities dwelling on cut meadows characterized by different moisture content and noticed more species on a very moist meadow lying by a water body. Such habitats, apart from species highly adaptable to different conditions, are inhabited by stenotopic Carabidae (JASKUŁA and STĘPIEŃ 2012).

The species composition of assemblages of Carabidae is often determined by the spatial differentiation of habitats (DUELLI et al. 1999, SKŁODOWSKI 2002). The composition and number of beetles translate directly to the structure of dominance of the examined assemblages. In the habitats analyzed in the current study, percentages of individual Carabidae species were quite even. There were no cases of extremely high shares of some of the dominant species, which could disturbe the structure of a whole community. The low rate Simpson's index, which pays more attention to common species while underestimating rare ones also indicates that. There were quite numerous species classified as recedent and subrecedent groups, a finding which – according to TROJAN (1999) – confirms that habitats located within a given territory are diverse. Small water ponds are most often associated with characteristic plants, shrubs and groups of trees. Ground beetles can use the examined habitats as a shelter, a place suitable for their development or a source of food in time periods with poorer food supplies (most often plant pests) on fields and in forests. Notable is the fact that some rare hygrophilous species e.g. *Oodes helopioides*, *Dicheirotrichus placidus* and *Agonum ericeti* were detected near the field pond. *Agonum ericeti* is recognized as a typically tyrphobiontic species (LINDROTH 1945, ALEKSANDROWICZ 2004, DREES et al 2007) so its presence in the studied habitat is all the more valuable. Therefore maintaining and protecting small water bodies on farmland or woodland may have a favourable influence on the epigeic ground beetles dwelling in such habitats, which could

manifest itself by a raised species diversity of Carabidae and the occurrence of rare species as well as the ones which have a narrow tolerance range in terms of habitat humidity.

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THE EFFECT OF ABOVE-WATER ARTIFICIAL LIGHT ON THE ZOOPLANKTON ABUNDANCE IN CAGES FOR FISH REARING

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K e y w o r d s: concentrating of zooplankton, illuminated cage, phototaxis, lake.

A b s t r a c t

The fish larvae rearing method in illuminated cages was originally dedicated to the coregonid spp. Its technical variants are currently applied in rearing other fish species. This method is based on the attraction of zooplankton by a light source placed inside a fish cage. Zooplankton is the sole or main food source for the fish inside such a cage, therefore an effective means of attracting the plankton is critical to effective fish rearing. The aim of this paper is to assess the influence of above-water illumination in the zooplankton abundance in lake-based fish rearing cages. The experiment was conducted in eutrophic Lake Maróz (Northeastern Poland). Observations were conducted starting at dusk in lit (24V, 60W electric bulb located just above the water surface) and unlit cages. The above-water illumination significantly increases the abundance of the Cladocera and adult Copepoda forms inside the cage. At the same time, a significantly reduced attraction to visible light was noted for the juvenile Copepoda and Rotifera forms. Overall, the above-water illumination is an effective method. The level of zooplankton density and its overall abundance might be dependent on the zooplankton's qualitative structure.

WPŁYW NADWODNEGO ŚWIATŁA NA LICZEBNOŚĆ ZOOPLANKTONU W SADZACH DO PODCHOWU RYB

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Abstrakt

Sadze oświetlone pierwszy raz zastosowano przy podchowie larw koregonidów. W różnych wariantach technologicznych jest obecnie wykorzystywana przy podchowie innych gatunków ryb. Metoda ta jest oparta na wabieniu zooplanktonu przez światło umieszczone we wnętrzu sadza. Dla ryb w sadzach zooplankton jest głównym źródłem pokarmu. Dlatego skuteczność jego wabienia jest kluczowa dla efektywności podchowu. Celem niniejszej pracy jest ocena wpływu nadwodnego oświetlenia na liczebność zooplanktonu w środowisku jeziorowych sadzów do podchowu ryb. Eksperyment przeprowadzono w jeziorze Maróz (północno-wschodnia Polska). Obserwacje prowadzono w sadzach oświetlonych od zmierzchu żarówką elektryczną umieszczoną tuż nad powierzchnią wody (24V, 60W) oraz w sadzach nieoświetlonych. Oświetlenie nadwodne w porze nocnej istotnie zwiększa liczebność Cladocera oraz dorosłych form Copepoda w sadzach. Jednocześnie wykazano istotnie mniejsze wabiące oddziaływanie światła widzialnego na młodociane formy Copepoda oraz Rotifera. Nadwodne źródło światła jest rozwiązaniem efektywnym. Zagęszczenie zooplanktonu pod wpływem światła i ogólna liczebność organizmów pokarmowych dla ryb mogą być znacząco uzależnione od struktury jakościowej zooplanktonu.

Introduction

The influence of variable light source intensity on the dial vertical migration (DVM) of the zooplankton continues to be the subject of numerous studies (RINGELBERG and FLIK 1994, MARTYNOVA and GORDEEVA 2010). The method of fish rearing in illuminated cages was developed in Poland during the 1970s and was dedicated to the coregonid spp. (MAMCARZ 1995a). This method is based on the attraction of zooplankton by a light source placed inside a fish cage (MAMCARZ 1995b). The technical variants of this method included submerged and surface cages illuminated by an a submerged light source (MAMCARZ and NOWAK 1987, MAMCARZ 1995a). The effectiveness of zooplankton concentration using an a submerged light source was also assessed in several papers and range from twofold (ZILIUKENE 2005), 5–15 fold (CECCUZZI et al. 2010) to about 40-fold (SICHROVSKY et al. 2013). They indicate the multitude of factors influencing the zooplankton concentration in cages (MAMCARZ 1995b, FERMIN and SERONAY 1997).

Submerged incandescent 60W bulbs (ZILIUKENE 2005), submerged 12V halogen bulbs (SICHROVSKY et al. 2013), also 60W LED bulbs (CECCUZZI et al. 2010) were used for attraction of zooplankton. Above-water light sources were also utilized in experimental fish rearing cages (SKRZYPCZAK et al. 1998b, FURGALA-SELEZNIOW et al. 2014). Using above-water light sources, alternative systems of zooplankton accumulation and transport (pump) to the rearing cages were developed (SKRZYPCZAK et al. 1998a). The effectiveness of concentrating zooplankton is dependent on the intensity and range of the light source. It has been hypothesized that relative changes in light intensity trigger a migratory response in zooplankton (RINGELBERG and FLIK 1994, NESBITT et al. 1996, DODSON et al. 1997). It must be assumed that placing a light source

above the water surface restricts the illuminated zone because when light passes from air into water, the light is refracted (or bent) towards the normal (GOODMAN 1993).

Presently several zooplankton sampling methods are used. One of them is engine driven pump, which didn't revealed significant advantage over 10L Schindler-Patalas trap (SICHROVSKY et al. 2013). Another known and used trap is Ruttner's bottle. Traditional conical shape net for taking samples is still used (CECCUZZI et al. 2010, MARTYNOVA and GORDEEVA 2010). None of the zooplankton sample collection methods is universally-applicable. On the contrary, each of them is usually dedicated to specific environmental conditions (PAGGI et al. 2001). The effectiveness of sample collection and the reliability of the obtained results continues to be largely dependent on the accurate selection of methods and the researcher's manual abilities (LIVINGS et al. 2010).

The aim of this paper is to assess the influence of above-water illumination on the zooplankton abundance in lake-based fish rearing cages.

Materials and Methods

The experiment was conducted in May 2013 in Lake Maróz, Poland (eutrophic, max. depth 41.0 m, 53° 32'N, 20°25'E, 3.32 km). The experimental environment were cuboid shape net cages (side length 1.0 m; volume 2.0 m^3) for fish rearing, made of nylon with mesh size 1.2 mm. The zooplankton samples were collected every three days in empty cages (no fish were present): one illuminated (CI) and the other unlit (CU). The distance between the two cages was 8 meters. In the CI cage, the light source was an electric bulb (24V, 60W), located just above water's surface and switched on 2 hours before sample collection. The samples were collected simultaneously in both cages (usually between 23:00–23:30) using a plankton net (mesh size 30 μm, round intake with diameter 2.2 dm, 3.8 dm^2, filtration surface 24 dm^2, volume 9.0 dm^3) hauled vertically from the bottom of the cages to the water surface (2.0 m). Each haul penetrated 76 dm^3 of water column volume. The average haul velocity was about 0.05m s^{-1} (total haul time of about 40s). The samples were condensed to the volume of 0.1 dm^3, preserved in Lugol solution and conserved in a 4% formaldehyde solution. The zooplankton identification was performed until the lowest possible taxonomic unit was identified in accordance with the following methodologies: FLÖSSNER (1972), KIEFER and FRYER (1978), KOSTE (1978). The quantitative analysis was performed using the Sedgewick-Rafter counting chamber and reported in the volume unit (ind. dm^{-3}). The zooplankton was observed at the level of three taxonomic groups: Cladocera, Copepoda

(adult and juvenile forms) and Rotifera. The level of light-induced plankton density was expressed using the concentration ratio of organisms (ind. dm^{-3}) in the lit and unlit cages (CI/CU ratio). To assess the general differences in the CI/CU ratios of the analyzed zooplankton groups, non-parametric analysis of variance was applied (Statistica 10.0 for Windows, Statsoft; Tulsa, UK). The results were processed by ANOVA with the non-parametric Kruskal-Wallis test to determine the statistically significant differences ($p \leq 0.05$).

Results

Large variability of the zooplankton abundance was noted in the fish rearing cages. The largest concentration of the Rotifera was noted on May 31st: 1065 ind. dm^{-3} in the illuminated cage (CI) and 816 ind. dm^{-3} in the unlit cage (CU) (Figure 1). The qualitative analysis of that day's samples revealed 8 species, with *Pompholyx sp.* being the most common. On the day of Rotifera's least abundance (May 16th, CI 142 ind. dm^{-3} and CU 119 ind. dm^{-3}), the species structure was dominated by the *Keratella sp*. Whereas on May 19th the Rotifera abundance in the CU was slightly greater than in the CI, 365 and 332 ind. dm^{-3}, respectively. On the same day, the greatest abundance of the Cladocera was noted (CI 554 ind. dm^{-3} and CU 140 ind. dm^{-3}) (Figure 2). Among the five Cladocera species, *Daphnia sp.* was the most abundant in both cages. Whereas the Cladocera were the least abundant on May 28th (CI 35 and 9 ind. dm^{-3}). The Copepoda were also the least abundant on that day: CI 66 ind. dm^{-3} and CU 44 ind. dm^{-3}) (Figure 3). Whereas the greatest Copepoda abundance was noted on May 4th: CI 908 ind. dm^{-3} and CU 761 ind. dm^{-3}. In both cases the juvenile forms (nauplius and copepodit) had the greatest influence on the total abundance of the Copepoda. Furthermore, it is the Copepoda forms that was noted to have the most instances of lack of accumulation in the illuminated cage. The values of CI/CU≤1.0 were noted on a total of five sample collection days (Figure 4). In four other cases, the CI/CU ration oscillated between 1.2 and 1.3. Only on May 25th its value reached 2.8 and it was noted in the conditions of decreased total plankton abundance (348 ind. dm^{-3} and 209 ind. dm^{-3}, respectively). The mean CI/CU ratio for the juvenile Copepoda (CP-J) was 1.26 (±0.57) and was not statistically different from the mean CI/CU ratio for the Copepoda (CP-T) and Rotifera (Table 1). This last taxonomic group was noted to have the smallest mean CI/CU ratio: 1.16 (±0.15). However, the values CI/CU≤1.0 were noted only twice for the Rotifera: May 13th and May 19th (Figure 4). The greatest density indicators were noted for the Cladocera and the adult Copepoda (CP-A): 5.5 on May 22nd and 4.6 May 4th. For both forms, the noted CI/CU ratio did not fall below 2.1. The CI/CU ratios for the Cladocera and

Copepoda adults were statistically larger than the CI/CU ratios for the rest of the zooplankton forms ($P<0.01$). The mean CI/CU ratio for the Cladocera was 3.96 (±0.99), and for the Copepoda 3.38 (±0.91). However, no statistically significant differences were noted between them (ANOVA, Kruskal-Wallis test, *P<0.01*).

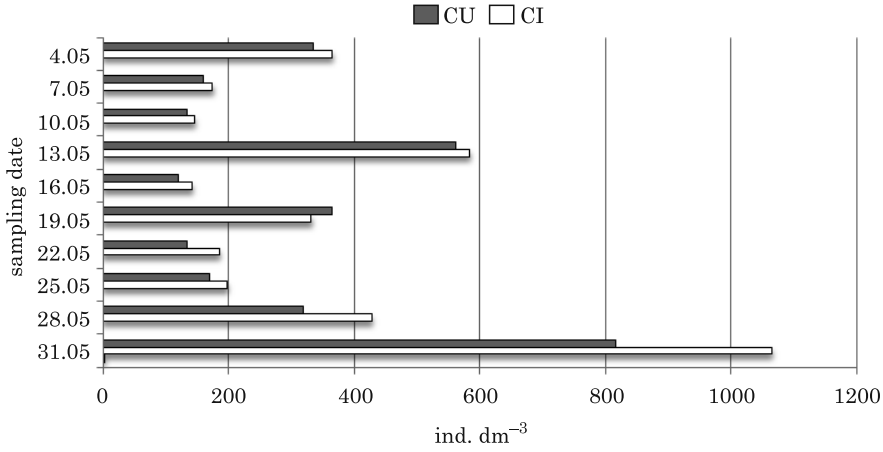

Fig. 1. The Rotifera density in fish rearing cages (CI- cage illuminated; CU- cage unlit)

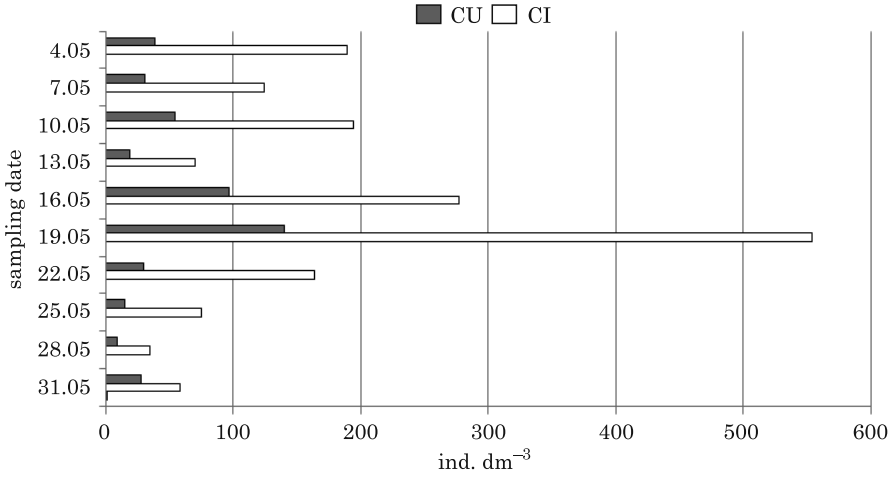

Fig. 2. The Cladocera density in fish rearing cages (CI- cage illuminated; CU- cage unlit)

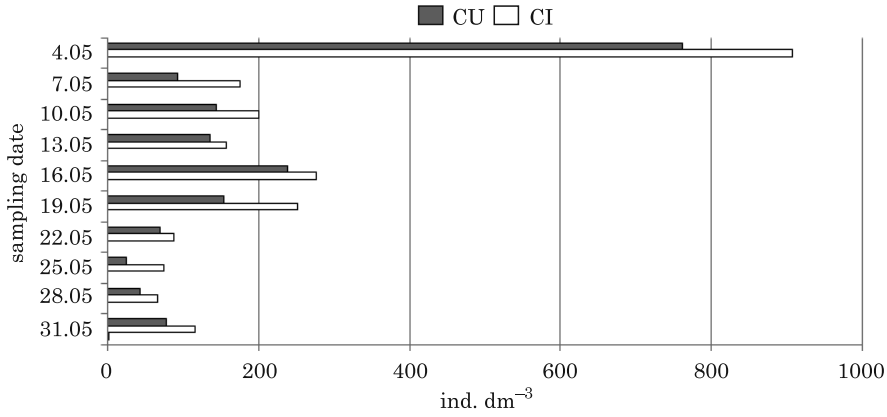

Fig. 3. The Copepoda density in fish rearing cages (CI- cage illuminated; CU- cage unlit)

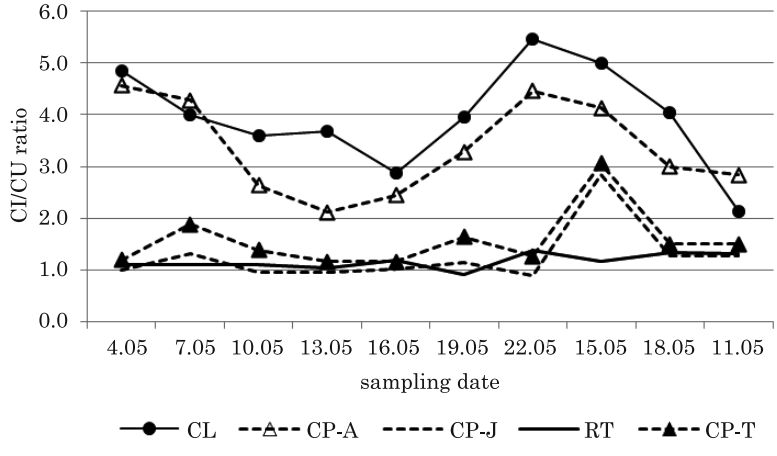

Fig. 4. The zooplankton density ratio (CI/CU) during the experiment (CI- cage illuminated; CU- cage unlit; CL- Cladocera; CP-A – Copepoda adult forms; CP-J – Copepoda juvenile forms; CP-T – Copepoda total; RT – Rotifera)

Table 1. Mean density of zooplankton (CL – Cladocera; CP-T – Copepoda total; CP-A – Copepoda adults forms; CP-J – Copepoda juvenile forms; RT – Rotifera) in fish rearing cages (CI – illuminated; CU – unlit). The means of ranks CI/CU ratio with the different letter index are statistically different (Kruskal-Wallis test H=36.19; df4; N=50; P<0.01)

Parameter	Unit of measure	CL	CP-T	CP-A	CP-J	RT
Mean CI abundance	ind. dm^{-3} (±SD)	174 (±153)	231 (±248)	70 (±54)	162 (±200)	362 (±286)
Mean CU abundance	ind. dm^{-3} (±SD)	46 (±41)	174 (±215)	21 (±14)	153 (±205)	311 (±227)
Mean CI/CU ratio	$\bar{x}$ (±SD)	3.96 (±0.99)	1.57 (±0.57)	3.38 (±0.91)	1.26 (±0.57)	1.16 (±0.15)
Mean of ranks CI/CU ratio	$\bar{x}$	41.7^{A}	22.5^{B}	3.2^{A}	11.9^{B}	13.2^{B}

Discussion

During conducted experiment were ascertained dynamic qualitative and quantitative changes in the lake zooplankton. The seasonal changes in the zooplankton abundance are caused by numerous biotic and abiotic factors which were extensively studied (WANG et al. 2007, SUTHERS and RISSIK 2009). We demonstrated an influence of the above-water light source on the zooplankton density that is partially consistent with the observations by MAMCARZ (1995b). In that study, the highest indicators of zooplankton density were noted in a shallow pond (2.0 m), the lowest were noted in a stratified lake and the greatest increase in abundance was noted in the Cladocera but also was observed in the Copepoda and Rotifera (MAMCARZ 1995b). Therefore, a pronounced reaction to light must be expected in plankton organisms which are sensitive to light, such as many of Cladocera and Copepoda which undergo vertical migration. Light is one of the key factors guiding their migration behavior during the 24-hour cycle (LAMPERT and SOMMER 2001, KUCZYŃSKA-KIPPEN 2008). Some of the representatives of the *Daphnia spp.* demonstrate negative phototaxis in response to UV-emitting light sources and positive phototaxis in response to visible light (MOORE 1912, STORZ and PAUL 1998). Whereas some of the Copepoda spp. do not demonstrate sensitivity to UV light and the basis of their migration behaviors remains unexplained (WILLIAMSON et al. 2011). The *Eudiaptomus sp*. and *Cyclops sp.* demonstrate diverse migration behaviors but their concentration in the surface layers during night time is undisputed (PASTERNAK et al. 2006). At the same time, no migration activity has been noted in the Copepoda nauplius forms, which may result from their reduced sensitivity to light stimuli (LAMPERT 1992, LOOSE 1993). Phototaxis among the Rotifera is also equivocal and controversial (KIM et al. 2014). Various species of zooplankton evolve different ways to avoid predation pressure (descent into depth or diapauses in life cycle), vertical or horizontal migrations, compensation of elevated mortality with increasing feeding and reproduction output, changes in habitat use (PASTERNAK et al. 2006). This may partly explain why we observed a significantly reduced level of Rotifera and juvenile Copepoda accumulation in the illuminated cage. PASTERNAK et al. (2006) suggest that movement potential of nauplii and copepodite stages I and II of *Eudiaptomus graciloides* and *E.gracilis* is much less than that of the older individuals.

Conclusions

The above-water illumination significantly increases the abundance of the Cladocera and adult Copepoda forms inside the cage. At the same time, a significantly reduced attraction to visible light was noted for the juvenile Copepoda and Rotifera forms. Our results indicate that above-water illumination might be effective in concentrating the zooplankton in surface cages. The level of zooplankton density and its overall abundance might be dependent on the zooplankton's qualitative structure. Reduced zooplankton density in illuminated cages should be expected in case of dominance of taxa less sensitive to light stimuli. The analysis of this phenomenon requires further study.

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THE EFFECT OF MAGGOTS ADDITION TO THE COMMERCIAL FEED AND A NATURAL DIET OF EUROPEAN GRAYLING (*THYMALLUS THYMALLUS* L.) SPAWNERS ON EMBRYOS SURVIVAL TO THE EYED-EGG STAGE

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K e y w o r d s: diet supplementation, insects, eggs quality, farmed broodstock, fish feeding, reproduction.

A b s t r a c t

A rearing experiment was focused on the effect of the type and diet composition on the European grayling (*Thymallus thymallus* L.) eggs quality, expressed by the embryos survival to the eyed-egg stage. Three feeding variants were analyzed. Spawners from group I were fed exclusively trout feed, group II received trout feed with maggots and fish in group III foraged on natural feed. A statistically significantly higher survival rate was determined for the eggs produced by group III spawners (84.57%) compared to the other two variants: 48.44% for group I and 56.00% for group II (the Tukey's *post hoc* test, $P < 0.05$).

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WPŁYW DODATKU LARW MUCH DO PASZY KOMERCYJNEJ ORAZ NATURALNEJ DIETY TARLAKÓW LIPIENIA EUROPEJSKIEGO (*THYMALLUS THYMALLUS* L.) NA PRZEŻYWALNOŚĆ EMBRIONÓW DO STADIUM ZAOCZKOWANIA

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S ł o w a k l u c z o w e: wzbogacanie diety, robaki, jakość ikry, hodowlane stado rozrodcze, żywienie ryb, rozród.

A b s t r a k t

Przeprowadzono eksperyment hodowlany dotyczący wpływu rodzaju i składu diety na jakość ikry lipienia europejskiego (*Thymallus thymallus* L.), wyrażoną przeżywalnością embrionów do stadium zaoczkowania. Analizowano trzy warianty żywieniowe. Tarlaki z grupy I karmione były wyłącznie komponowaną paszą pstrągową, z grupy II – paszą pstrągową z dodatkiem larw much, natomiast III grupa ryb odżywiała się wyłącznie pokarmem naturalnym. Wykazano istotnie wyższą przeżywalność embrionów z ikry pozyskanej od tarlaków z grupy III (84,57%) w stosunku do pozostałych wariantów – 48,44% i 56,00% odpowiednio w I i II wariancie (Tukey *post hoc* test, $P < 0.05$).

Introduction

Stocking material production of endangered fish species and the reinforcement of natural fish populations with material obtained under controlled conditions is an important element of active fish protection. These activities, carried out *ex situ* mostly involves implementation of the breeding methods for a given species and maintaining fish broodstocks in pond facilities (GORYCZKO et al. 2001). Fish breeding stations should play a dominant role (WITKOWSKI and BARTEL 1999), as they guarantee the suitable conditions for fish rearing, which can ensure regular stocking actions (WITKOWSKI 1992). Successful production of stocking material in pond facilities requires, among others, optimum feeding strategies for fish during the successive development stages (IZQUIERDO et al. 2001, SPURNÝ et al. 2004, WUNDERLICH et al. 2011, CZERNIAWSKI et al. 2011, CZERNIAWSKI et al. 2012, SZMYT et al. 2013).

Currently, some of the fish breeding stations continue to acquire spawners from natural habitats. However, when populations of the species become extremely limited, this approach is less effective and may hinder stocking

material production. On the other hand, acquisition of gametes from farmed broodstocks is not very popular, even though it seems to be a recommendable or even necessary solution with respect to many species (ZAKĘŚ and DEMSKA-ZAKĘŚ 2009, SZCZEPKOWSKI et al. 2010, WITKOWSKI et al. 2013), including the European grayling. In Europe, e.g. in Poland (SZMYT 2011) or the Czech Republic (TUREK 2010), the above method may help to stabilize fish stocking material production on a satisfying level. Nonetheless, in the late 20th century, only four European grayling broodstocks were reared in pond facilities in Poland (AUGUSTYN et al. 2000), and the situation has not improved since then. The main problems are: the complicated breeding biotechnology (KOWALEWSKI 1987, 2002, GORYCZKO et al. 1995), burdened with such problems as the high embryos mortality during incubation and, more importantly, the lack of suitable feed dedicated for the European grayling spawners.

Apart from the rate of breeding success, the efficiency of fish breeding depends on the quality of gametes. While state-of-the-art innovative biotechnological solutions are increasingly often implemented, much attention is still being paid to the right selection of feeds. The overall goal is to obtain good quality gametes, which in turn is a deciding factor for the survival and growth of larvae. The gametes quality depends on external conditions (the genotype, age and size of spawners, size and development stage of gametes) as well as internal ones, including technical manipulations and the nutrition of spawners (CRAIG and HARVEY 1984, BALLESTRAZZI et al. 2003). Feeding not only affects the age of fish maturation, their fecundity, the size and chemical composition of eggs, but it also influences the development of embryos (IZOQIUREDO et al. 2001, SARGENT et al. 2002, KOWALSKA et al. 2006). The spawning success and survival of the offsprings are largely dependent on the polyunsaturated fatty acids and carotenoids supplied with a diet (CRAIK 1985, TVERANGER 1986, VERRETH et al. 1994, GEORGE et al. 2001).

Due to their relatively small size, grayling individuals in a broodstock cannot be fed typical feed for trout spawners. Out of necessity, they are fed grower feed. However, the excessive content of fat combined with the insufficient levels of vitamins and minerals relative to the requirements of potential reproducers in such feeds have an adverse impact on the condition of fish and consequently on the quality of gametes they produce (JENSEN 1996, CIERESZKO et al. 2002). As a result, the embryos survival rate to the eyed-egg stage is deteriorated. An alternative solution is to reduce the amount of grower feed and to enrich spawners diet with components which will counteract the deficiencies of primary nutrients. A right diet for spawners can be designed based on the chemical composition of fish gametes originating from the natural environment (SARGENT et al. 2002, JANKOWSKA et al. 2003). Selection of a suitable feed is one of the most difficult steps in fish rearing, especially

concern fish species for which feed recipes have not been worked out yet. This is the obstacle encountered in the rearing of the European grayling, a fish seen as an important element in the species diversity of ichthyofauna.

The objective of this study has been to determine exclusively an effect of the type and feed composition on the quality of European grayling (*Thymallus thymallus* L.) eggs, expressed by the embryos survival rate until the development of eyed-egg stage.

Material and Methods

The research on the effect of the European grayling spawners diet supplementation was composed of two steps. The first step was to arrive experimentally at the preliminary biotechnological assumptions, primarily the type of tested food and the rearing conditions for spawners (SZMYT et al. 2004). The second step, described in this article, is the continuation and optimization of our previous investigations.

Fish rearing and feeding

The study material comprised a farmed broodstock of the European grayling reared at the Department of Salmonid Research in Rutki, Inland Fisheries Institute in Olsztyn (GORYCZKO et al. 1995). The experiment was carried out in production scale. After the 2004 spawning season, the whole broodstock was divided into three treatments, without determination of spawners sex ratio. Two groups of spawners were kept indoors, in concrete tanks with the bottom area equal 9 m^2 and the working capacity 5.4 m^3 each. The third group of fish was kept in a semi-natural environment such as a sedimentation pool, dimensioned on 50m long x 20m width and 2m depth, used water from a fish farm.

Each experimental group was composed (in the same ratio 60 and 40% respectively) of fish aged 2+ and older, which had spawned at least once (Table 1). Because of the very high incidence of poaching (SZMYT and GRUDNIEWSKA 2005a), 66 grayling individuals aged 1+ were introduced to the stock of fish living in the sedimentation pool. Due to lack of specific data about size of poaching and the inability to compare of spawners losses in particular experimental groups were not taken into account. In this group, all mature fish regardless age were used for artificial reproduction although quality of eggs obtained from two years old females is much worse than from older fish (GRUDNIEWSKA et al. 2001, SZMYT and GRUDNIEWSKA 2005b). The idea was that environmental and food conditions will compensate it.

Table 1.

Age and initial number of fish in each variant of the experiment

Age group	Feeding variants		
	Extruded trout feed	Extruded trout feed + maggots	Natural feed
1 +	–	–	66
Older*	80	100	106
Total	80	100	172

* include 2 and 3 years old fish

Fish from first group were fed with extruded trout feed Dan-Ex 2446 (Dana Feed A/S) and Aller Safir (Aller Aqua A/S), in a 1:1 ratio. Size of dose was not constant and was not correlated with fish biomass. The exact quantities of feeds given daily were adjusted to the foraging effort of spawners. Fish from the second group additionally were fed with maggots. It was assumed by Author that the amount of maggots added to the feed was constant during experiment and was 200g daily. In September and October spawners with this group were fed the maggots only. The content of the basic chemical components (total protein, crude fat, crude ash) in maggots was determined with the standard methods (AOAC 1996), (Table 2).

Table 2

Chemical composition of diet used in European grayling spawners feeding

Component (%)	Feed		
	Dan-Ex 2446	Aller Safir	Maggots
Crude protein	46.0	45.0	14.68
Crude fat	24.0	20.0	7.25
N-free extractives	14.3	16.0	2.55
Crude fibre	1.2	2.0	–
Crude ash	7.5	8.0	1.32
Gross energy (kcal)	5531.0	5171.0	1677.0
Digestibility energy (kcal)	4554.0	4145.0	*
Vitamin and minerals	+	+	not determined

* digestible energy was no determined due to the absence of data concerning the physiology of maggots digestion

Fish from third group were not fed but foraged themselves on macroinvertebrates, living naturally in the water reservoir. This experimental variant consisted of an *in situ* identification of the qualitative and quantitative composition of the fish food. Samples of macrozoobenthos were taken once, in May 2004, into a tube sampler, at 13 stations set along the longitudinal axis of the sedimentation pool, on a line running from the inflow to the outflow. The collected material was separated on a sieve with the mesh size of 0.5 mm bar

length. The analysis of the chemical composition of maggots as well as the analysis of the quality and quantity of the food ingested by fish in the sedimentation pool served as the basis for our determination of the effect of the type and composition of food on the quality of the European grayling's eggs.

Methodology of artificial reproduction and especially fertilization was conventional for rainbow trout and other salmonids (SEDGWICK 1973). Details of this method in the case of grayling, under trout farm conditions, was described by GRUDNIEWSKA et. al. (2009) and GRUDNIEWSKA et. al. (2013). For reproduction were used all mature fish in each group, regardless age of spawners. For eggs fertilization in particular feeding groups were used exclusively males from the same groups.

Prophylactic treatments

During the whole fish rearing season, spawners kept indoors were washed in a disinfecting bath twice weekly, alternately in copper sulphate solution (0.8 g per 1 m^3 of water) and in chloramine T solution (9.0 g per 1 m^3 of water). Any handling of spawners was carried out having first anaesthetized the fish with solution of Propiscin (IFI, Olsztyn, Poland), diluted to a concentration of 0.5 ml per 1 dm^3 of water (KAZUŃ and SIWICKI 2001).

Eggs incubation

Eggs from the different variants were incubated in cupboard incubators with round trays, separately in three replicates. The incubation was accomplished in deep water at the constant temperature of 8°C. In order to prevent formation of mould on eggs, prophylactic baths in a solution of formalin (1:500 concentration, for 30 min.) were given at two-day intervals (WITKOWSKI et al. 1984). At the eyed-eggs stage, dead embryos were separated from live ones for each feeding variant, their volume was measured (ml) and a sample (30 ml) was taken, in which all grains were counted. Dead eggs were separated manually, by rubber hose or bulb with glass tube. Based on these results, the total amount of live and dead spawn was calculated, from which the survival rate was derived. The eggs quality was evaluated at the eyed-egg stage.

Statistical analysis

In order to determine the effect of food ingested by spawners on the eggs quality, the results of the experiment were submitted to one-way analysis of variance (ANOVA) followed by the Tukey (HSD) *post hoc* test at a significance level of $P < 0.05$. The data were log-transformed to avoid violations of normality and homoscedesticity assumptions. The statistical analysis of the results was performed with the STATISTICA 10.0 software (Stat Soft®, Inc., USA).

Results

The fish maturity check done immediately before spawning showed varied numbers of mature fish in the particular variants. The group of fish fed only trout feed (variant I) comprised 27 ovulating females, which corresponded to 51.92% of all fish (males and females together) initially stocked. In the variant where maggots supplementation was applied, the number of females ready to spawn was 47 (67.14% of all the fish in that stock), and the fish inhabiting the sedimentation tank (variant III) presented 30 ovulating females, which equalled 30.61% of all the fish in that variant. The total duration of spawning period by the European grayling in the 2004/2005 season was 136.8°D (18 days).

The taxonomic composition of the natural food ingested by grayling living in the sedimentation tank included 7 classes of invertebrates (tab. 3). The mean density of benthic fauna in the tank was 14.459 individuals per m^2, and the mean biomass was 400.97 g per m^2.

The most numerous class were Malacostraca (76.6% of the total number). They were represented by two taxa: *Asellus aquaticus* (Isopoda) and *Gammarus sp*. (Amphipoda). *A. aquaticus* was the dominant species, making up 98.0% of the total number of this class and 75.0% of the total benthic fauna. Malacostraca were also a dominant contributor to the total biomass (38.8%, including 37.8% composed of *A. aquaticus*).

The influence of a diet on the reproduction success was found. The highest embryos survival rate, almost 85% (84.57%), was achieved by the eggs obtained from spawners feeding on natural food, and that result was statistically significantly higher than in the other variants (the Tukey *post hoc* test, $P < 0.05$). Table 4 shows the current results juxtaposed with the input data of 2004.

Table 3
Taxonomic composition, mean number (N indiv. m^{-2}) and biomass (B g m^{-2}) in the sedimentation pool.

Taxon	N	B
Turbellaria:		
Dugesia sp.	340	2.89
Clitellata:		
Oligochaeta	20	0.04
Hirudinea:		
Erpobdella octoculata (Linnaeus, 1758)	700	38.03
Malacostraca:		
Asellus aquaticus (Linnaeus, 1758)	10859	151.56
Gammarus sp.	220	4.15
Arachnida:		
Hydracarina	20	0.04
Insecta:		
Ephemeroptera:		
Caenisdae	20	0.10
Beatidae	580	2.35
Diptera:		
Simulium sp.	20	0.08
Chironomidae	80	0.22
Gastropoda:		
Ancylus fluviatilis Müller, 1774	20	0.05
Bithynia tentaculata (Linnaeus, 1758)	680	68.27
Physa fontinalis (Linnaeus, 1758)	380	23.67
Planorbis planorbis (Linnaeus,1758)	60	95.02
Valvata piscinalis (Müller, 1774)	400	5.44
Bivalvia:		
Sphaerium sp.	60	9.05
TOTAL	14459	400.97

Table 4
The survival of embryos (%) to the eyed eggs stage from spawners in different feeding groups. Statistical differences between groups are indicated by different letter superscripts (ANOVA, $P < 0.05$)

Year	Feeding variants		
	Extruded trout feed	Extruded trout feed + maggots	Natural feed
2004*	41.37^a	52.22^a	77.36^b
2005	48.44^a	56.00^a	84.57^b

* preliminary results of studies presented in Szmyt et al. 2004

Discussion

A broodstock of the European grayling is the most valuable element of fish stocking programmes, which contribute to 'active species protection'. Therefore, fish breeding should be conducted in conditions as close as possible to the

natural (SZMYT 2011). An adequately composed diet should enable spawners to achieve good growth and condition, but prevent excessive fat content (BIENIARZ and EPLER 1991). Otherwise, it may prove very difficult or even impossible to obtain high quality gametes, and this makes questionable any protection or restitution programmes based on the reproduction and rearing up of fish under controlled conditions (KUCHARCZYK 2002). Full quality feed for spawners are unlikely to be offered commercially for fish species with no economic importance, such as the European grayling. It is therefore necessary to enrich the feed with supplements which will minimize the deficiencies of some nutrients.

Presumably, one of the reasons for a higher survival rate of embryos obtained from spawners fed commercial extruded feed with maggots supplementation could be their chemical composition. Fish spawners demonstrate a higher demand for proteins than sexually immature individuals. Standard trout feeds contain about 45% of protein, while feeds dedicated for spawners are at least a few per cent richer in this nutrient. This explains such a distinct rearing effect obtained when standard feeds were supplemented with protein from maggots. Moreover, the composition of protein and other ingredients content like vitamins and minerals could be the other reason of better quality of eggs.

Supplementation of fish diets is a popular solution. Feeds used in the nutrition of juvenile fish are enriched, for example, with fortified rotifers or artemia nauplii (ROBIN 1998). Various substances are used for this aim, e.g. polyunsaturated fatty acids (HAN et al. 2000, HANAEE et al. 2005) or free amino acids (TONHEIM et al. 2000). A purified mixture of nucleotides can also be used as a fish dietary supplement (WALKER et al. 2011).

A diet-enriching supplement, especially when fed to non-commercial fish species, should have a beneficial influence on the production output, yet be economically viable. The dietary supplement given to the European grayling such as frozen maggots seems to fulfill the above criteria.

Regular supplementation of a diet fed to the huchen (*Hucho hucho* L.) with small quantities of fresh and frozen fish had a positive effect on rearing parameters (GORYCZKO 1993). This is very important finding because eggs from the huchen fed only commercial trout feeds had a very low biological value, and monotonous diet impairs the growth rate of fish (GORYCZKO et al. 2001). The beneficial influence of natural food on the reproduction success of the huchen broodstock has also been described by AUGUSTYN et al. (2003). Spawn obtained from spawners fed live fish caught from a river was very good in quality, and the best effects were achieved when live fish were released directly into a pond with spawners. Using reared rainbow trouts (*Oncorhynchus mykiss* Walbaum, 1792) as live food for huchen spawners failed to produce satisfying outcome and the resulting eggs were of inferior quality.

Positive effects of dietary supplementation were also demonstrated in the carp, for example CEKOW and TOMASIN (1982) added ground carrot to a diet given to carp spawners, achieving a 12% higher survival of larvae and its better food conversion rate. BLOM and DĄBROWSKI (1995) found out that rainbow trout spawners could have a higher demand for ascorbic acid than sexually immature individuals. Thus, for attaining the best possible reproduction success in this species, it is recommended to supplement their diet with ascorbic acid to a level eight-fold higher than the previously suggested dose of 50 mg per kilogram of feed.

Optimization of the reared spawners nourishment does not always correspond into a complete reproduction success, which the current results support. The best eggs quality were obtained from fish kept in a sedimentation tank, which foraged on natural food only. The results on the survival rate of embryos to eyed-egg stage correspond to the data of WITKOWSKI et al. (1984) and RYŠAVÝ (2000) on the embryos survival from grayling specimens obtained from the natural environment. The authors would argue that the loss should not exceed 15–25% if the eggs incubation was carried out properly. This survival rate was most probably a consequence of the availability as well as the right composition and food quality ingested by spawners from the sedimentation tank. The benthic fauna in the tank was dominated by crustaceans of the orders Isopoda and Amphipoda, which in nature can make up as much as 90% of the intestinal contents of fish (STAŃCZYKOWSKA 1986). Amphipoda are very important in the fish diet, especially of salmonids. Because of the high nutritive value of amphipods, crustaceans of foreign origin have been introduced to these biocenoses where the native freshwater shrimps have become extinct (SCHMITZ 1960).

Another dietary component which can potentially affect the eggs quality consists of carotenoid compounds. GABRIELSEN and AUSTRENG (1998) demonstrated that the biological activity of carotenoids from natural sources is much higher than synthetic ones. In nature, fish usually obtain these compounds from crustaceans (SYNOWIECKI and SHAHIDI 1997). Contents of carotenoids from natural sources such as crustaceans is high and can range from 30 mg per kilogram in crayfish meal to 1,300 mg per kilogram in crab meal (GUPTA et al. 2007). Carotenoids play a role in the eggs pigmentation, as shown in several studies, e.g. WOŹNIAK et al. (2003). In addition, Szmyt et al. (2004) observed a clearly visible differences in eggs coloration obtained from grayling spawners foraged on natural feed only (Figure 1). Furthermore CZECZUGA (1975) and CZECZUGA et al. (1985) found the highest concentration of carotenoids in European grayling eggs during a spawning season. PROTASOWICKA and DOMAGAŁA (1989) claim that the presence of this group of compounds in the sea trout (*Salmo trutta* m. *trutta* L.) eggs may play a role in reproduction success.

GEORGIEV (1971) detected an extremely high mortality of rainbow trout eggs with a low content of carotenoids during the period of their highest sensitivity. CRAIK (1985) as well as TVERANGER (1986) imply a positive influence of carotenoids during the embryonic and post-embryonic development of fish.

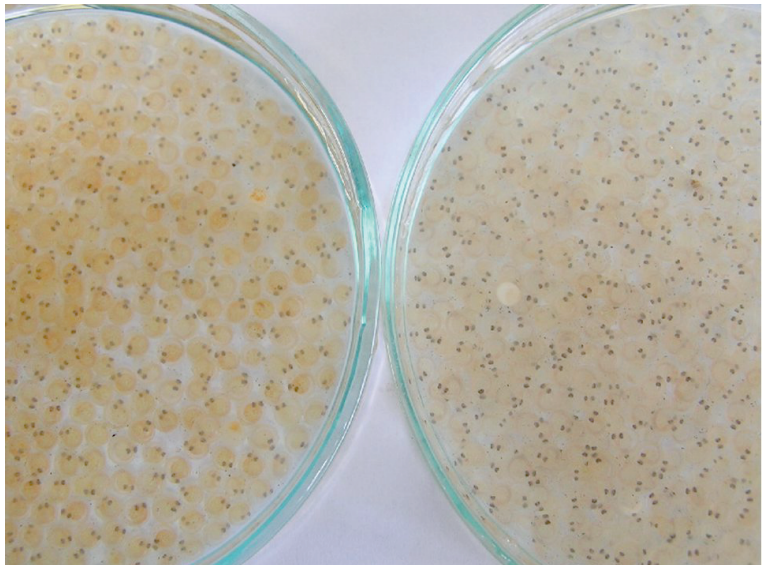

Fig. 1. Grayling embryos at the eyed-egg stage, obtained from spawners foraged on natural feed (left) and fed with extruded trout feed only (right), (SZMYT et al. 2004)

Optimization of fish breeding methods, especially those used to rear fish with no commercial importance, will often call for non-standard solutions and needs additional studies. Supplementation of a diet of the European grayling farmed spawners with maggots is a good example of such an innovative approach.

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EFFECTS OF STOCKING DENSITY AND WEANING AGE ON CANNIBALISM, SURVIVAL AND GROWTH IN EUROPEAN PERCH *PERCA FLUVIATILIS* LARVAE

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K e y w o r d s: cannibalism, European perch, growth, larvae, stocking density, survival, weaning age.

A b s t r a c t

The present study was designed to investigate the effects of stocking density and weaning age on growth, survival and cannibalism in Eurasian perch (*Perca fluviatilis*) larvae under controlled conditions. Two weeks experiment was conducted with 7 day post-hatch perch larvae (mean weight – 2.9 mg). Effects of two different stocking density (low 'L'- 13 and high 'H'- 26 larvae dcm^{-3}) and three weaning age treatments (7, 10 and 14 days post-hatching) on cannibalism, survival and growth in perch larvae under controlled conditions were evaluated. Type I (incomplete) cannibalism was significantly affected by weaning age but not by initial stocking density. The significantly lowest losses due to type I cannibalism were observed in both densities weaned at 14 dph and were 8.0 ± 0.5% and 8.6 ± 1.2% for low and high density, respectively. In other groups losses due to type I cannibalism exceeded 12% and did not differ significantly between stocking densities and other weaning age treatments. None of the factors had a significant impact on type II (complete) cannibalism. Survival and mortality other than cannibalism were significantly affected by both: stocking density and weaning age. The later weaning was found to impact positively on the survival of perch larvae, mainly through a reduction of cannibalism type I and mortality other than cannibalism. The significantly highest mean survival rate was obtained in both low (61.5 ± 2.1%) and high (57.0 ± 3.9%) density groups weaned at 14 dph. In other groups survival not exceeded 51% and did not differ significantly between stocking densities and other weaning age treatments. No significant effects of stocking density or weaning age on growth or size heterogeneity were found. The final body weight (FBW), growth rate (SGR) and coefficient of variation of FBW (CV_{FBW}) ranged from 0.025 ± 0.016 g to 0.032 ± 0.020 g, from 15.82 ± 0.42% day^{-1} to 17.16 ± 0.76% day^{-1}, and from 39.02 ± 3.32% to 48.58 ± 9.04%, respectively.

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TEMPO WZROSTU, PRZEŻYWALNOŚĆ I KANIBALIZM LARW OKONIA EUROPEJSKIEGO *PERCA FLUVIATILIS* (L.) W ZALEŻNOŚCI OD POCZĄTKOWEGO ZAGĘSZCZENIA OBSAD ORAZ CZASU PRZEJŚCIA NA ŻYWIENIE WYŁĄCZNIE PASZĄ KOMERCYJNĄ

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S ł o w a k l u c z o w e: kanibalizm, okoń europejski, larwy, tempo wzrostu, przeżywalność, wielkość obsady, przejście na żywienie paszą.

A b s t r a k t

Niniejsze badanie miało na celu zbadanie wpływu początkowego zagęszczenia i czasu przejścia na żywienie wyłącznie paszą komercyjną na przeżywalność, kanibalizm i tempo wzrostu larw okonia europejskiego (*Perca fluviatilis*) w warunkach kontrolowanych. Dwutygodniowy eksperyment przeprowadzono na 7 dniowych larwach okonia (średnia waga – 2,9 mg). Zastosowano sześć wariantów eksperymentalnych w zależności od początkowego zagęszczeniach (małego „L" – 13 i dużego „H"- 26 larw dcm^{-3}) i momentu przejścia na żywienie wyłącznie paszą (7, 10 i 14 dni po wykluciu). Na wzrost zachowań kanibalistycznych obserwowanych u larw okonia z dwóch badanych czynników istotnie wpływał tylko moment wyeliminowania z karmienia pokarmu żywego. Najniższe straty spowodowane tym typem kanibalizmu obserwowano w obu grupach ryb niezależnie od początkowego zagęszczenia obsady (odpowiednio 8.0 ± 0.5 i 8.6 ± 1.2% dla obsady L i H), których żywienie wyłącznie paszą komercyjną rozpoczęto najpóźniej tj. w 14 dniu po wykluciu. W pozostałych grupach straty spowodowane kanibalizmem typu I przekraczały 12% i nie różniły się w zależności od wielkości obsad oraz od zastosowanych wariatów żywieniowych. Zarówno czas przejścia na karmienie wyłącznie paszą, jak i zastosowane wielkości obsad nie wpłynęły znacząco na intensywność kanibalizmu typu II, miały natomiast istotny wpływ na końcową przeżywalność okoni oraz na obserwowaną śmiertelność nie spowodowaną kanibalizmem. Najwyższą przeżywalność obserwowano w grupach ryb, których żywienie wyłącznie paszą komercyjną rozpoczęto najpóźniej tj. w 14 dniu po wykluciu, niezależnie od początkowej wielkości obsady, która wyniosła odpowiednio 61.5 ± 2.1 i 57.0 ± 3.9% dla grup L i H. Zarówno początkowe zagęszczenie larw, jak i moment przejścia na żywienie okoni z wykorzystaniem wyłącznie paszy komercyjnej nie wpłynęły istotnie na końcowe parametry zootechniczne ryb. Średnia końcowa masa ryb (FBW), ich względne tempo wzrostu (SGR) oraz współczynnik zróżnicowania masy (CV_{FBW}) mieściły się odpowiednio w zakresach od 0.025 ± 0.016 g do 0.032 ± 0.020 g dla FBW, od 15.82 ± 0.42% $dzień^{-1}$ do 17.16 ± 0.76% $dzień^{-1}$ dla SGR oraz od 39.02 ± 3.32% do 48.58 ± 9.04% dla CV_{FBW}.

Introduction

European perch (*Perca fluviatilis*) is a strong candidate for percid fish species and product diversification in the European freshwater aquaculture due to its high flesh quality and high market value (KESTEMONT and MÉLARD 2000, LJUNGGREN et al. 2003, STEJSKAL et. al 2009). Aquaculture of perch has been developing rapidly in recent years with many new methods of reproduction (KUCHARCZYK et al. 1996; 1998, KOUŘIL et al. 1997, MIGUAD et al. 2004,

SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2011a,b). The larval and juvenile production of perch has been recognized as one of the main bottlenecks in the industrial production of this species, mainly because of high cannibalism and mortality at weaning time (BRABAND 1995, BARAS et al. 2003, KESTEMONT et al. 2003, BABIAK et al. 2004).

The cannibalism occurs in many cultured fish species, including non-predatory species (VAN DAMME et al. 1999), however it is most prevalent in piscivorous taxa, especially at larval and juvenile stages (SMITH and REAY 1991, HECHT and PIENAAR 1993, BARAS and JOBLING 2002). In European percid larviculture, intra-cohort cannibalism is an important issue that can induce losses exceeding 50% of the initial density during the first weeks of rearing (BARAS et al. 2003, KESTEMONT et al. 2003, BABIAK et al. 2004, KESTEMONT et al. 2007, KRÓL et al. 2015, KRÓL and ZAKĘŚ 2015). During the first days of life, when the size heterogeneity of larvae is low, type I cannibalism (incomplete – biting the abdomen or tail) occurs. Later, as size dispersal of larvae increases, type I cannibalism is replaced by type II cannibalism (complete – total prey ingestion) (CUFF 1980, HECHT and APPELBAUM 1988, HECHT and PIENAAR 1993, BARAS and JOBLING 2002, BARAS et al. 2003). Nowadays, cannibalism is regarded as an alternative feeding strategy, influenced rather by environmental than genetic factors (HECHT and PIENAAR 1993, SVENNING and BORGSTRØM 2005, BARAS and LUCAS 2010, BARAS et al. 2014, KRÓL et al. 2014). BARAS and JOBLING (2002) identified several population factors, as well as extrinsic factors that regulate size heterogeneity and cannibalism in fish. The previous results indicate that in European perch larvae, increasing stocking density did not impair growth, but significantly decreased of losses due to cannibalism (BARAS et al. 2003, KESTEMONT et al. 2003).

Food availability and quality are the most important factors which can account for the emergence and intensity of cannibalism within a larvae cohort (HECHT and APPELBAUM 1988, KUBITZA and LOVSHIN 1999, HECHT and PIENAAR 1993, BARAS and JOBLING 2002, BARAS 2013). Feeding levels have a significant effect on survival of perch post-larvae with increasing impact of cannibalism at lower doses of food (FIOGBÉ and KESTEMONT 2003). The larvae of European percid fish species are very small and must be fed with live food first (KESTEMONT et al. 2007), however, relevant study has been demonstrated that pikeperch larvae can be successful reared with feeding by formulated feed only (OSTASZEWSKA et al. 2005). Furthermore it is rather important to wean larvae at the earliest possible stage of rearing in order to decrease the cost of production (BASKERVILLE-BRIDGES and KLING 2000, PALIŃSKA-ŻARSKA et al. 2014). Weaning is a critical step for all cultured fish as the transition from live food to commercial feed often results in high mortality (FLETCHER et al. 2007, POLICAR et al. 2007). Success of weaning is dependent on several factors such

as feed quality and feeding procedure (WOCHER et al. 2013) but also on correlation of time in substitution of live food by formulated diet with the development of the digestive system of the larvae (KESTEMONT et al. 1996, 2007). European percid fish larvae ingest dry food after yolk sac absorption so fish can be trained to accept and to use a dry diet efficiently starting at the end of maturation processes of the digestive system, both in terms of morphological structures and enzymatic activities (HAMZA et al. 2007).

Therefore, the present study was designed to investigate the effects of stocking density and weaning age on growth, survival and cannibalism in perch larvae under controlled conditions.

Material and Methods

Experimental larvae origin

Spawners were captured in Sasek Wielki Lake (North-Eastern Poland) in early April. Immediately after catching, the fish were transported to the recirculating aquaculture systems (RAS) in Aquaculture and Ecological Engineering Center, University of Warmia and Mazury in Olsztyn, Poland. The gametes of perch were obtained in accordance with the previously established procedure (ŻARSKI et al. 2011b). Egg-ribbons from two females were fertilized with pooled semen from four males. Eggs were incubated in a flow-through water recirculating system at 14°C. Just before hatching, ribbons were placed in 500 dcm^{-3} tank at 17°C. Larvae started to hatch on day 8 post fertilization. After 24 hours from the first hatching, ribbons with unhatched embryos were removed from the tank. Perch larvae were reared in the same tank for the next 6 days. Temperature was progressively increased to 21°C and dissolved oxygen was maintained to saturation at 9.5 mg dcm^{-3}. Before the experiment, larvae were co-fed with *Artemia* nauplii and dry diet in *ad libitum* from 3 days post-hatching (dph).

Experimental design, data collection and analysis

Two weeks experiment was conducted with 7 dph perch larvae (with swim bladder inflation – ISBI at 67%). Before the experiment, 30 perch larvae were sacrificed by using an overdose of anesthetic (2-phenoxyethanol – 2 $ml \cdot dcm^{-3}$) then individually weighed (nearest to 0.0001 g) and measured (nearest to 0.01 cm). ISBI (%) was estimated on observation of larvae using stereo microscope in terms of inflation or not their swim bladders. Six experimental groups with

two different stocking density (low 'L' – 13 and high 'H' – 26 larvae dcm^{-3}) and three weaning age treatments (7, 10 and 14 dph) were set in triplicates. Newly-hatched *Artemia* nauplii (AF ± 430 micron size, INVE, Belgium) were used as live preys. For co-feeding procedure, daily feeding level for live prey were 1.5 g and 3.0 g per aquarium for L and H stocking density, respectively. For co-feeding and weaning procedure, commercial diet (Aller ArtEX 2, Aller Aqua, Poland) containing 50% protein, 14% lipid and 22,5% carbohydrates was used. A daily feeding level for Aller ArtEX 2 increasing from 100% of fish initial biomass on day 1 to 200% of fish initial biomass on day 14. During the all experiment, larvae were fed by hand six times a day (8:00, 10:00, 12:00, 14:00, 17:00 and 20:00), in co-feeding time *Artemia* were added first, before commercial feed. The weaning ages were applied on days 7, 10 and 14 post-hatch in both stocking densities. The perch larvae were reared during 14 days in a recirculating aquaculture system consisting of 18 rectangle glass aquaria with a volume of 15 dcm^{-3} each. The recirculating system was equipped with UV lamp, a lamellar filter for mechanical water purification and a trickling filter for biological water purification. The daily water exchange amounted to 10% of the total unit volume (1.5 m^3). The water flow through the rearing aquaria was 1.5 dcm^{-3} min^{-1}. The fish were exposed to a 24 h light and 0 h dark photoperiod, and the light intensity measured just above the water surface in the rearing aquaria ranged from 60 to 80 lx. The water temperature and dissolved oxygen were measured daily and totaled, on average, 21.5 ± 0.5°C and 8.1 ± 0.7 mg l^{-1}, respectively. Nitrogenous compounds were measured semiweekly in rearing volume of water and did not exceed 0.15 and 0.07 mg dcm^{-3} for $N\text{-}NH_4^+$ and $N\text{-}NO_2^-$, respectively. The rearing aquaria were cleaned of unconsumed food and fish waste once daily in the morning before feeding. Dead fish were removed daily and ranked as truncated (posterior damaged or tailed-off) or non-truncated (starving, intact, or with traces of bites on the abdomen or head) using Stereo Microscope LEICA MZ16A with Qwin Pro software. Truncated individuals were considered as victims of type I cannibalism and non-truncated fish as deaths by other causes. A bitten abdomen or head are typical secondary postmortem damages in cultured percids, therefore individuals having such damages were not classified as type I cannibalism victims (BARAS et al. 2003, BABIAK et al. 2004, KRÓL and ZAKĘŚ 2015).

At the end of the experiment all survivors per tank were counted and 30 fish per aquarium were weighed and measured. Other relevant parameters were assessed as a follows:

– Missing fish were considered as victims of type II cannibalism [(%) = 100 INF- (FNF + DF) INF^{-1}, where INF is initial number of fish, FNF is final number of fish, DF is number of truncated and non-truncated death fish].

– Coefficients of variation in body weight [CV(%) = $100 \cdot SDBW \cdot BW^{-1}$, where BW is average body weight and SDBW is standard deviation of body weight], were calculated for the first and last day of measurement (respectively, CV_{IBW} and CV_{FBW}).

– Overall specific growth rates [SGR (% day^{-1}) = $100 \cdot (Ln\ FBW - Ln\ IBW) \cdot t^{-1}$, where FBW is average final body weight and IBW is average initial body weight (g); t is14 days] were also calculated.

Statistical analysis

The data were analyzed using one-way ANOVA. The following validation of the normal distribution of the data, post hoc LSD Fisher test was used. Probability data in percentages were arcsine transformed before analysis. A two-way ANOVA was also used to test the effects of stocking density (13 and 26 larvae dcm^{-3}) and weaning age (7, 10 and 14 dph) and the interactions of both factors on survival, both types of cannibalism and other counted mortality. Differences were considered significant at $p<0.05$.

Results

Cannibalism and survival

The significantly highest mean survival rate was obtained in both low and high density groups weaned at 14 dph (Figure 1). Significant differences in survival between low and high stocking density groups were found at 7 dph weaning age, only (Figure 1). The significantly lowest losses due to type I cannibalism were observed in both densities weaned at 14 dph and were 8.0 ± 0.5 and 8.6 ± 1.2% for low and high density, respectively (Figure 2). In other groups losses due to type I cannibalism exceeded 12% and did not differ significantly between stocking densities and other weaning age treatments (Figure 2). Losses to type II cannibalism ranged from 12 to 21%, but did not differ significantly between treatments (Figure 3). The significantly lowest mortality other than cannibalism (15,5%) was found in group with low density weaned at 14 dph (Figure 4). The highest counted mortality values were observed in both densities weaned at 10 dph and were 24.5 ± 1.2% and 24.7 ± 0.3% for low and high density, respectively (Figure 4). Based on two-way ANOVA, survival was significantly affected by stocking density and weaning age at $p = 0.0191$ and $p = 0.0012$, respectively. Also mortality other than cannibalism was significantly affected by stocking density ($p = 0.0087$) and

weaning age (p = 0.0015). Cannibalism type I was significantly affected by weaning age (p = 0.0079) but not by initial stocking density (p>0.05). None of the factors had a significant impact on cannibalism type II (p>0.05). Significant interaction between the two parameters was found only in case of mortality other than cannibalism (p = 0.0169).

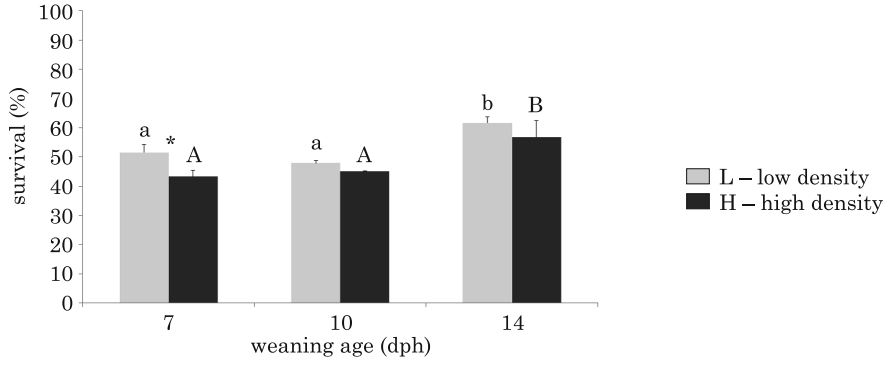

Fig. 1. Survival (mean ± SD) of perch larvae (22 dph) in relation to weaning age (7, 10 and 14 dph) and initial stocking density (L – 13 and H – 26 larvae dcm^{-3}). Lowercase letters indicate statistical differences among low density groups following different weaning age. Capital letters indicate differences among high density groups following different weaning age. Asterisks indicate significant differences between low and high density at the same weaning age (p<0.05)

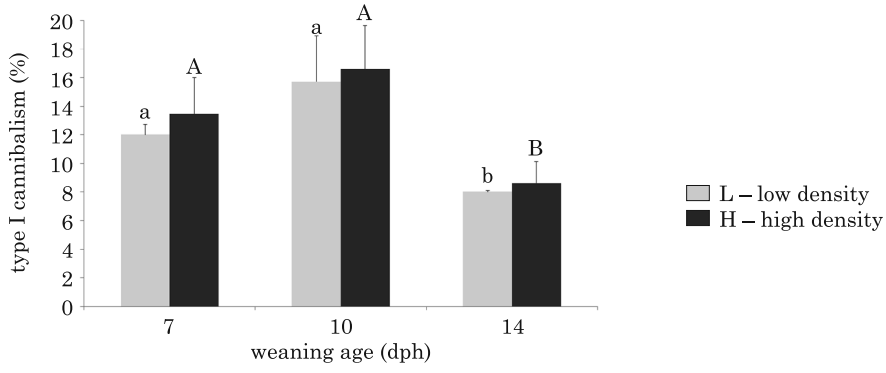

Fig. 2. Incomplete (type I) cannibalism (mean ± SD) of perch larvae in relation to weaning age (7, 10 and 14 dph) and initial stocking density (L – 13 and H – 26 larvae dcm^{-3}). Lowercase letters indicate statistical differences among low density groups following different weaning age. Capital letters indicate differences among high density groups following different weaning age (p<0.05). No significant differences between low and high density at the same weaning age were found (p>0.05)

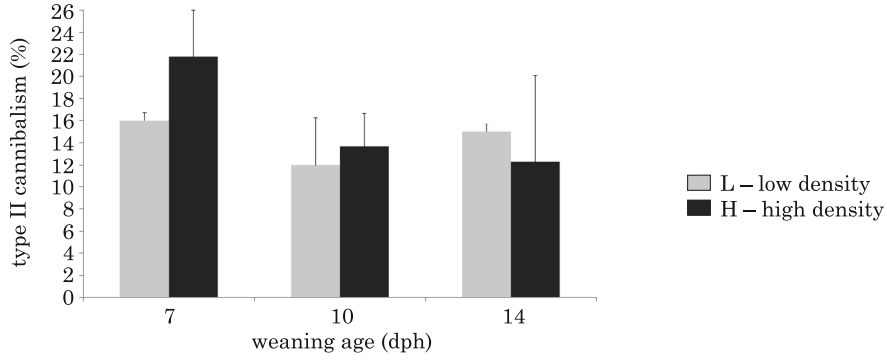

Fig. 3. Complete (type II) cannibalism (mean ± SD) of perch larvae in relation to weaning age (7, 10 and 14 dph) and initial stocking density (L – 13 and H – 26 larvae dcm^{-3}). No significant differences among treatments were found ($p>0.05$)

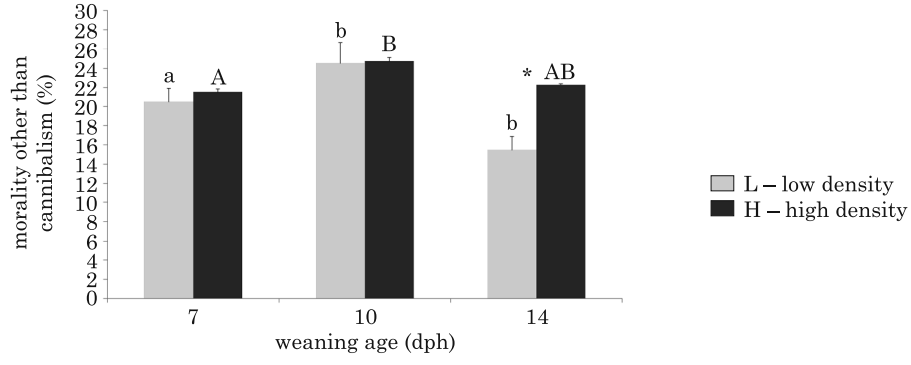

Fig. 4. Mortality other than cannibalism (mean ± SD) of perch larvae in relation to weaning age (7, 10 and 14 dph) and initial stocking density (L – 13 and H – 26 larvae dcm^{-3}). Lowercase letters indicate statistical differences among low density groups following different weaning age. Capital letters indicate differences among high density groups following different weaning age. Asterisks indicate statistically significant differences between low and high density at the same weaning age ($p<0.05$)

Growth performance

Mean of FBW and SGR did not differ among all experimental groups (Table 1). CV in weights increased by almost 2 times from the beginning (CV_{IBW}) to the end of the experiment (CV_{FBW}), but did not differ among all groups (Table 1).

Table 1. Mean values (±SD) of initial (n = 30 fish) and final (n = 3 replicates) parameters of perch larvae reared with two different stoking density (L – 13 and H – 26 larvae dcm^{-3}) and three weaning age treatments (7, 10 and 14 dph) during 14 days. No significant differences were found among treatments (One-way ANOVA, LSD post-hoc test, p>0.05)

Parameter	Group					
	L7	L10	L14	H7	H10	H14
Initial Body Weight (g) – IBW	0.0029 ± 0.0007					
Coefficient of variation of IBW (%) – CV_{IBW}	23.91					
Initial Swim Bladder Inflation (%) – ISBI	67					
Final Body Weight (g) – FBW	0.028 ± 0.012	0.028 ± 0.014	0.032 ± 0.020	0.025 ± 0.018	0.025 ± 0.016	0.031 ± 0.013
Coefficient of variation of FBW (%) – CV_{FBW}	41.40 ± 2.91	42.66 ± 2.03	48.58 ± 9.04	45.20 ± 0.50	41.66 ± 6.58	39.02 ± 3.32
Specific Growth Rate (% day^{-1}) – SGR	16.19 ± 0.33	16.17 ± 0.65	17.16 ± 0.76	15.82 ± 0.42	15.85 ± 0.30	16.80 ± 0.75

Discussion

In case of the European perch, rearing larvae at higher densities usually resulted in the better survival rate (BARAS et al. 2003, KESTEMONT et al. 2003), contrary to the pikeperch where growth and survival in the final phase of rearing decreased with increasing initial larval stocking density (SZKUDLAREK and ZAKĘŚ 2007). The higher rates of cannibalism at lower stocking densities can be accounted for the development of dominance hierarchies in perch larvae, resulted from available territories within a limited space, because in this species cannibalism is facilitated by territorial behavior (BRABAND 1995, MÉLARD et al. 1996). In contrast to the results obtained by the aforementioned studies, higher density did not result in reduction of cannibalistic behavior here, however both densities used in this study were lower than mentioned before, rather on experimental not industrial scale.

Beside several population factors as well as extrinsic factors that regulate cannibalism in fish larvae the quantity of feed, method of its providing and reducing frequency of feeding may enhance the intensity of cannibalism (HECHT and APPELBAUM 1988, HECHT and PIENAAR 1993, BARAS and JOBLING 2002, SZCZEPKOWSKI 2009, TRABELSI et al. 2011). Weaning is a critical rearing period for all fish species, and on several occasions, the supplementation of diet with live prey was found to reduce agonistic and cannibalistic behaviours (HECHT and PIENAAR, 1993). In both cultured, European percid species, several authors suggested that further studies are required for optimization of the weaning protocol at the earliest possible stage of larval rearing (KESTEMONT

et al. 2003, 2007, SZCZEPKOWSKI et al. 2011). Moreover, commercial feed has a higher energy content than live prey, and the determination of age at which perch larvae can be weaned is a pivotal, for reducing feeding costs. The results of our experiment for the first time indicate that procedure of weaning, preceded by co-feeding may significantly reduce cannibalism type I in perch larviculture. The later weaning was found to impact positively on the survival of perch larvae, mainly through a reduction of cannibalism type I and mortality other than cannibalism. That is probably due to the fact that perch larvae in late weaned groups had more time to the sudden change to an artificial diet. Our results are consistent with previous observations in other fish species, that co-feeding strategies can improve survival of fish larvae especially in early stages of their life (BASKERVILLE-BRIDGES and KLING 2000, LIU et al. 2012, LJUBOBRATOVIĆ et al 2015).

The our study confirmed that cannibalism is an important factor in European perch larviculture and a similar impact of both types of cannibalism may stem from a short duration of the experiment, usually type II cannibalism is more intense than the type I in long-lasting rearing (MANDIKI et al. 2007, KRÓL et al. 2015). The low difference between contributions of type I and type II cannibalism here can also resulted due to the fact that our study started with larvae with incomplete inflated swim bladder (67%) and thereby accelerated emergence of complete cannibalism. This occurs because European percid larvae with non-inflated swim bladders are easy victims for cannibals due to the impairment of motor skills (SZCZEPKOWSKI et al. 2011, KRÓL and ZAKĘŚ 2015). However, swim bladder inflation in pikeperch can occur on day 5 after hatching and can last until days 11 or 12 (DEMSKA-ZAKĘŚ et al. 2003), so it is conceivable that also some percent of perch larvae can inflated swim bladder in the first week of our experiment.

The effect of rearing strategy on larval growth is also an important parameter although it may not be as critical as survival, since larval size dispersal may be compensated at further rearing of fish, e.g.: high size variation of larvae can facilitate the emergence of cannibalism (BARAS and JOBLING 2002, KRÓL et al. 2015). Moreover, minimize growth heterogeneity in the cohort is a good aquaculture practice in order to minimize food wastage and water quality degradation (KESTEMONT et al. 2003). High initial stocking density of perch larvae does not adversely affect larval growth (MÉLARD et al. 1996 BARAS et al. 2003, KESTEMONT et al. 2003). Also no growth positive effects were observed in Eurasian perch larvae when *Artemia nauplii* were distributed over longer periods (KESTEMONT et al. 2003). In the present study, growth parameters of perch larvae were not significantly affected by either weaning age or initial stocking density. However, co-feeding procedure improved the nutritional status of larvae to avoid high size variation of larvae in other taxa (CURNOW et al. 2006, ALVES et al. 2006, ENGROLA et al. 2007).

In conclusion, the results of our experiment indicate that procedure of weaning, preceded by co-feeding may significantly enhance survival in perch larviculture *via* reducing the intensity of cannibalism type I within first weeks of rearing.

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COMMON SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) AS AN ALTERNATIVE ORCHARD PLANT

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K e y w o r d s: sea-buckthorn, varieties, crop productivity, use value.

A b s t r a c t

Due to the wealth of nutrients contained in the sea buckthorn (*Hippophae rhamnoides* L.) fruit, it is a raw material attracting increasing interest from the food and pharmaceutical industries. Sea buckthorn is also a plant with low soil requirements while being drought-resistant. Therefore, it may offer an alternative to farmers who have either soils poor in minerals or degraded land in need of soil values being restored to it. Sea-buckthorn, due to its ability to fix nitrogen, excellently fertilises poor and degraded soils.

**ROKITNIK ZWYCZAJNY (*HIPPOPHAE RHAMNOIDES* L.)
JAKO ALTERNATYWNA ROŚLINA SADOWNICZA**

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S ł o w a k l u c z o w e: rokitnik, odmiany, plonowanie, wartość użytkowa.

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Abstrakt

Bogactwo składników odżywczych i zdrowych owoców rokitnika (*Hippophae rhamnoides* L.) sprawia, że jest owocem cieszącym się coraz większym zainteresowaniem w przemyśle spożywczym oraz farmaceutycznym. Rokitnik to również roślina o niewielkich wymaganiach glebowych a jednocześnie odporna na suszę. Tym samym może stanowić alternatywę dla rolników posiadających gleby ubogie w składniki mineralne bądź też grunty zdegradowane, które wymagają przywrócenia im wartości glebowych. Rokitnik dzięki swym zdolnościom do wiązania azotu doskonale użyźnia ubogie i zdegradowane gleby.

Common sea buckthorn (*Hippophae rhamnoides* L.), also referred to as the 'Siberian pineapple', is a valuable medicinal plant. First notes concerning the therapeutic properties of the sea buckthorn were written in 7th century C.E. in Tibetan medical books. The origin of the plant name comes from ancient times when Greeks fed horses with sea buckthorn in order for their hair to be shiny, and thus the common name has been established (*hipp* – horse, *phaos* – shiny). In ancient times, all structural parts of the shrub were used for therapeutic purposes: fruits, leaves, bark, and even the roots.

Sea buckthorn fruit is among the most nutritious and rich in vitamins, polyphenols, minerals, and organic acids. The plant, similar to olives or avocado, contains fats in the pulp of the fruit. This fat is distinct from other fats because it is considered to have significant therapeutic properties. The oil is used for the treatment of frostbite, poorly healing wounds, or eczemas. Sea buckthorn oil was used for the treatment of radiation burns caused by the Chernobyl explosion.

The therapeutic properties of sea buckthorn fruit have contributed to an increasing interest in the production and processing of these fruits over the recent years.

Sea-buckthorn, in its natural state, occurs as a wild plant in Central Asia, China, Tibet, Mongolia, the Caucasus, Turkey and Russia, and grows in the areas of Siberia, Central Europe, and the Baltic Sea and North Sea coasts. Its range of occurrence reaches as far as Great Britain (Figure 1) (LI and BEVERIDGE 2003, SZAŁKIEWICZ and ZADERNOWSKI 2006, BATOOL 2011). Due to the variety of climatic conditions, various species and varieties of this plant grow in particular areas of Eurasia (Figure 1). So far, approx. 150 species of sea buckthorn have been identified within Eurasia (Table 1), differing not only in the habitat of the shrub but also in the appearance and use-value of the fruit.

Table 1

Species of sea buckthorn – Part 1

Lp.	Cultivar	Yield kg·skrub^{-1}	Lp.	Cultivar	Yield kg·skrub^{-1}
1	Awgustinka	12,5–20,5	45	Diujmowoczkaja	7,9
2	Alej	–	46	Ełizaweta	7,1
3	Altajechka	7,6	47	Żołtaja rannaja	30
4	Altajskaja	8	48	Żiwko	13–20
5	Ananasnaja	6,7	49	Zariewo	11,6–14,5
6	Atsula	14	50	Zarnica	7–8,5
7	Ayula	15,5	51	Zarja Dabat	6,7–9,0
+–					
8	Ayaganga	7,5–10,2	52	Zarja Meszczery	6,0–7,0
9	Bazeliskaja	7,0	53	Zołatoja kosa	5,8–12
10	Bajkal	7,8	54	Załatistaja Sibiri	6,8–14,0
11	Bajkaliskij rubin	8,5	55	Zołotoj kackad	10
12	Bajan Gol	8,5–12,2	56	Zołotoj kliuczik	4,8
13	Bijczanka	6,8	57	Zyrjanka	–
14	Bogatyrskaj	9,6	58	Iwuszka	8
15	Botaniczeskaja	20,5	59	Inja	8–12
16	Botaniczeskaja aromatnaja	9,8–15	60	Kapriz	–
17	Botaniczeskaja luczistaja	10	61	Karamelika	7
18	Botaniczeskaja liubitelickaja	7–15	62	Karotinnaja	6–8
19	Businka	9	63	Katunskaja –24	6–10
20	Batutinskaja	10	64	Kenigsbergskaja	–
21	Welikan	9	65	Kostior	18
22	WIL-5(15–1–36)	3,0–7,0	66	Krasawica Niecziernoziemja	8,5
23	WIL-7	4,0	67	Krasnopłodnaja (17397-20)	20
24	WIL-8	2,8–4,1	68	Krasnyj karlik	5
25	WIL-9	5	69	Krasnyj fakieł	8–9
26	WIL P-2	10	70	Kudrina	14,3
27	WIL P-3	11	71	Lilła kaług inna	8–9
28	WIL P-4	11	72	Lisiczka	8–13
29	Witaminnaja(B29)	4,3–13	73	Lomonocowskaja	14
30	Wladimipka	3,8–6,5	74	Luczezapnaja	8–9
31	Wopobiockaja	11–18	75	Ljubimaja	8–13
32	Wostocznaja	–	76	Ljudmila	–
33	Galerit	10	77	Majutka	3
34	Gibrid Perczika	11–23	78	Marinka	4–5
35	Gomełiskaja	25	79	Marija	10–12
36	Guś-Hructalinaja	7,7	80	Masłicznaja	11
37	Dar-Kazakowu	17,5	81	Mendieleewskaja	15
38	Dar MGU	20	82	Meszczerskaja krupnopłodnaja	7
39	Desert maslicznyj	6,5	83	Minusa	12–20
40	Desertnaja	7,3	84	Moria czka	11
41	Donczanka	8	85	Mockwiczka	14–20
42	Drużina	9	86	Moskowskaja ananasnaja	14–17
43	Dubowczanka	12–15	87	Moskowskaja krasawica	30
44	Dyet	12–18	88	Moskowskaja prozracznaja	–

Source: (SKALIJ 2007)

Table 1

Species of sea buckthorn – Part 2

Lp.	Cultivar	Yield kg·skrub^{-1}	Lp.	Cultivar	Yield kg·skrub^{-1}
89	Niżiegorodskaja suwienir	14,2	125	Sajana	10
90	Nariadnaja	12–15	126	Sewernyj diesiert	5,3
91	Niwieliena	35	127	Sejanec Welikana	5,7
92	Niżiegorodskaja cladkaja	8–10	128	Sibirskaja	9–11
93	Nadieżda	5–8	129	Sibirskij rumianec	10
94	Nowinskaja	10	130	Slawnaja	8–11,8
95	Nowosti Ałtaja	10	131	Solnecznaja	7–8
96	Obilynaja	20	132	Solnyszko	9–11
97	Ognistaja	–	133	Startowaja	5,9
98	Omska ja–27	6,5	134	Stoliczna ja	14,5
99	Oranżewaja	22	135	Studenczeskaja	20
100	Otradnaja	94–16,6	136	Sjurnpiz Baltiki	9,7
111	Poliwitaminnaja	7,7	137	Talickaja	12
112	Populiarnaja	7–104	138	Timipiazewskaja krasawica	5–77
113	Priewoshodnaja	7,1	139	Trofimowskaja	–
114	Prieliesti	7,2	140	Ułała	10
115	Prima Dona	–	141	Uniwiersitetskaja	12
116	Primorczanka	–	142	Urożajnaja	–
117	Priokskaja	9–18	143	Fantastika	–
118	Rannaja stołowaja	–	144	Finskaja	19
119	Rossijanka	20	145	Czeczek	10–25
120	Ruet	8	146	Czujskaja	23
121	Ryżik	1–18	147	Czulyszmanka	10–17
122	Riabinka	15,7–25,2	148	Szczerbinka	10
123	Riabinowaja	5	149	Jantarnaja	9,7
124	Samorodok	5–12	150	Jantarnoje ożerelie	11,2

Source: (SKALIJ 2007)

Sea buckthorn can be grown almost everywhere: in parks, gardens, on slopes, hillsides, on riverbanks, and on littoral dunes. When growing under the diversified climatic conditions of Eurasia, it is resistant to extreme temperatures from -43°C to 40°C. In addition, sea buckthorn is a plant with minimum soil quality requirements and, at the same time, it is drought-resistant (LI and BEVERIDGE 2003, KAWECKI et al. 2007). Therefore, it may offer an alternative to farmers who have either soils poor in minerals or degraded land in need of soil texture being restored to it. Sea-buckthorn, due to its ability to fix nitrogen, excellently fertilises poor and degraded soils. In addition, the plant exhibits natural resistance to pests, therefore the use of pesticides harmful to the environment may be reduced to a great extent. This means that sea buckthorn shrubs are suitable as a crops because they are both environmentally friendly and suitable for land reclamation (GIEJBOWICZ and WOŁEK 2008).

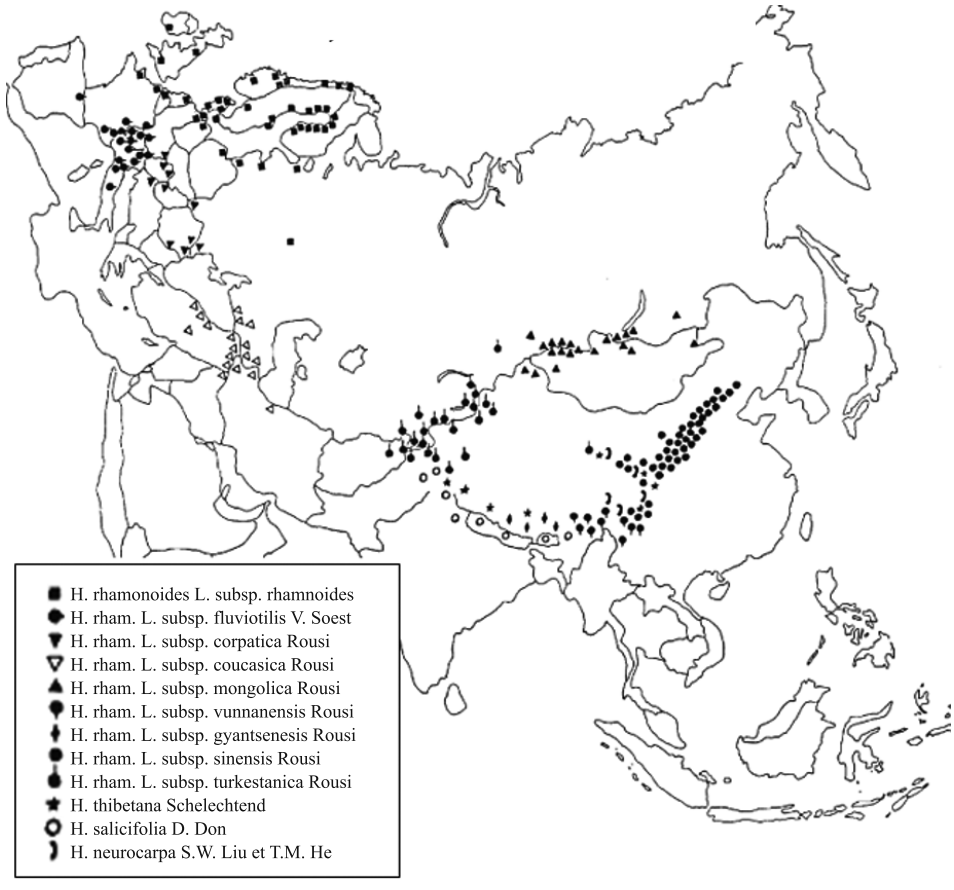

Fig.1. The presence of sea buckthorn (Hippophae rhamnoides) in Eurasia
Source: Li and Beveridge (2003)

As mentioned earlier, sea buckthorn is a wild plant. In Poland, it is only found in its natural state on the Baltic coast, where it grows on littoral dunes. In 1983, it was subjected to strict species protection, and currently, pursuant to Article 48 of the Act of 16 April 2004 on nature protection (Journal of Laws of 2013, item 627, as amended), it is under partial protection. Since the 1950s, two institutes have been involved in the improvement and adaptation of the plant to the orchard and commodity cultivation: the Russian Institute in Moscow, and the Belarusian Fruit-Farming Institute in Samokhvalovichi near Minsk. After years of research, several dozen improvedsea buckthorn varieties have been created, which primarily differ in crop productivity, harvest ease (due to increased pedicel length), and the quality of fruit. Improvements in fruits, include their size, colour, texture, structural and morphological charac-

teristics, and, primarily, the chemical composition have been considered (SHALKIEVICH et al. 2009).

This focus on cultivation research into the plant arises from the fact that the main asset of the sea buckthorn is its fruit with its high nutritional and health-promoting properties. Also, the oil derived from sea buckthorn fruit and seeds is an extremely valuable resource which is in demand from the pharmacological and cosmetics industries. The industrial use of sea buckthorn fruit was initiated in Russia in 1940 when scientists discovered and identified the wealth of biologically active substances found in the fruit, leaves, and bark. The first Russian plant manufacturing sea-buckthorn-based products is situated in Bisk (Altai Krai). These products have been used in both the diets of Russian astronauts and as a cream protecting against cosmic radiation (DELABAYS and SLACANIN 1995, XU et al. 2001). Chinese experiences in the industrial use of sea buckthorn fruit are more recent, even though they have been traditionally used for centuries (LU 1992). The research and establishment of commodity plantations in China were initiated in 1980. Since 1982, sea buckthorn has been planted on 300.000 ha. In addition, it has been found that approximately 150 plants process the fruits of the plant, and manufacture over 200 articles.

In Poland, there are very good climatic and soil conditions that provide Polish farmers the opportunity to grow sea buckthorn as an alternative crop (GIEJBOWICZ and WOŁEK 2008). At the same time, sea buckthorn crops may contribute to fulfilling numerous needs of specific sectors of the food, pharmaceutical, and cosmetics industries.

In Poland, research work was carried out in the years 2011–2014 as part of the programme NN312170939 financed by the Ministry of Science and Higher Education in Warsaw, and then by the National Science Centre (*NCN*) in Cracow. As part of the project, waste-free technology for processing the fruit of sea buckthorn (*Hippophae rhamnoides L.*) was developed, and the bioactive properties of the resulting products were assessed. The project was implemented under the supervision of Prof. Ryszard Zadernowski, at the University of Warmia and Mazury in Olsztyn, at the Department of Processing and Chemistry of Plant Materials. As mentioned earlier, sea-buckthorn is cultivated on a commercial scale primarily in Eurasia, e.g., in Russia, China, Finland, Germany, Lithuania, the Czech Republic, Slovakia, and Estonia. In Poland, small sea buckthorn plantings were established mainly in the Suwałki Region and near Łomża (Photo 1). These plantations have shown that such plantings may be also attempted in other parts of the country. In the 20th century, an attempt was made to cultivate this plant in North America, in the eastern, central and western provinces of Canada (LI and BEVERIDGE 2003). In Canada, sea buckthorn is cultivated in the following provinces: British Columbia,

Photo. 1. The experimental plantation of sea buckthorn (2ha) Amaranth Companies in Lomza and its owner John Hołownia
Source: B. PIŁAT

Alberta, Saskatchewan, Manitoba, Quebec, and Newfoundland (HARRISON and BEVERIDGE 2002). Currently, Canada is among the global leaders in the cultivation of sea-buckthorn. In the province of Quebec, a pilot area for the cultivation and processing of this plant has been designated in the town of Matane. In this region, approx 36 manufacturers and approx. 78.000 plants are found. The variety most often used for plantings, and recommended by the Ministry of Agriculture, Fisheries and Food of Quebec, is 'Indian summer' (MAPAQ, 2002). Under the climatic conditions of Canada, female plants start producing fruit in the 4th to 6th year after planting (LI and BEVERIDGE 2003), and in the first few years, they can yield a crop of fruit at a level of 0.75-1.5 t per ha (LU 1992). SCHROEDER and YAO (1995) recorded production of fruit at a level of 4–5 t per ha in Saskatchewan, while in British Columbia, cases of crop productivity at a level of 20–25 t·ha^{-1} were noted (LI and BEVERIDGE 2003). Research into the acclimatisation and use of sea buckthorn is carried out by the Pacific Agri-Food Research Centre in Summerland, British Columbia. The aim of the research that has been carried out for several years is to develop manufacturing technologies for commodity sea buckthorn varieties that would be suitable for cultivation in the climate prevailing in Canada, and to develop technologies of manufacture of valuable products (with an added value) in North America (LI and BEVERIDGE 2003). The research and

production issues being addressed by the Centre include, *inter alia*, the timeliness of ripening and the associated difficulties with harvest. Difficulties with harvest, as regards the traditional varieties, result from the short length of the berry pedicel, and the firm attachment of the fruits to branches. In addition, cultivation work is carried out with an aim to decrease the size and spread of trees, the development of a method and system for cutting shoots, and control of diseases, insects and pests, and the development of a technique of combine harvesting.

It is known that the basis for the development of food, pharmaceutical and cosmetic products is the profound knowledge of the chemical composition of raw materials. In the Polish market, there are products manufactured mostly based on imported sea buckthorn oil. Only in recent years has the manufacture of sea buckthorn oil from domestic fruit been initiated.

Sea buckthorn as an unconventional orchard plant

Common sea buckthorn *(Hippophae rhamnoides* L.) is a plant that grows and develops in a form of a shrub or a tree, whose height exceeds 3, and sometimes even 7 metres, while according to the literature, certain varieties may grow as tall as 20 metres. The plant has narrow and lanceolate leaves with a silvery bloom on the upper surface (ROUSSEAU 2002), and a green lower surface. Sea-buckthorn is a dioecious, anemophilous plant of the oleaster family (*Elaegnaceae*) (GIEJBOWICZ and WOŁEK 2008). Sea buckthorn flowers are produced in the early spring. They are inconspicuous, and yellowish in colour. Since the sea buckthorn is a dioecious plant, it has unisexual flowers, which means that female flowers grow on certain shrubs on which the fruits are set, while on different shrubs, male flowers grow (Figure 2) and their task is to produce pollen for the fertilisation of female flowers. The fruits are classified as non-climacteric. Depending on the variety, ripe fruits are oval-shaped and most often yellow, orange or red in colour. The weight of the fruit is usually within the range of 4–60 g 100^{-1}, and for certain Russian varieties, it exceeds 60 g (PIŁAT 2014). LI and BEVERIDGE (2003) report that the average weight of fruit of the Canadian variety *Indian summer* is between 20 and 40 g·100^{-1} fruit. Each sea buckthorn fruit contains a brown seed with a weight of approximately 16 mg (HARRISON and BEVERIDGE 2002).

On commodity plantations, sea buckthorn is cultivated in the form of shrubs or trees with a height of 2–4 m. Female plants are dominant, and male individuals, which are planted as pollinators, should account for approx. 7–8% of the total. It is most preferred to plant the males in the first row from the side of the most frequent winds, and then in every third row, every 6^{th} or 7^{th} seedling. In practice, the percentage of pollinating varieties may be reduced to

Fig. 2. Sea buckthorn; female flowers and masculine
Source: „Das Buch vom Wurzen", Leipzig, Verlag fur die Frau, 1989

4–5%, which, as a result, allows several extra tonnes to be harvested from each hectare (PLUTA 2014). The most preferred time for planting is either autumn (October) or early spring. The planting stock should consist of 1-year or 2-year-old shrubs which are planted at a spacing of 4 m x 2 m (1,250 plants/ha). When planting poorly-growing varieties, and applying the cultivation in the form of low-growing trees, there is the possibility for increasing the density of the plantings (4 m x 1.5 m). In Poland, on industrial plantations, the average yield is 7–9 tonnes per 1 ha. This is similar to the yields obtained in other European countries. These yields are obtained every second year due to the harvest method applied (cutting shoots with fruit). Therefore, the actual average annual yield per 1 ha is approx. 4 t/ha (PLUTA 2014). When managing the plantation, it is necessary to control weeds, mow the grass, apply mineral fertilisation, sanitary pruning of the plants as well as irrigation during drought, and to protect the plants against diseases and pests. Plants may be infected by fungal diseases, e.g., fusarium wilt (*Fusarium oxysporm*) and verticillium wilt *(Verticillium sp.)*, causing massive drying off of plants as well as trunk gangrene and black cancer. The pests posing the greatest risks include *Rhagoletis batata*, sea buckthorn moth; *Eriophyoidea*, the green sea buckthorn aphid; and sea buckthorn fly.

Characteristics of the sea buckthorn varieties

Sea-buckthorn, as a plant growing in many climatic zones, has undergone natural modification over the centuries, which has resulted in the creation of species differing in numerous characteristics, e.g., the habitat of trees or shrubs, the size and colour of fruits, the length of pedicel, the density of fruit on the shoots, the tendency to drop fruit, and resistance to pests and diseases.

The first sea buckthorn plantations were established in Russia, where 60 varieties were selected from among approx. 150 varieties growing in the wild (Table 1) (Lipowski et al. 2012). The area under commodity plantations in Poland is small. Most often, sea buckthorn shrubs are planted for aesthetic purposes, on allotments in the form of hedges, and in parks. For this purpose, a biotype commonly referred to as 'Nadbałtycki' (or ''Baltic'') is used. As a result of cultivation research carried out at the Research Institute of Horticulture in Skierniewice, the variety 'Jozef' was selected, which requires it to be promoted and introduced to cultivation. Currently, on commodity plantations of sea-buckthorn, German varieties and those registered in Belarus and Russia are cultivated. These varieties include: 'Podarok Sadu', 'Avgustinka', 'Botanicheskaya', 'Nivelena', 'Trofimovskay', and 'Plamiennaya'.

'Podarok Sadu' (Photo 2) is a mid-late variety with slightly ellipsoidal fruits that are orange to orange-red in colour. The fruits ripen at the turn of August and September. The content of vitamin C is 85–166 mg/100 g, sugars 2.6–4,97%, organic acids 1.75–3.6%, and fats 4.6–4,91%. The shrubs have umbrella-like crowns, and reach a height of up to 3 m. It is a frost-resistant variety, yielding a crop of 15–24 kg from a shrub (Szałkiewicz and Zadernowski 2006, Skalij 2007, Lipowski et al. 2012, Piłat 2014).

Photo. 2. Podarok sadu

Source: A. Bieniek

'Avgustinka' (Photo 3) is a variety with spherical, slightly ellipsoidal, orange fruits. The of vitamin C falls within the range of 95–174 mg·100^{-1} g, the content of sugars is 1.1–4.5%, organic acids 1.5–4.3%, and fats 2.7–4.8%). The crop obtained from one shrub is within a range of 12.5–20.5 kg (Skalij 2007, Lipowski et al. 2012).

Photo. 3. Avgustinka

Source: SKALIJ (2007)

'Botaniczeskaya' (Photo 4) is a variety characterised by small, spherical fruits that are dark-yellow in colour. The content of vitamin C is 120–140 $mg \cdot 100^{-1}$ g fruits, sugars 3.1–5,67%, acids 1,60–3.1%, and fats 3,67–5.6%. Up to 2.5 kg of fruit may be harvested from one shrub (SKALIJ 2007, LIPOWSKI et al. 2012, PIŁAT 2014).

Photo. 4. Botaniczeskaya

Source: A. BIENIEK

'Nivelena' (Photo 5) is a mid-early variety. The harvest date is 15–20 August. Fruits are yellow-and-orange in colour. The content of vitamin C is approx. 81 mg/100 g fruit, and the content of fats is 3%. This variety yields excellent crops. Up to 35 kg of fruits may be harvested from one shrub (SZAŁKIEWICZ and ZADERNOWSKI 2006, SKALIJ 2007).

Photo. 5. Nivelena
Source: SZAŁKIEWICZ and ZADERNOWSKI (2006)

'Trofimovskaya' is a mid-late variety. The fruit harvest date is at the turn of August and September. It is frost-resistant. Fruits are oval and oblong, orange in colour. The content of vitamin C is 91,78–183 mg·100^{-1} g, sugars 3.1–5,00%, fats 3,99–4.8%. A crop harvested from one shrub is, depending on the year, from 1.79 to 10.43 kg (SZAŁKIEWICZ and ZADERNOWSKI 2006, SKALIJ 2007, PIŁAT 2014).

'Plamiennaya' is a mid-early variety. Fruit is harvested on 15–20 August. Fruits are oval-shaped, red-and-orange in colour. A crop harvested from one shrub is, on average, 19 kg, up to a maximum of 29 kg from one plant (SZAŁKIEWICZ and ZADERNOWSKI 2006).

Under the climatic and soil conditions of the city of Olsztyn (north-eastern Poland), in experiments carried out at the Department of Horticulture at the University of Warmia and Mazury in Olsztyn, the Ukrainian varieties 'Podarok Sadu', 'Botanicheskaya', 'Trofimovskaya' and 'Otradnaya' were cultivated and assessed. Results for the crop productivity and the quality of fruit were compared to those for the 'Nadbałtycki' biotype, and presented in the following publi BIENIEK et al. 2001, BIENIEK et al. 2007, KAWECKI et al.

2001, KAWECKI et al. 2004ab, KAWECKI et al. 2010, SZAŁKIEWICZ et al. 2007, PIŁAT et al. 2012.

Due to the scientific recognition of the therapeutic properties of sea buckthorn fruit, in many European and Asian countries as well as in Canada, commodity sea buckthorn orchards have been established for several dozen years. Currently, sea buckthorn is cultivated on an industrial scale in Russia, Germany, China, Finland, and Estonia. It is estimated that the area of natural occurrence of sea buckthorn shrubs in Russia, China and Mongolia is approx. 810,000 ha. Commodity plantings in that region have an area of 300,000 to 500,000 ha, while the area under plantation in Finland is approx. 200 ha (SZAŁKIEWICZ and ZADERNOWSKI 2006, KAUPPINEN 2013). According to SZAŁKIEWICZ and ZADERNOWSKI (2006), small plantings have also been established in Poland, particularly in the Suwałki Region, and near Łomża.

Conclusion

The implementation of innovative solutions in agriculture through the introduction of new, novel crops may contribute to the stabilisation and increase in small farm income from agriculture. It should be remembered that sea buckthorn fruit, despite being rich in nutrients, are rarely consumed raw but are primarily a component of many processed multi-fruit products, or a raw material in the manufacture of medicinal products. Therefore, the establishment of the national plantation area must be closely correlated with the needs of the domestic market and export capacity for fruits or processed products. At the same time, sea buckthorn crops may contribute to fulfilling the demand for raw and primary processed products by specific sectors of the food, pharmaceutical, and cosmetics industries.

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EFFECT OF SELECTED STARTER CULTURES ON CONTENTS OF *cis*9*trans*11 C18:2 (CLA) AND *TRANS* C18:1 AND C18:2 ISOMERS IN FERMENTED MILK DRINKS

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K e y w o r d s: fermented dairy drinks, yoghurt, starter cultures, *cis*9*trans*11 C18:2 (CLA), *trans* isomers.

A b s t r a c t

This study was aimed at evaluating the effect of selected starter cultures on the content of *cis*9*trans*11 C18:2 acid and contents of *trans* isomers of C18:1 and C18:2 acids in fermented milk drinks. Analyses were carried out for normalized milk used to produce fermented milk drinks and for fermented drinks produced from this milk with three different yoghurt starter (Ceska-star Y508, YC-X11 and ATB-1).

The study demonstrated that the type of starter culture applied affected the content of *cis*9*trans*11 C18:2 acid (CLA) as well as contents of *trans* isomers of C18:1 and C18:2 acids in the fermented dairy drinks.

In all analyzed fermented dairy drinks produced with various starter cultures, analyses showed slightly lower contents of conjugated linoleic acid that in normalized milk used as a raw material for their production.

WPŁYW WYBRANYCH KULTUR STARTEROWYCH NA ZAWARTOŚĆ KWASU *cis*9*trans*11 C18:2 (CLA) ORAZ IZOMERÓW *TRANS* C18:1 I C18:2 W FERMENTOWANYCH PRODUKTACH MLECZARSKICH

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S ł o w a k l u c z o w e: fermentowane produkty mleczarskie, jogurt, kultury starterowe, CLA, izomery *trans*.

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Abstrakt

Przedmiotem badań była ocena wpływu wybranych kultur starterowych na zawartość kwasu *cis*9*trans*11 C18:2 oraz na zawartość izomerów *trans* kwasu C18:1 i C18:2 w mlecznych napojach fermentowanych. Analizie poddano mleko normalizowane przygotowane do produkcji mlecznych napojów fermentowanych oraz wyprodukowane z niego produkty fermentowane. Objęte badaniem mleczne napoje fermentowane zostały wyprodukowane przy użyciu trzech różnych starterowych kultur jogurtowych (Ceska-star Y508, YC-X11 oraz ATB-1).

Przeprowadzone badania wykazały, że rodzaj zastosowanej kultury starterowej wpływa na zawartość kwasu *cis*9*trans*11 C18:2 (CLA) oraz na zawartość izomerów *trans* kwasu C18:1 i kwasu C18:2 w wyprodukowanych jogurtach.

We wszystkich objętych badaniem napojach fermentowanych wyprodukowanych z udziałem różnych kultur starterowych stwierdzono nieco niższe zawartości sprzężonego kwasu linolowego niż w mleku normalizowanym stanowiącym surowiec do ich produkcji.

Introduction

Conjugated dienes of milk fat (CLA) constitute a group of positional and geometric isomers of linoleic acid (C18:2), in which two double bonds are separated with only one single bond. In fat of the ruminants, the main representative of this group of isomers is *cis*9*trans*11 C18:2 acid which in milk fat constitutes from 75 to over 90% of total isomers of C18:2 acids with conjugated bonds (CHIN et al. 1992, LIN et al. 1998, PRECHT and MOLKENTIN 2000). The *cis*9*trans*11 C18:2 acid displays a variety of health-promoting properties, including: antioxidative, anticarcinogenic and antimutagenic ones (MOLKENTIN 1999, PARIZA 1991, PARODI 1994, 1997, 1999, PRZYBOJEWSKA and RAFALSKI 2003).

An important source of CLA in man's diet is milk and dairy products (butter, cheeses and fermented products). The content of this acid in milk depends, most of all, on the feeding regime and rearing method as well as on the breed, age and lactation period of an animal. Its average content in milk fat ranges from 3 to 6 mg/g fat (CHIN et al. 1992, LIN et al. 1995, JIANG et al. 1997). CLA content in dairy products may, however, differ from its content in milk, as in dairy products it may be influenced by parameters of technological processes conducted, additives applied, or capability of some lactic fermentation bacteria to synthesize CLA under appropriate conditions of the fermentation process (strain dose and duration of its action, conditions of incubation and composition of milk) (JIANG et al. 1998, KIM and LIU 2002, LIN 2003, SIEBER et al. 2004, CIOŁKOWSKA et al. 2012). According to a study by KIM and LIU (2002), nine out of eleven analyzed strains of lactic fermentation bacteria were capable of synthesizing CLA, with *Lactococcus lactis* IO-1 strain found to be the most effective. In turn, a research by JIANG et al. (1998) shows that among seven *Lactobacillus* strains, two *Streptococcus* strains and six *Propionibacterium*

strains cultured in vitro, only *Propionibacterium freudenreichii* was capable to transform free linoleic acid to *cis*9*trans*11 and *trans*9*cis*11 C18:2 isomers. DOMAGAŁA et al. (2009) determined the effect of seven different starter cultures on the level of conjugated linoleic acid in fermented cream and demonstrated that only the addition of a yoghurt culture ABY-2 caused an increase in CLA content in the finished product.

The aim of this study was to evaluate the effect of selected starter cultures on the content of conjugated linoleic acid (*cis*9*trans*11 C18:2) and contents of *trans* isomers of C18:1 and C18:2 acids in fermented milk drinks.

Material and Methods

Analyses were conducted for normalized milk prepared to produce fermented dairy drinks and for freshly-produced drinks. Three production series were run with three starter cultures: Ceska-star Y508 – containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles* bacteria, produced by CSK Food Enrichment, Poland; YC-X11 – containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles* bacteria, produced by Chr. Hansen; and ABT-1 – containing *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum* bacteria, produced by Chr. Hansen.

Fermented drinks were produced with the thermostat method, according to the following technological scheme: after collection, raw milk was cooled to a temp. of 6°C and stored for 4 h, next after heating to 45°C it was subjected to centrifugation and degasing (80 kPa; 60°C), and to HTST pasteurization (72°C/15 s), and finally it was cooled to a temp. of 6°C. Afterwards, milk was normalized to fat content of ca. 2% (addition of skim milk). The normalized milk was subjected to two-stage homogenization (18/5 MPa, temp. 65°C) and long-term VHT pasteurization (90°C/5 min). After cooling to 45°C, it was divided into three portions (about 10 liters each one) and inoculated with three different starter cultures. The fermented dairy drinks produced in this way were poured into unitary packages (250 mL cups) and thermostated at 43.5°C for ca. 3.5h, to pH 4,65.

Three samples were collected from each production series for analyses. All determinations were carried out in two parallel replications.

Methods

Fat content in produced fermented milk drinks was determined with the Roese-Gottlieb's method (PN-75/A-86130).

To determine CLA and *trans* C18:1 and C18:2 isomers, fat of normalized milk and fermented milk drinks was isolated with the Folch's method [Christie, 1973].

Methyl esters of fatty acids were prepared acc. to the IDF method, using a methanol solution of KOH [IDF Standard 182: 1999].

Determinations of fatty acid composition and contents of CLA and *trans* isomers of unsaturated fatty acids were carried out with gas chromatography (GC) method using an HP 6890 chromatograph with a flame-ionization detector. Chromatographic separation of fatty acid methyl esters was carried out on a capillary column (100 m x 0.25 mm i.d., film thickness 0.20 μm) with CP Sil 88 phase. Separation conditions were as follows: column temp.: 60°C (1 min) – 180°C, Δt = 5°C/min; detector temp.: 250°C; injector temp.: 225°C; carrier gas: helium, flow rate: 1.5 mL/min, injector: split 50:1.

For identification of positional *trans* isomers of C18:1, used the standards of methyl esters of those isomers (*trans* 6, Supelco and *trans* 9 and *trans* 11, Sigma-Aldrich) and literature data. The *trans* isomers of C18:2 acid (*cis*,*trans* and *trans*,*cis*) were identified with the use of a mixture of standards of C18:2 isomers (Supelco), *cis*9, *trans*11 CLA – with a mixture of CLA methyl esters (Sigma-Aldrich).

Quantitative computations of *cis*9*trans*11 C18:2 acid and *trans* isomers of C18:1 and C18:2 acids were made against the introduced standard (methyl ester of C21:0 acid). Statistical calculations were conducted with STATISTICA 10 software.

Results and Discussion

All analyzed fermented milk drinks were characterized 2 ± 0.1% fat content.

Results obtained for contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in normalized milk and fermented milk drinks produced using different starter cultures are presented in Table 1. The exemplary chromatogram separation of CLA and *trans* isomers C18:1 and C18:2 acids of fermented milk drink produced with YC-X11 starter is presented in Figure 1.

The content of *cis*9*trans*11 C18:2 acid in normalized milk prepared for the production of fermented dairy drinks reached 3.75 mg/g fat. LINA et al. (1995)

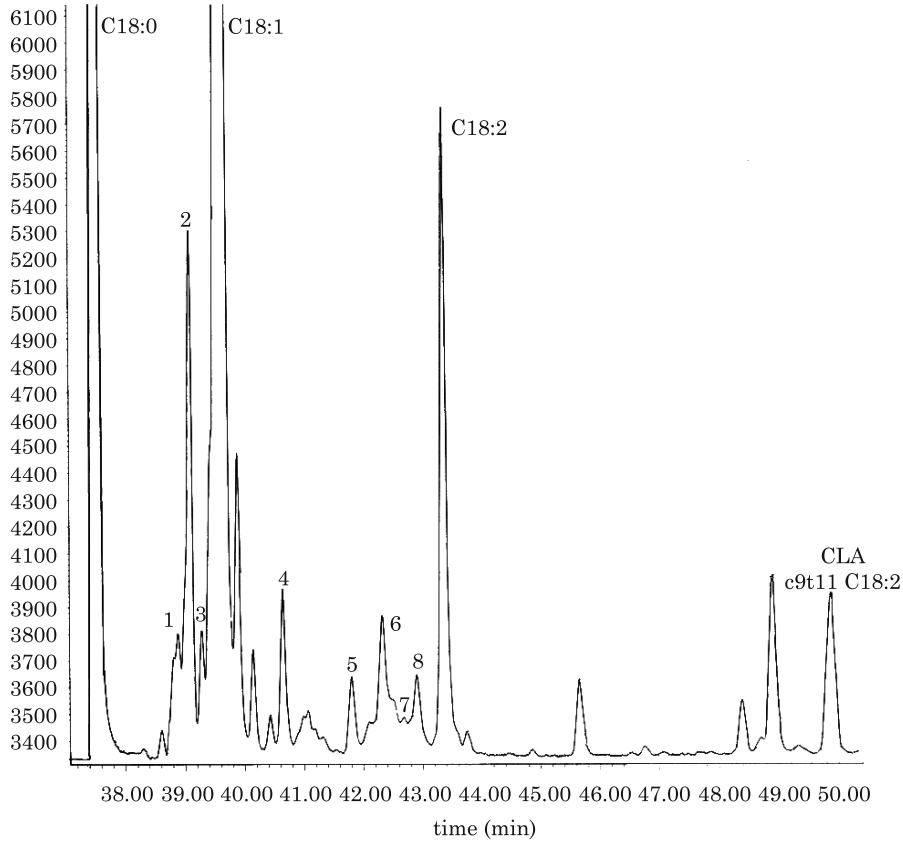

Fig. 1. Separation of CLA and *trans* isomers of C18:1 and C18:2 fatty acids of fermented milk drink produced with YC-X11 starter

reported that CLA content in full-fat milk was at 4.49 mg/g fat. In milk with 2% fat content, the level of this acid was at 4.14 mg/g fat. Based on investigations of other authors, KOWALSKA and CICHOSZ (2013) state that CLA content in full-fat milk may range from 3.4 to 6.8 mg/g fat. In milk with fat content of 2%, the CLA content accounts for 4.1 mg/g fat, whereas in condensed milk it may range from 6.3 to 7.0 mg/g fat. The fermented milk drinks produced with various starter cultures were characterized by a slightly lower content of this acid than milk (Table 1.). The content of *cis*9*trans*11 C18:2 acid in the analyzed fermented drinks reached: 3.68 mg/g fat in drinks produced with YC-X11 starter, 3.62 mg/g fat in drinks produced with ATB-1 starter, and 3.61 mg/g fat in drinks produced with Ceska-star Y508 starter culture.

Table 1

Content of CLA and C18:1 and C18:2 *trans* isomers in milk and fermented milk drinks (mg/g fat)

Trans isomers	Normalized milk prepared for production $\bar{x}$ ±s /SD	Type of starter culture		
		Ceska-star Y508 $\bar{x}$ ± s /SD	YC-X11 $\bar{x}$ ± s /SD	ATB-1 $\bar{x}$ ± s /SD
*cis*9*trans*11 C18:2 (CLA)	3.75^{a} ± 0,04	3.61^{a} ± 0.08	3.68^{a} ± 0.06	3.62^{a} ± 0.16
t6 – t9 C18:1	2.80 ± 0.04	2.83 ± 0.19	2.74 ± 0.08	2.71 ± 0.12
*t*10+*t*11 C18:1	9.16 ± 0.01	8.77 ± 0.07	8.99 ± 0.13	8.72 ± 0.34
t12 C18:1	1.83 ± 0.19	1.95 ± 0.12	1.95 ± 0.04	1.87 ± 0.08
t16 C18:1	2.60 ± 0.03	2.35 ± 0.45	2.46 ± 0.09	2.54 ± 0.14
Σ *trans* C18:1	16.39^{a} ± 0.16	15.90^{a} ± 0.76	16.14^{a} ± 0.27	15.85^{a} ± 0.57
c9 t13 C18:2	1.59 ± 0.24	1.40 ± 0.11	1.41 ± 0.08	1.51 ± 0.31
t9 c12 C18:2	2.41 ± 0.06	2.12 ± 0.08	2.19 ± 0.26	2.02 ± 0.20
t11 c15 C18:2	0.77 ± 0.07	0.62 ± 0.03	0.60 ± 0.03	0.70 ± 0.13
Σ *trans* C18:2	4.77^{a} ± 0.11	4.14^{b} ± 0.11	$4.20^{a,b}$ ± 0.31	$4.22^{a,b}$ ± 0.49

^{a,b} – values in the rows denoted by the same letter are not significantly different ($p > 0.05$).

Lower concentrations of CLA in yoghurts compared to milk they were made of were also reported by SANTOS JUNIOR et al. (2012). These authors conducted their study in Brazil in the summer period and demonstrated that CLA content reached 6.22 mg/g fat in pasteurized milk and 5.41 mg/g fat in yoghurts. Differences in *cis*9*trans*11 C18:2 acid content in Italian fermented milk drinks produced with the use of various starter cultures were reported by PRANDINI et al. (2007). In products analyzed by these authors, the mean content of CLA ranged from 4.42 mg/g fat in probiotic yoghurts to 6.15 mg/g fat in fermented milk produced from milk of cows grazed on a mountain pasture. A research by DOMAGAŁA et al. (2009) indicated that CLA content in fermented cream depended on the type of starter culture applied in the fermentation process. The CLA content in the samples analyzed by these authors ranged from 3.33 mg/g fat in fermented cream produced with YC-180+*Propionibacterium* starter to 4.03 mg/g fat in the product made with ABY-2 starter culture. Yoghurts analyzed by Lin *et al*., (1995) contained CLA at 3.82 mg/g fat, and buttermilk – at 4.66 mg/g fat. Based on investigations of other authors, KOWALSKA and CICHOSZ (2013) state the CLA content may range from 3.8 to 8.8 mg/g fat in yoghurts, from 5.4 to 6.7 mg/g fat in buttermilk, and from 4.6 to 7.5 mg/g fat in sour cream.

The total content of *trans* isomers of C18:1 acid in normalized milk prepared for production reached 16.39 mg/g fat. The production process and starter cultures applied caused a decrease in the content of these isomers. The

lowest decrease (to the value of 16.14 mg/g fat) was reported in drinks produced with YC-X11 starter culture. In the fermented drinks produced with Ceska-star Y508 and ATB-1 starter cultures, the levels of C18:1 *trans* isomers were at 15.90 mg/g fat and 15.85 mg/g fat, respectively.

In both milk and fermented milk drinks, among *trans* isomers of C18:1 acid the highest contents were noted for *trans* 10 + *trans* 11 isomers of C18:1. The contribution of the total sum of these isomers in the total fatty acid composition of normalized milk reached 9.16 mg/g fat. In the fermented dairy drinks, the total contents of these isomers were slightly lower (Table 1).

The content of *trans* isomers of C18:2 acid in normalized milk reached 4.77 mg/g fat. A significantly lower (4.14 mg/g fat) total content of these isomers was determined in fermented milk drinks produced with Ceska-star Y508 starter. Lower than in milk contents of C18:2 *trans* isomers acid were also noted in the other analyzed fermented drinks, however they were not statistically significant.

Conclusions

The study demonstrated that the type of the applied starter culture affected the content of *cis*9*trans*11 C18:2 acid (CLA) and contents of *trans* isomers of C18:1 and C18:2 acids in fermented products.

In all analyzed fermented dairy drinks produced with various starter cultures, analyses showed slightly lower contents of conjugated linoleic acid that in normalized milk used as a raw material for their production.

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THE INFLUENCE OF STORAGE TEMPERATURE ON OXIDATIVE STABILITY AND SHELF-LIFE OF PEANUTS

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Key words: Food Quality, Peanuts, Oxidative stability, Shelf-Life, Storage.

Abstract

The aim of this study was to evaluate the influence of storage temperature on oxidative processes and shelf-life of peanuts. Shelled roasted peanuts of two different origin, Argentina and Brazil, vacuumpacked in laminatepolyamide /polyethylene, were stored up to 6 months in practical (20 ± 1°C) and accelerated (28 ± 1°C) storage dark conditions. The measure of oxidative stability was peroxide value with proposed limiting value 20 meq O_2/kg of extracted oil and hexanal (proposed critical limit 3 ppm). The kinetic parameters from Arrhenius' equation were calculated. The temperature dependent oxidativeprocesses in peanuts from Argentina and Brazil do not occure xactly in the same way. Moreover, regardless of peanuts origin, there was higher temperature sensitivity for primary thansecondary oxidative changes observed. Uncontrolled increase of temperature from e.g. 20°C to 25°C would reduce shelf-life of peanuts by 20–30% which entails not only economic consequences but is also important because of nutritional value and food safety issues.

WPŁYW TEMPERATURY PRZECHOWYWANIA NA STABILNOŚĆ OKSYDACYJNĄ I TRWAŁOŚĆ ORZECHÓW ARACHIDOWYCH

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Słowa kluczowe: jakość żywności, orzechy arachidowe, stabilność oksydacyjna, trwałość, przechowywanie.

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Abstrakt

Celem pracy była ocena wpływu temperatury przechowywania na przebieg procesów oksydacyjnych i trwałość orzechów arachidowych. Zapakowane próżniowo w laminat poliamid/polietylen orzechy arachidowe, pochodzące z Argentyny i Brazylii, przechowywano przez 6 miesięcy bez dostępu światła w warunkach praktycznego składowania (20 ± 1°C) oraz przyspieszonego starzenia (28 ± 1°C). Jako limity krytyczne wyróżników stabilności oksydacyjnej badanych orzechów przyjęto zawartość nadtlenków na poziomie 20 meq O_2/kg wyekstrahowanego tłuszczu i zawartość heksanalu na poziomie 3 mg/kg orzechów. Na podstawie równania Arrheniusa wyznaczono parametry kinetyczne procesów utleniania. Mimo, iż temperaturowa zależność procesów oksydacyjnych nie była jednakowa w przypadku próbek z Argentyny i Brazylii, w obu rodzajach wykazano większą zależność od temperatury zmian pierwotnych niż wtórnych. Kontrolowanie i przestrzeganie założonej temperatury podczas przechowywania orzechów ma ogromne znaczenie dla wartości odżywczej i bezpieczeństwa produktu. Wykazano, że niekontrolowany wzrost temperatury przykładowo z 20°C do 25°C skraca trwałość orzechów o 20–30%.

Introduction

Oxidative processes of lipid fraction during processing and storage contribute food quality. They may change nutritional value of food because of impact on essential fatty acids, proteins and vitamins (Chun et al. 2005, HĘŚ and KORCZAK 2007). Some of the arising products of oxidation such as free radicals or oxysterols have been described to have negative or even toxic health effect (GUILLEN and GOICOECHEA 2008). Degradation of the unstable primary oxidation products, hydroperoxides, leads to the formation of a variety of volatile compounds, such as aldehydes, ketones, hydrocarbons, alcohols, acid compounds and furans. Formed secondary products with low threshold values are responsible for the off-flavors development (MIN and BOFF 2002, OLMEDO 2012).

Several internal and external factors may have an impact on storage stability of peanuts. The works on influence of storage conditions on oxidative stability of different nuts proved that the most important factor influencing shelf-life was temperature which had the accelerating effect on the deteriorative processes (VANHANEN and SAVAGE 2006, MEXIS et al. 2009, WAMBURA et al. 2012, WILKIN et al. 2014).

The effect of temperature on the rate of reaction can be described by the Arrhenius equation (SCHMIDL and LABUZA 2000):

$$k = k_a \exp(-E_a / RT) \quad (1)$$

where:
k – the rate constant,
k_a – the pre-exponential constant,

E_a – the activation energy (kJ/mole),
R – the gas constant (8,315 J/mol·K),
T – the absolute temperature (K).

The activation energy can be used as the measure of temperature dependence of processes – the higher the E_a, the faster the reaction as temperature increases.The increase of temperature has various impacts on different kinds of changes in food. The chemical reactions are usually highly temperature dependent (MAN 2011).

Another method of expressing the temperature dependence of reaction is the Q_{10} (temperature quotient) approach. Q_{10} can be defined as the reaction rate at one temperature compared to that at a temperature 10°C lower (SCHMIDL and LABUZA 2000):

$$Q_{10} = k_{(T+10)}/k_T \quad (2)$$

where:
Q_{10} – the temperature quotient,
T – the absolute temperature (K).

The relationship of Q_{10} and Ea can be described by the means of the following equation:

$$\ln (Q_{10}) = 10E_a/RT(T+10) \quad (3)$$

where:
Q_{10} – the temperature quotient,
E_a – the activation energy (kJ/mole),
R – the gas constant (8,315 J/mol·K),
T – the absolute temperature (K).

The most important mechanism of lipid oxidation is chemical process of autoxidation. It is influenced by temperature (KERRIHARD et al. 2015). Inappropriate storage conditions, such as temperature abuse, would increase the rate of oxidation in peanuts. Taking into consideration high temperature dependence of oxidative processes, it is important to recognize the way the changes of storage temperature influence peanuts' shelf-life. The aim of this study was to evaluate the influence of storage temperature on oxidative processes and shelf-life of peanuts.

Materials and Methods

Materials

Shelled roasted peanuts of two different origins sample A – from Argentina, and sample B – from Brazil, were supplied by a local importer. The samples were vacuum packed in plastic laminate PA (polyamide) / PE (polyethylene). The total fat was 52,7% (sample A) and 53,1% (sample B) and the moisture content 1,9% (A) and 1,4% (B), as determined using AOAC Official Methods (948.22, 925.40).

The samples were stored in closed 100 g packages up to 6 months in practical (20°C ± 1°C) and accelerated (28°C ± 1°C) storage dark conditions. After and at the end of each month the samples were withdrawn to perform the analysis of hydroperoxides and after every two months for hexanal measurements. The experiment was repeated twice.

Lipid extraction

Lipids were extracted from peanuts according to the method described by FOLCHET et al. (1957) with modifications. About 10 g milled peanuts were homogenized twice with 50 ml chloroform/methanol mixture (2:1 vol/vol). The obtained mixture was filtered and combined into a separatory funnel. The extract was then diluted with distilled water and the lower phase was collected and passed through anhydrous sodium sulfate. Solvent was removed in a rotary vacuum evaporator (BüchiLabortechnik, Flawil, Switzerland). The extracted lipids were used forhydroperoxides evaluation.

Peroxide value

PV (peroxide value) in extracted lipids was determined by iodometric method according to Polish Standard (PN-EN ISO 3960:2009) and expressed in meq O_2/kg of extracted oil.

Hexanal analysis

Hexanal measurements in peanuts were performed with the use of the SHS-GC (static headspace gas chromatography). The analyses were carried on the Varian 3800 gas chromatograph (Varian Inc., Lake Forest, CA, USA)

equipped with an autosampler Tekmar 7000 (Tekmar-Dohrmann, Cincinnati, USA).

Sample preparation

A standard stock solution containing hexanal in the concentration around 2 mg/ml of freshly refined rapeseed oil (Z.T. Kruszwica S.A., Kruszwica, Poland) was used to prepare subsequent solutions. The hexanal has not been detected in rapeseed oil as measured by SHS-GC. Then, a standard addition method was applied: 4 g ± 0.2 g of peanuts were placed in glass vials (22 mL) and 0.5 mL of rapeseed oil without any standard (zero sample) or 0.5 mL of rapeseed oil containing increasing concentration of hexanal was added (KOLB and ETTRE 2006). Vials were closed with the use of septum and left in darkness overnight to equilibrate.

Static headspace conditions

Samples were agitated for 30 min at 50°C to reach equilibrium. The headspace conditions were as follows: vial pressurization 34.5 kPa, pressurize time 0.5 min, sample equilibration 0.1 min, loop fill 0.6 min, loop equilibration, 0.1 min, injection 0.1 min, loop temperature 110°C, transfer line temperature 120°C, vial needle flow 55 mL/min. The gas phase (the headspace) was introduced into the carrier gas stream- helium (Linde, Kraków, Poland) and carried into the column.

Gas chromatography

A gas chromatograph Varian 3800 was equipped with a flame ionization detector and a capillary column CP Sil 8CB (30 m x 0.53 mm x 1.5 μm; Varian Inc., Lake Forest, CA, USA). The initial column temperature was 40°C (2 min), then it was raised to 100°C (8°C/min) and to 200°C (20°C/min), then held 5,5 min (SAMOTYJA and MAŁECKA 2010). Hexanal was determined by comparison of retention time with that of a known hexanal standard.

Chemicals

Hexanal (98% GC) was from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Other chemicals were of reagent quality grade and were provided by POCh (Gliwice, Poland).

Estimation of shelf-life

The kinetic parameters were calculated for measured primary and secondary oxidative processes. The loss of food quality can be represented by the kinetic equation:

$$dC/dt = kC^n \tag{4}$$

where:
C – the quality factor measured,
t – time,
k – the rate constant,
n – the reaction order.

The rate constant $k$of each temperature was determined separately for hydroperoxides and hexanalby plotting ln C versus time. Then, the rate constant – temperature dependence was established on the basis ofconverted Arrhenius equation:

$$lnk = lnk_0\text{-}(E_a/RT) \tag{5}$$

Subsequently, the activation energy was determined (Gallagher et al. 2011). The temperature quotient Q_{10} was calculated from the Eq. (3) (MAN 2011).

The shelf-life of peanuts was estimated by plotting the linear relation between the log of end-point and the tested temperatures. The results were presented as the shelf-life linearcurves directly showing the end-point of oxidative stability versus temperature (°C).

Statistical analysis

All analyses were done in duplicate and the results were expressed as average values. The data were fitted to the mathematical models and the regression analyses were carried out. The calculations were performed using Statistica 8.0 software (StatSoft, Inc., Tulsa, USA).

Results and Discussion

Oxidative stability

The course of oxidative changes in peanuts is presented in Figure 1. The lower stability of lipid fraction, the higher is the extent of primary and secondary oxidative processes as measured by PV and hexanal concentration (respectively).

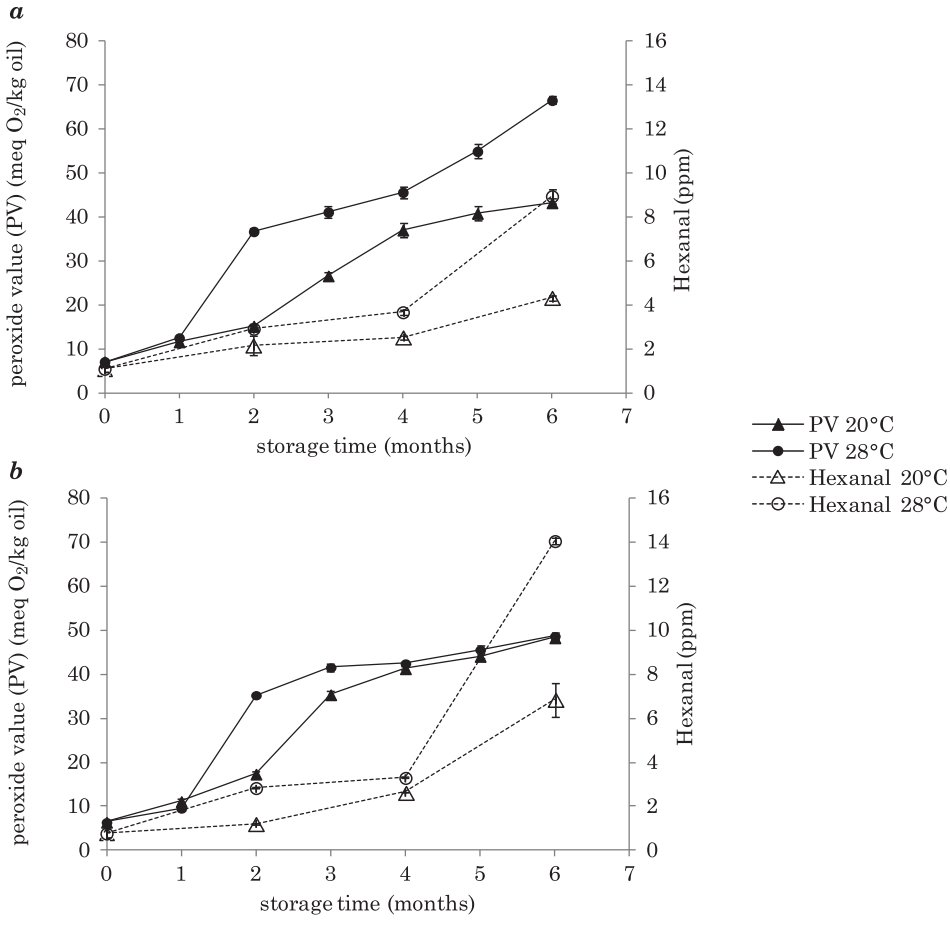

Fig. 1. The extent of primary and secondary oxidative changes in peanuts during 6-months storage at 20°C and 28°C. A – sample A, B – sample B

The amount of hydroperoxides increased from starting point to the end of experiment. PV increased from the initial level of 7.1 meq O_2/kg to 15.2 meq O_2/kg (sample A) and from 6.5 meq O_2/kg to 17.4 meq O_2/kg (sample B) in the two first months of storage at 20°C. After this period there wasa sudden change in PV observed. During the third month of the test at 20°C PV of peanuts reached the level of 26.6 meq O_2/kg (A) and 35.4 meq O_2/kg (B). Storage at elevated temperature resulted in higher degree of primary oxidation products formation. The analyses showed rapid increase of PV during second month of storage at 28°C to the values 36.7 meq O_2/kg (A) and 35.3 meq O_2/kg(B).

Initial concentrations of hexanal were 1.1 ppm (sample A) and 0.8 ppm (sample B). Hexanal concentration increased slowly till the end of fourth month of storage, and then rapidly increased to 4.3 ppm (A) and 6.8 ppm (B) at ambient conditions and to 8.9 ppm (A) and 14.0 ppm (B) at 28°C as measured at the end of storage.

Shelf-life considerations

The shelf-life limiting value (end-point) of PV was established on the level of 20 meq O_2/kg. There is no standard critical limit of hydroperoxides in peanuts and in literature different values are assumed, but usually they are within the range of 20–30 meq O_2/kg (EVRANUZ 1993). Taking into consideration the course of oxidative processes, the established critical limit corresponds to the propagation phase excerpt of the PV curves.

Rapid decomposition of primary products, yielding sharp increase of hexanal was observed after fourth month of samples storage and corresponded to the avera gealdehyde contents of about 3 ppm – this value was used as the critical limit during further considerations in this study. For comparison, MEXIS et al.(2009) who studied the effects of storage conditions on the quality of walnuts, proposed limit for PV close to 10 meq O_2/kg of walnut oil with respective values for hexanal 1–2 ppm. ROBARDS et al.(1988) correlated hexanal concentration with flavor score of corn chips and found out that samples unacceptable according to the hedonic scale corresponded to concentration of hexanal exceeding 5 ppm. Nepote et al. (2006) found out that the end point of consumer acceptance of rancid flavor in peanuts corresponded values over 80 meq O_2/kg, what was much beyond the accepted limit of hydroperoxides. The difficulty for arbitrary assignation of a limiting value of a given quality criterion in shelf-life studies is a result of complexity of deteriorative changes in food. The kind, amount and the rate of secondary oxidation products formation depend on the rate of their precursors' decomposition which in turn depend on food composition and mechanism of deterioration.

From adjustments of experimental data to different kinetic models the first order reaction model was selected to describe the oxidative changes in peanuts during storage. The kinetic parameters (the rate constant k and the activation energy E_a) are presented in Table 1.

Table 1

Rate constant (k) for hydroperoxides and hexanal formation, the activation energy (E_a) and temperature quotients Q_{10}

Criterion of shelf-life	Sample	Temperature (°C)	Rate constant [a]k (1/month)	Coefficient of determination (R^2)	Activation energy E_a (kJ/mol)	Temperature quotient Q_{10}
Hydroperoxides formation	A	20	0.413 ± 0.023	0.991	63.0	2.4
		28	0.821 ± 0.148	0.969		
	B	20	0.553 ± 0.037	0.991	39.1	1.7
		28	0.846 ± 0.263	0.912		
Hexanal formation	A	20	0.232 ± 0.042	0.940	36.2	1.6
		28	0.345 ± 0.057	0.949		
	B	20	0.364 ± 0.040	0.977	17.6	1.3
		28	0.441 ± 0.087	0.928		

Explanation to Table 1: [a]k ± confidence intervalat 95%

Increasing rate constant with rising temperatures confirms temperature dependence of measured processes. Regardless of the sample origin, there was higher temperature dependence for PV than hexanal observed. It means that increase in temperature would result in higher degree ofhydroperoxidesformation than hexanal. On the contrary, a reduction in temperature would have greater impact on inhibition of hydroperoxides formation.

Q_{10} values for reactions in peanuts A and B were found to be different. These values were also differentiated in relation to primary and secondaryoxidation products formation. Increase of temperature of 10°C would cause increase of hydroperoxides formation 2,4 – fold in peanuts A and 1,7 – fold in sample B, whereas hexanal would be formed 1,6 and 1,3 times faster (in peanuts A and B, respectively).

Higher values of E_a and Q_{10} for peanuts A mean that with increase of temperature their shelf-life would be reduced more than that of B samples.

Similarly, the temperature decrease would prolong shelf-life of peanuts A more than peanuts B.

On the basis of storage trials the shelf-life can be estimated at any temperature range in which the mechanism of reactions does not change. The influence of temperature on the predicted time to reach PV = 20 meq O_2/kg and 3 ppm of hexanal is presented in Figure 2. This period is exponentially reduced with increasing temperature and indicates quality deterioration resulting reduction of shelf-life.

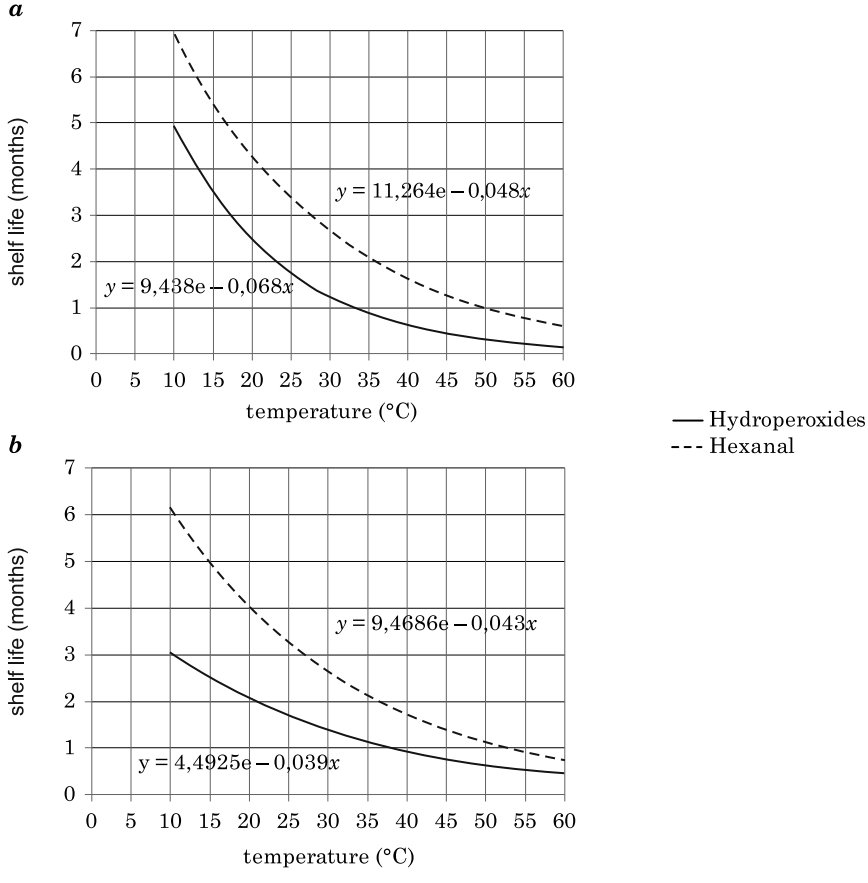

Fig. 2. Predicted shelf-life of peanuts as a function of temperature. A – sample A, B – sample B

Presented shelf-life curves show that oxidative stability of peanuts according to storage temperature does not change exactly in the same way and depends on the origin of the product. Moreover, during chain of distribution

and storage, some additional factors (such as temperature fluctuations through time) can influence the shelf-life and therefore they also should be taken into consideration.

Conclusions

The storage temperature influences oxidative processes in peanuts and thus decrease their shelf-life. Uncontrolled increase of temperature from e.g. 20°C to 25°C would reduce shelf-life of peanuts by 20–30% which entails not only economic consequences but is also important because of nutritional value and food safety issues. It can be assumed that the retailers are not always aware of this fact, the peanuts are often stored in unsuitable conditions and, finally, lose their quality before they reach the consumer.

The temperature dependent oxidative processes in peanuts do not change in the same way. Different activation energies mean that increase of temperature would have different impact on samples which have the same state of oxidation after production (packaging). Q_{10} values available in literature should be used only for approximation or experiment design purposes as there is no universal value for a certain kind of food.

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DERMANYSSUS GALLINAE STILL POSES A SERIOUS THREAT FOR THE REARING OF LAYING HENS

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K e y w o r d s: *Dermanyssus gallinae*, poultry, laying hen, infestation, pest control.

A b s t r a c t

Dermanyssus gallinae (poultry red mite) is a temporary parasitic mite which feeds on the blood of many species of domestic, wild and exotic birds. It can also attack mammals and humans. It is widely dispersed throughout the world and is a major problem in poultry production, mainly for laying hens. Under the suitable conditions of farms, the parasite population grows quickly and becomes difficult to control. Invasion of *D. gallinae* in chickens causes chronic stress, anxiety, irritability, increased cannibalism and mortality (6–8%), and a decrease in laying performance (approx. 15–20%). The possibility of the transmission of many pathogens can affect the spread of epidemiological risks in poultry. *D. gallinae* has exhibited resistance to adverse environmental conditions and eradication formulas. It is estimated that losses caused by the invasion of *D. gallinae* and eradication costs in the EU amount to about EUR 130 million annually. To combat *D. gallinae*, synthetic acaricides, products containing natural or synthetic silica, and the Thermo-kill method are commonly applied. Due to some limitations, alternative methods are still being sought, e.g. substances of natural origin (thuringiensin, spinosad, garlic extract and neem tree, geraniol, eugenol and citral) and vaccinations (subolesin and protein Bm86), as well as the biological control of natural enemies, or the introduction of a condensed light cycle. It seems that today the best results can be achieved by the application of the principles of IPM (Integrated Pest Management). One of the actions which should be jointly implemented by specialists from various fields is the construction of sheds to hinder the settlement of the parasite and to facilitate its liquidation.

DERMANYSSUS GALLINAE NADAL POWAŻNYM ZAGROŻENIEM W CHOWIE KUR NIOSEK

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Abstrakt

Dermanyssus gallinae (poultry red mite) to okresowe pasożytnicze roztocze, które odżywia się krwią wielu gatunków ptaków domowych, dzikich oraz egzotycznych. Może atakować ssaki oraz ludzi. Jest szeroko rozpowszechniony na świecie i stanowi poważny problem w chowie i hodowli drobiu, głównie kur niosek. W warunkach ferm populacja pasożyta szybko narasta i jest trudna do zwalczenia. Inwazja *D. gallinae* u kury powoduje stan stresu chronicznego, niepokój, rozdrażnienie, wzrost kanibalizmu i liczby upadków (6–8%) oraz spadek nieśności (ok. 15–20%). Ze względu na możliwość transmisji wielu czynników chorobotwórczych może wpływać na szerzenie się zagrożeń epidemiologicznych wśród drobiu. Wykazano oporność *D. gallinae* na niekorzystne warunki środowiska oraz preparaty do jego zwalczania. Straty wywołane inwazją *D. gallinae* oraz koszty zwalczania w UE szacowane są na ok. 130 mln euro rocznie. Do zwalczania *D. gallinae* powszechnie stosowane są syntetyczne akarycydy, preparaty zawierające naturalną lub syntetyczną krzemionkę oraz metoda Thermo-Kill. Ze względu na pewne ograniczenia ciągle poszukuje się metod alternatywnych jak: substancje pochodzenia naturalnego (thuringiensyna, spinosad, ekstrakt z czosnku oraz drzewa neem, geraniol, eugenol i citral), a także szczepienia ochronne (subolesina i białko Bm86), jak również kontrolę biologiczną z wykorzystaniem naturalnych wrogów, czy wprowadzanie skróconego cyklu świetlnego. Wydaje się, że najlepsze rezultaty można osiągnąć stosując zasady IPM (Integrated Pest Management). Jednym z działań powinno być wspólne opracowanie przez specjalistów z wielu dziedzin konstrukcji kurników w celu utrudnienia zasiedlenia ich przez pasożyta i ułatwienie jego likwidacji.

Introduction

Dermanyssus gallinae (De Geer, 1778) (*D. gallinae*), Poultry Red Mite (PRM), is a parasitic mite belonging to the class: Arachnoidea, row: Acarina, suborder: Mesostigmata, family: Gamasidae, type: Dermanyssus. *Dermanyssus gallinae* feeds primarily on the blood of chickens, but also on many other species of domestic birds, as well as approx. 30 species of wild birds and exotic birds (CHMIELEWSKI 1982, CENCEK et al. 2000, 2002, ROMANIUK and OWCZARZAK-PODZIEMSKA 2002, ROY and CHAUVE 2007, SMITH et al. 2014). In the absence of specific hosts, it also attacks mammals, mostly rodents, dogs, cats and farm animals, especially horses (MIGNON and LOSSON 2008). There are also numerous reports of an invasion of mites in humans, primarily in poultry farms workers, in whom the mite can induce severe itching, allergies, dermatitis and lesions of the skin (AUGER et al. 1979, ARENDS 1997, ROSEN 2002, AKDEMIR et al. 2009, HAAG-WACKERNAGEL and BIRCHER 2010).

Dermanyssus gallinae is widely distributed in the world, and occurs in the areas north to 60° latitude, where it poses a significant threat to poultry production and hen health (HOGLUND et al. 1995, WOJCIK et al. 2000, CENCEK 2003, GUY et al. 2004, FIDDES et al. 2005, SPARAGANO et al. 2009, 2014). The *D. gallinae* population grows very quickly, especially under conditions of factory farms, and is difficult to control due to the specific behaviour and favourable environmental conditions and facilities of farm, such as the large number of hiding places, high density of birds in the limited space, and permanent high

temperature and humidity (CHAUVE 1998, SOKÓŁ and ROMANIUK 2007, SPARAGANO et al. 2009). Mite invasion is difficult to control due to the colonisation sites, which are poorly accessible, resistance to adverse environmental conditions, and used preparations (CHAUVE 1998).

Dermanyssus gallinae occurs in all types of farming system, independently of the destination and size of the flock (SPARAGANO et al. 2009). The mite population in laying hen factory farms reaches a higher level than in flocks of broilers due to the longer production cycle, which usually takes 80–90 weeks (ROY et al. 2010). The battery cage system promotes *D. gallinae* invasion due to it being a more favourable habitat for mites and a greater challenge for decontamination between flocks. It is the system which currently predominates in the poultry industry (HARRINGTON et al. 2011). For example, annual production of poultry in this system in Denmark, France and Italy is about 56%, 76.5%, and 96.4%, respectively (SPARAGANO et al. 2009). Damage caused by the mites, and associated costs of control only in the EU amount to about 130 million euros per year (SPARAGANO et al. 2009, 2014). In poultry flocks in the USA and in some countries in South America and Asia the following ectoparasites of poultry are found more common: *Ornitonyssus sylviarum* (Canestriniand Fanzago 1877), Mesostigmata: Dermanyssoidea: Macronyssidae, known as Northern fowl mite (AXTELL, ARENDS 1990, MULLENS et al. 2001). *Dermanyssus gallinae* density in battery cage system can be up to approx. 50 000 parasites per bird, and in severe cases even approx. 500 000 per bird. The most massive invasions have been observed during warm and humid months (KILPINEN et al. 2005, OTHMAN et al. 2012).

The scale of the problem of *D. gallinae* is very serious, which is proved by the fact that in November 2014 an international research consortium was formed to combat PRM. The project, called COREMI (Improving current understanding and research for sustainable control of the poultry red mite *Dermanyssus gallinae*), brings together scientists from over 17 European countries (http://www.cost.eu/COST_Actions/fa/Actions/FA1404 February 2015).

Characteristics, biology and behaviour of *Dermanyssus gallinae*

The adult *D. gallinae* is from 0.7 to 1 mm long and from 0.4 to 0.5 mm wide. The body is pear-shaped, dorsoventrally flattened, and covered with transparent, greyish chitinous armour. After sucking blood it is red, and when the blood is digested it turns brown and the intestines are clearly visible. It has four pairs of legs, ending with two claws, and oral apparatus of the piercing and sucking type, ending with long stiletto chelicerae, which puncture the skin of the host (SIKES and CHAMBERLAIN 1954).

Dermanyssus gallinae is a periodic ectoparasite which spends on the host usually from 0.5 to 1.5 h only during sucking blood. It feeds every 2–4 days, mainly at night, when birds are less active (WOOD 1917, NAKAMAE et al. 1997, CHAUVE 1998, ROMANIUK and SOKÓŁ 2007). *Dermanyssus gallinae* locates its host using a combination of several stimuli: temperature, chemical signals, vibration, and carbon dioxide (ZEMAN 1988, KILPINEN and MULLENS 2004, KILPINEN 2005). Led by the smell of pheromones secreted by other individuals, an engorged individual mite goes back to the hideout, where all the parasites aggregate together (ENTREKIN and OLIVIER et al. 1982, KOENRAADT and DICKE 2010). *Dermanyssus gallinae* prefers inaccessible places, shielded from light, mainly cracks and crevices in the construction of poultry houses, where it proliferates. Its development cycle consists of 5 stages: egg, larva, protonymph, deutonymph and adults (male or female). Protonymph, deutonymph and mature females must feed on blood. Males feed on the blood occasionally. Adult mites mate after the last moult, and the females lay eggs within 3 days after sucking blood. The number of eggs laid depends on the environmental conditions and reaches up to eight clutches. (WOOD 1917, MOSS 1978). The egg is oval, smooth, pearl white coloured, and it is 400x270μ. After approx. 1.5–2 days it hatches into a larva, which after approx. 12 h undergoes moulting and turns into a protonymph. The protonymph, within 24 h after sucking blood, moults again and turns into a deutonymph. It moults and becomes mature after approx. 2 days after the last feeding. The larva is of a white-grey colour and has 3 pairs of legs, while the nymph and imago has 4 pairs of legs (WOOD 1917).

The length of the *D. gallinae* lifecycle depends on the availability of the host, the ambient temperature and relative humidity. It usually takes about 2 weeks. Under favourable conditions, such as 20–25°C and high relative humidity (> 70%), this may be shortened to approx. 7–10 days. As a result, the population may double in one week (MAURER and BAUMGARTNER 1992, HOGLUND 1995). In less favourable environmental conditions its lifecycle may be extended, and in the absence of the host it does not take place. *Dermanyssus gallinae* is very resistant to adverse environmental conditions, e.g. in anticipation for the host. While waiting for the host it can survive 8–9 months at temp. 5°C, and at 25°C – approx. 6 weeks. Temperature below -20°C and above 45°C is fatal. The female lays eggs in temperatures from 5 to 45°C. At 5°C the time required for the larvae to hatch exceeds 50 days if the egg maintains the proper humidity, and they can hatch into larvae under appropriate conditions. Relative humidity (RH) is also important for the development of *D. gallinae*, e.g. at a temp. of 20°C and 23% RH it can survive for only 6 weeks, and at 11% RH larvae hatch is inhibited due to the drying of the eggs (MAURER and BAUMGARTNER 1992, NORDENFORS et al. 1999).

Analysis of the COI mitochondrial gene (cytochrome oxidase subunit1) and the 16S rRNA gene showed genetic variation between closely related species of the genus Dermanyssus and geographically distant populations of the species *D. gallinae*. The nuclear gene ITS was also submitted to the analysis. COI proved to be the best marker in phyleogeographic studies, as it allowed the differentiation of genetically low taxonomic levels. Differences within the ITS gene were not significant. The genetic diversity of the population of the species *D. gallinae* may be a consequence of the development of resistance to pesticides used in individual countries or geographical regions (ROY et al. 2009, MARANGI et al. 2009). It is believed that due to genetic variation *D. gallinae* can exhibit certain plasticity in relation to the host (but still remaining associated primarily with birds, and in particular laying hens) and shows tolerance to changing environmental conditions and adaptation to selecting factors (CHAUVE 1998, ROY et al. 2009, SPARAGANO et al. 2014).

Dermanyssus gallinae invasion impact on hens

Dermanyssus gallinae is the most serious ectoparasite affecting laying hens. Upon puncturing the skin of the host, it introduces a toxic saliva that can cause itching and irritation. Under heavy invasion, the welfare of the laying hens significantly decreases, which is reflected in the birds being restless and irritable. Consequently, they develop characteristic behaviour called self-grooming, or the ability to clean the skin and feathers, and increase the frequency of feather pecking (CHAUVE 1998, KILPINEN et al. 2005). An increased prevalence of cannibalism and mortality is also observed. It is estimated that the mortality ratio ranges from 6 to 8% (CENCEK 2003, KILPINEN et al. 2005). There has been a case of a tenfold increase in death rates following severe infestation (COSOROABA 2001).

Intense infestation of *D. gallinae* on hens gives rise to chronic stress condition which activates the hypothalamic-pituitary-adrenal axis. As a result, hormones which suppress the hypothalamic-pituitary-gonadal axis responsible for egg laying are secreted. This results in reduced egg laying. PILARCZYK et al. (2004) reported an approx. 15–20% reduction in egg laying. In addition, depending on the severity of stress, especially in young chickens, infestation can cause small, tangible physiological and pathological changes that lower overall health status and immunity. In infested chickens reduction in the level of corticosterone, β- and γ- globulin (KOWALSKI and SOKÓŁ 2006, 2009) is reported. Heavy infestations may adversely affect the development of the immune response against pathogens in chickens, reduce post-vaccination antibody titre, or inhibit the production of antibodies (KOWALSKI and SOKÓŁ 2009, KAOUD 2010, SPARAGANO et al. 2014). An increase in the consumption of

food and water, with simultaneous loss of weight have been observed (CHAUVE 1998, MUL et al. 2009).

Dermanyssus gallinae attacks the hen once every 2–4 days, and feeds on approx. 0.2 mg of blood, which, with an estimated 25 000–50 000 mites per bird, leads to a loss of approx. 4 g of blood per day, i.e. approx. 3% of the total hen blood volume (VAN EMOUS 2005). *Dermanyssus gallinae* can thus contribute to the development of anaemia and, in extreme cases, to severe anaemia (KIRKWOOD 1967, COSOROABA 2001, WOJCIK et al. 2000, KILPINEN et al. 2005). In other studies, the haematological blood indices of infected chickens were not significantly different from hens free from invasion, and did not prove the mites to be the main factor causing anaemia (KOWALSKI and SOKÓŁ 2006).

Dermanyssus gallinae as a vector of pathogenic agents

Dermanyssus gallinae may contribute to increased epidemiological risks due to the probable transmission of many pathogens (VALIENTE-MORO et al. 2005, 2009, DE LUNA et al. 2008, SPARAGANO et al. 2009).

In 1944, Smith was the first to isolate the *Dermanyssus gallinae* St. Louis encephalitis virus (SLEV- Flaviviridae). From that moment investigations into the possibility of the transmission of various pathogens commenced. It has been shown that the mite is a reservoir and vector of pathogenic bacteria for chickens and other animals and humans: *Salmonella gallinarum*, *Listeria monocytogenes, Erysipelothrix rhusiopathiae, Chlamydia* spp., *Escherichia coli*, *Staphylococcus* spp., *Streptomyces* spp., as well as a virus: Avian paramyxovirus type1 (Newcastle disease). It was experimentally demonstrated that the mite can transmit other bacteria: *Pasteurella multocida*, *Coxiella burnetii*, Spirochetes, *Salmonella enteritidis,* and viruses: Fowl poxvirus smallpox, Eastern equine encephalitis virus – EEEV, Western equine encephalitis virus – WEEV, Venezuelan Equine Encephalitis Virus, Russian spring virus, and summer tick-borne encephalitis. (DE LUNA et al. 2008, VALIENTE-MORO et al. 2009, SPARAGANO et al. 2009).

Methods and agents proposed to control and combat invasion of *D. gallinae*

The basis for the control of *D. gallinae* is to follow the rules of hygiene in the poultry house, and prevention of the introduction of the parasite from the outside. Poultry houses should be carefully disinfected after each production cycle. Structural elements of the poultry house should be washed with warm water with the addition of oil-penetrating agents and egg-lethal additives.

Newly introduced flocks of hens and means of transport should be free of mites. Wild birds and rodents can be potential carriers of *D. gallinae,* especially in barn, free-range or another traditional breeding system (VAN EMOUS 2005). It is also important to regularly monitor *D. gallinae* populations. Different systems of traps placed on the structural elements of the poultry house can be used for this purpose (NORDENFORS and CHIRICO 2001, ZENNER et al. 2009). The structure of the population can be determined based on the number of individual developmental forms of the parasite (SOKÓŁ 2006). These proceedings make the following deacarization easier.

Synthetic acaricides are commonly used in cases of *D. gallinae* infestation. Thirty-five acaricidal compounds were tested and proved effective in cases of *D. gallinae,* and they included: organochlorines, organophosphates, pyrethrin, pyrethroids, carbamates, amitraz, and endectocides (CHAUVE 1998). They are effective, but their use is limited due to food safety, because their residues or metabolites may accumulate in the meat and eggs of hens (MUL et al. 2009). Most of these products are dedicated for empty poultry houses, but a few of them, which have received marketing authorisation, can be applied directly to the birds. It is important to abide by the grace period because incompliance poses a threat to the life and health of hens, as well as the consumers of the eggs and meat (MARANGI et al. 2012). In addition, long-term use of these agents, associated with the application of high concentrations, leads to the resistance of *D. gallinae* to these agents (CHAUVE 1998, NORDENFORS et al. 2001, CENCEK et al. 2011, ZDYBEL al. 2011). Products containing foxim (foxim-based) were authorised for marketing in 2010. A case of the development of resistance of *D. gallinae* to this substance was reported a year later (ZDYBEL et al. 2011). Effective acaricides should penetrate into the gaps, stay active on the exposed surfaces, act selectively on the parasite, and should induce resistance (CHAUVE 1998).

Other agents which are used as often as acaricides are products comprising natural or synthetic silica (silicon dioxide, SiO_2), which fall into the category of physical methods. Their acaricidal activity is based on the absorption properties of silica particles that adhere to the body shells of *D. gallinae* and absorb lipids from the exoskeleton. This leads to drying out, and results in the death of the parasite. The effectiveness of these measures depends on the quality of the applied chemicals and the relative humidity (RH). It has been shown that a high level of RH reduces the efficacy of the silica products. In poultry houses where RH is high the efficacy of these products can be reduced (MAURER and PERLER 2006, KILPINEN and STEENBERG 2009, ZDYBEL et al. 2011).

The thermal method of parasite elimination is also a physical method. This method consists in applying high temperatures in an empty hen house for several days. On the first day the temperature in the house rises gradually to at least 45°C due to the use of a heating device, and it is maintained at this level

constantly for another two days. Then it slowly decreases (VAN EMOUS 2005). This method is also very efficient, and eliminates most of the *D. gallinae* eggs, but does not allow for limitation of the invasion during the cycle. In addition, excessive heat can destroy the shed's structure and equipment.

The above-described methods of elimination and controlling the *D. gallinae* invasion are among the most efficient, but due to their limitations, researchers are still seeking better, more effective, cheaper and less toxic methods.

Alternatives to synthetic acaricides are substances of natural origin (HARRINGTON et al. 2011, SPARAGANO et al. 2014). SPARAGANO et al. (2014) describes these as new acaricides, which include biopesticides and plant-derived products. Biopesticide formulations are based on the natural properties of bacteria and their components. The insecticidal properties of thuringiensin *from Bacillus thuringiensis* bacteria have long been known and successfully used in agriculture to control pests of agricultural crops (VAN DER GEEST et al. 2000). The toxicity of these bacteria was confirmed with respect to ticks and *O. sylviarum* (MCKEEN 1988, HASSANAIN et al. 1997). Bacterial exotoxin was also shown to be toxic for vertebrates, and therefore it cannot be used against *D. gallinae* (SPARAGANO et al. 2014). In 2010, another natural acaricide based on spinosad, which is produced as a result of fermentation carried out by bacteria *Saccharopolyspra spinosa,* was approved for use. The brand name of the product is Elector (HOLT 2005, GEORGE et al. 2010). Spinosad has also been used in agriculture for the control of many species of pest insects. In contrast to thuringiensin, it has shown a low toxicity to mammals and birds, as well as for insects which play a positive role in the ecosystem (ANASTAS et al. 1999, HARINGTON et al. 2011).

Among the plant-derived substances garlic extract has been shown to be very effective. It is the basis of garlic preparations such as Barrier and Breck-a-Sol (BIRRENKOTT et al. 2000, FAGHIHZADEH et al. 2014). There is also a product based on an extract of the neem tree, which is available under the brand name MiteStop, and it has shown higher efficiency than foxim (LUNDH and CHIRICO 2005, ABDEL-GHAFFAR et al. 2009). Strong acaricidal properties have also been shown by geraniol, eugenol and citral (SPARAGANO et al. 2013).

Attempts to develop a vaccine have failed. A marginal protective effect was obtained by immunizing chickens with a vaccine based on the somatic antigens of mites and other arthropods – a recombinant protein called subolesin (Subolesin SUB) or the Bm86 protein. Ingestion of blood with the antibodies by mites was expected to hinder the development of the mites. *D. gallinae* mortality was 23% after immunization with Bm86, and 35% after subolesin immunization. However, at present no *D. gallinae* vaccines have been authorised for marketing (HARRINGTON et al. 2009).

Biological control of *D. gallinae* involves the use of their natural enemies, such as predatory mites and entomopathogenic fungi (SPARAGANO et al. 2014).

Predatory mites occur naturally in the nests of wild birds, and can also spontaneously colonize hen houses. This method of control relies on the introduction of selected species of mites into the environment infested by *D. gallinae* A few mite species, such as *Androlaelaps casalis, Stratiolaelaps scimitus, Hypoaspis aculeifer, Hypoaspis miles* (LESNA et al. 2009, 2012, ALI et al. 2012), have shown the potential to destroy *D. gallinae*. This method has been commercially applied but research is still needed to confirm its efficacy in the field, consequences of its long-term use, and the limiting impact of high temperature and alternative preys on its efficacy (SPARAGANO et al. 2014).

Fungi such as *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces album and Trichoderma fumosoroseus* also show a detrimental impact on *Dermanyssus gallinae.* Satisfactory results have been obtained after inoculation with a high dose of conidia under laboratory conditions, but experiments under semifield conditions have revealed unsatisfactory results (STEENBERG et al. 2006, TAVASSOLI et al. 2011, STEENBERG and KILPINEN 2015).

One of the methods to control *D. gallinae* infestation consists in intramuscular or intraperitoneal administration of ivermectin and moxidectin to birds. This method has been referred to as a systemic application, i.e. through the host body and the ingested blood. The effective dose was too toxic in birds. In addition, the operating time was too short. Control of mite infestation based on this method would be risky and unprofitable due to the necessity to apply a high dose of the active substance, time consumption, and repeated applications. (ZEMAN 1987, POPIÓŁ and OLIVER 1989).

High potential has been seen in the introduction of short-cycle intermittent light/dark periods in poultry houses. This method could reduce *Dermanyssus gallinae* invasion, probably by disrupting its normal nocturnal feeding cycle. The law, however, prohibits shortening the period of darkness below 8 hours, which in practice prevents the introduction of this type of control (STAFFORD et al 2006).

One idea is to develop the IPM (integrated pest management) system, incorporating the whole variety of the above *D. gallinae* elimination methods, which are described as using a combination of the methods and bringing tangible benefits (AXTELL 1999, CHATTERTON 2000).

In 2013, in accordance with an EU Council Directive (EU 1999.74/EC) on the welfare of laying hens, conventional cages were withdrawn from use and are now replaced by enriched cages incorporating more complex environments. It should be mentioned that enriched cages for laying hens entered under the supervision of the EU to poultry production farms have definitely improved the welfare of the birds; however, it appears to contribute to quite a significant growth of *D. gallinae* populations due to the extensive system of cracks and crevices which allow mite populations to grow more rapidly. One of the actions which should be jointly undertaken is the development of a cage design which

will allow the elimination of hiding places while facilitating washing the surface. The same applies to air ventilation systems. To summarize, the construction of poultry houses must be carefully thought out and designed by specialists from various fields.

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