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Estimation the effectiveness of probiotics as a factor influencing the results of fattening rabbits

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Abstract: *Estimation the effectiveness of probiotics as a factor influencing the results of fattening rabbits.* The aim of this study was to establish the influence of addition *Bacillus cereus* var. *toyoi*, as probiotics factor, on young rabbits fattening results. The level of 400 mg per 1 kg of probiotic preparation was used in experimental group (EG, $n = 34$) as factor affecting fattening results. The control group (CG, $n = 32$) was fed commercial feed. Experiment started at weaning (35 days) and finished at 84 days. The following data were collected: body weight at weaning; body weight every week up to 84 days of age; feed intake during fattening, health status fattened rabbits, dressing percentage. The positive impact of used preparation on health status and yield results of fattened fryers was observed.

Key words: rabbits fattening, probiotics factor, *Bacillus cereus* var. *toyoi*

INTRODUCTION

The digestive process is very complex and fragile in rabbits. Young rabbits are especially exposed to negative impacts of pathogen bacteria. Breeders need any factor, that can to prevent pathogen bacteria growth in digestive tract. Probiotics are the usual bacteria that all animals need for their digestive well-being. By probiotics using we can improve the growth and development of the normal, desirable microbial population in the gut, allowing them to maintain domination over the undesirable

organisms (Fuller 1989, Bielecka et al. 2002). There are studies showing positive effect of probiotics addition as a diet supplement in poultry and swine feeding (Barrow 1992, Jin et al. 1997, Jadamus et al. 2000, Jadamus et al. 2002). There are also many studies show positive effect of probiotics using on young rabbits productivity – growth results and health condition (Gippert et al. 1992, Maertens et al. 1994, Kamra et al. 1996, Tachikawa et al. 1998, Voros and Voros 1998, McNitt et al. 2000, Kustos et al. 2004, Kermauner and Struklec 2005, Brzozowski et al. 2007a, Brzozowski et al. 2007b, Combes et al. 2012).

The aim of this study was to establish the influence of addition *Bacillus cereus* var. *toyoi* as probiotics factor, on young rabbits fattening results.

MATERIAL AND METHODS

New Zealand White young rabbits were used in the study. There were two groups of fattening rabbits: control group (CG, $n = 32$ kits) and the experimental group (EG, $n = 34$ kits).

The rabbits from CG were fed by standard feed mixture produced by De Heus Koudijs Hima. EG was fed by the same feed with addition *Bacillus cereus* var. *toyoi*, as probiotics factor. The use

of *Bacillus cereus* var. *toyoi* is ideal because it has an advantage due to its good heat stability that is important because relatively high values of temperature occur during pelletizing of animals' feed. The vegetative form of *B. toyoi* spores quickly germinates in the intestinal tract. The rapid germination rate of *B. toyoi* has been demonstrated in piglets (Thelen and Pallauf 1996). 1 g of used preparation contains $1 \cdot 10^9$ cfu (colony forming units) *Bacillus cereus* var. *toyoi*. The level 400 mg of preparation per 1 kg feed was used in experimental group.

Experiment started at weaning (35 days) and finished at 84 days.

The following data were collected:

1. Body weight at weaning and than every week up to 84 days of age.
2. Surviving rate from weaning to the end of fattening.
3. Feed intake during fattening.
4. Dressing percentage.

The results were statistically evaluated by analysis of one-way variance using SPSS 14.0 PL for Windows (SPSS 2006).

RESULTS AND DISCUSSION

The body weight changes during fattening are presented in Table 1.

Animals, that were randomized to the experimental group (EG), were heavier than those in the control group (CG). The difference was statistically significant and was maintained during the first period of fattening (up to age of 56 days). The average weight of animals at the end of fattening proved to be similar in both groups. Weight gain during the fattening period were significantly higher in the control group. This results indicates, that the used preparation did not have positive impact to the rabbits growth and body gain.

The fattening results of rabbits are presented in Table 2.

On rabbit farms, it is estimated that the mortality of young weaned is about 10–12% (Bielański et al. 2002). In the experimental group this result was much more favorable (3%), which may result from the application of probiotics. It was also observed better health status of the

TABLE 1. Body weights and gain during fattening ($\bar{x} \pm \text{sd}$)

Rabbits age (days)	Average body weight (g)	
	Control group	Experimental group
35	771 ^a ±120	869.30 ^a ±165
42	1 016.32 ^A ±152	1 138.94 ^A ±180
49	1 218.84 ^a ±229	1 350.73 ^a ±256
56	1 395.68 ±283	1 468.06 ±268
63	1 629 ±278	1 680.76 ±232
70	1 874.64 ±277	1 891.09 ±247
77	2 119.40 ±290	2 125.55 ±259
84	2 387 ±310	2 342.55 ±266
Body gain from 35 to 84 days (g)	1 616 ^a ±256	1 473.25 ^a ±203

A – data in rows differ statistically at $p < 0.01$; a – data in rows differ statistically at $p < 0.05$.

TABLE 2. Health status of fattening rabbits in control and experimental groups

Items	Estimated groups	
	Control	Experimental
Number of weaned kits	32 (100%)	34 (100%)
Number of kits with diarrhea	9 (28.12%)	8 (23.53%)
Number of kits, which falls	4 (12.5%)	1 (2.94%)
Number of fattened kits	28 (87.5%)	33 (97.06%)

animals in the experimental group (less cases of diarrhea).

The results of feed intake during fattening are presented in Table 3.

TABLE 3. Feed intake

Group	Control	Experimental
Average feed intake during fattening (kg per 1 kg of gain)	3.66 ±0.32	3.73 ±0.27

There was no impact on the efficiency of the preparation of the feed conversion: in both groups the average feed consumption per 1 kg of gain did not differ statistically.

The group of 21 animals in the CG and 27 in the EG were slaughtered; for this group of animals were calculated average final mass and dissection's indicators (Table 4).

TABLE 4. Dissection results of fattening rabbits (average for groups, x ±sd)

Items	Estimated groups	
	Control (n = 21)	Experimental (n = 27)
Body weight before slaughter (g)	2 298 ±260	2 295 ±258
Weight of the head (g)	152 ±17	146 ±13
Weight of the skin (g)	356 ±53	351 ±58
Weight of the gastrointestinal tract (g)	338 ± 26	334 ±49
Weight of meat offal (g)	94 ±13	92 ±15
Weight of carcass (g)	1 243 ±159	1 258 ±160
Weight of cooled carcass (g)	1 137 ±161	1 157 ±155

Dressing percentage (yield) was calculated according to the formula:

$$\frac{\text{weight of cooled carcass}}{\text{body weight before slaughter}} \times 100 (\%)$$

The results are presented in Table 5.

TABLE 5. The results of average dressing percentage of fattening rabbits

Group	Control	Experimental
Average dressing (%)	51.81 ^a ±1.8	52.94 ^a ±24

a – data in rows differ statistically at p < 0.05.

There was observed a higher dressing percentage in experimental group at the same body weight in both groups: it shows the higher share of edible parts in the experimental group of rabbits. The difference in slaughter efficiency may also result in higher average body weight

at weaning of young rabbits in the experimental group.

CONCLUSIONS

The used preparation, containing *Bacillus cereus* var. *toyoi* as probiotics factor, improved:

- the health status rabbits from experimental group,
- the yield results fryers from experimental group.

The used preparation did not influenced to:

- final weight of fattened rabbits,
- feed intake.

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- Streszczenie:** Ocena efektywności probiotyku jako czynnika poprawiającego wyniki tuczu królików. Celem badań było określenie wpływu dodatku *Bacillus cereus* var. *toyoi*, jako czynnika probiotycznego, na wyniki tuczu młodych królików. W doświadczeniu zastosowano poziom 400 mg preparatu na 1 kg paszy w żywieniu królików z grupy doświadczalnej (EG, grupa liczyła 32 osobniki). Grupa kontrolna (CG, licząca 34 osobniki) była żywiona mieszanką pełnoporcjową bez dodatku preparatu. Doświadczenie rozpoczęto u królików w wieku 35 dni wraz z odsadzeniem i zakończono w wieku 84 dni. Zbierano następujące dane: masa ciała przy odsadzeniu; masa ciała co tydzień do wieku 84 dni; spożycie paszy podczas tuczu; stan zdrowotny młodych w czasie tuczu, wydajność rzeźna. Stwierdzono pozytywny wpływ stosowanego preparatu na stan zdrowotny i wydajność rzeźną.

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Comparison of the laying and egg weight of laying hens in two types of cages

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Abstract: *Comparison of the laying and egg weight of laying hens in two types of cages.* This study was aimed at comparing production results of laying hens kept in two types of cages: furnished cages and conventional cages. It covered the period from the 36th till the 54th week of hens life that was divided into two experimental stages: stage I – from week 36 till week 44, and stage II – from week 46 to week 54. Till week 44, 190 layers were reared in groups (10 hens each) in furnished cages (F) and 190 layers were kept individually in conventional cages (C). In week 45, the hens from furnished cages (F) were moved to conventional cages (FC), whereas these from conventional cages (C) were randomly merged into groups of 10 hens and transferred to furnished cages (CF). Egg laying (%) and egg weight (g) were controlled as well as percentage contribution of eggs in standard egg weight classes was determined in both experimental stages. The study showed a significant ($P \leq 0.01$) effect of cage type on the laying performance of the hens but only in the second stage of the study, as well as a significant ($P \leq 0.01$) effect of hens moving to different cages. In both cases, higher egg laying was reported for the hens from the conventional cages. Egg weight in the first and the second stage of the experiment was significantly ($P \leq 0.01$) higher in the groups housed in the furnished cage. A higher egg weight ($P \leq 0.01$) was determined in the layers in the second stage of the study. Both in the first ($P \leq 0.01$) and in the second ($P \leq 0.05$) stage, analyses showed a significant effect of cage type on the contribution (%) of eggs in particular egg weight classes. A higher percentage of eggs in the L class was obtained from the hens housed in the furnished cages. Differences in laying perfor-

mance after hens moving suggest that the layers adapt more easily and faster to conditions of the C type cages. The egg weight was, probably, more dependent on general laying performance and age of the hens than on cage type.

Key words: laying hens, types of cage, laying performance, egg weight

INTRODUCTION

Rearing conditions of laying hens are recently arising much controversies, which is due to the implementation of EC Directive 1999/74/EC that stipulates rearing standards for layers in the EU Member States (European Commission 1999). This Directive obliges egg producers to replace traditional battery cages by new furnished cages with an increased area (750 cm² per 1 hen) and possessing additional equipment. With no explicit evidence that hens rearing in furnished cages contributes to their improved welfare (Barnett et al. 2009, Tactacan et al. 2009, Lay et al. 2011), producers had to incur vast expenses to modernize their hen houses according to the Directive and in some cases were forced to eliminate their flocks. Too little time has gone since the final deadline of cages replacement (1.01.2012) to

conclude on any consequences of these changes to production farms that had adjusted their production standards to Directive guidelines as well as to the laying hens themselves that were the focus of interest in this fight for “rearing conditions improvement” between producers and animal rights defenders. Problems in reconciling these two sides may result from difficulties in the unequivocal determination of animal welfare (Rodenburg et al. 2008). In the case of laying hens, the level of welfare may be determined based on observations of their behavior (Appleby and Hughes 1991), changes in their plumage (Sherwin et al. 2010), their ability to absorb calcium from feedstuff and its further use in the calcification process (Nasr et al. 2012) as well as the incidence of cannibalism symptoms (Gunnarsson et al. 1999). Also production performance may be indicative of the birds adaptation to rearing conditions. Simultaneously, this performance is of the key significance to producers as it determines poultry production profitability (Sosnowka-Czajka et al. 2010). Another important information may as well be

provided by observations of birds ability to adapt to altered rearing conditions.

The aim of this study was to compare two production parameters: laying performance and egg weight, of laying hens reared in conventional cages and furnished cage, as well as to compare the impact of a rapid change in rearing conditions on these two parameters.

MATERIAL AND METHODS

The experiment was conducted with two types of three-store cages. Furnished cages (F), with area for 10 hens, equipped according to guidelines of Directive 1999/74/EC (European Commission 1999) – Figure 1, and individual cages, i.e. conventional cages (C), adjusted for individual housing of hens, with area of 1,196 cm², height of 44 cm, and equipped only in one nipple drinker and 26 cm long feeders (Fig. 2).

The study included 380 ISA Brown hens: 190 layers kept in groups in furnished cages and 190 layers kept individually in conventional cages. The housing



FIGURE 1. Furnished cages for laying ISA Brown hens at the RZD Wilanów-Obory experimental farm, SGGW (photo J. Riedel)



FIGURE 2. Conventional individual cages for laying ISA Brown hens at the RZD Wilanów-Obory experimental farm, SGGW (photo J. Riedel)

conditions (light program, temperature and air humidity) were consistent with the ISA Brown Management Guide (www.hendrix-genetics.com 2008). All birds were receiving the same powdered feed mixture in the quantity of 114 g per hen daily. The nutritive value of the feed mixture was provided in Table 1.

TABLE 1. Nutritive value of basal diet applied in ISA Brown laying hens

Nutritive value	Unit	Content in diet
EMN	kcal	2 750.00
EMN	MJ	11.60
Total protein	%	17.00
Crude fiber	%	4.40
Crude fat	%	3.90
Ash	%	12.10

In week 45 of hens life, their housing conditions were changed as follows: the laying hens from furnished cages (F) were moved to conventional cages (FC) and housed individually, and the laying hens from conventional cages (C) were randomly merged into groups of 10 birds and transferred to furnished cages (CF).

The other housing conditions and feed mixture remained unchanged.

Observations were continued in two stages (9 weeks each). The first stage (from week 36 to week 44) covered the period when the hens were staying in the same cage as at the beginning of production, whereas the second stage (from week 46 to week 54) covered the period since hens transfer to different cages till the end of experiment. The week when the cages were changed (45th week) was not included into any of the stages in order to eliminate the impact of direct stress induced by hens transfer.

Since week 44 to week 54 of hens life, laying performance and egg weight were controlled in hens from both types of cages. The percentage of egg laying was calculated on an everyday and weekly basis. Eggs were weighed 3 times a week, next day after laying. Data achieved enabled calculating: the mean laying performance (%), the mean egg weight (g), and the percentage contribution of eggs in particular egg weight classes (F, C, FC, CF).

The statistical analysis of results was carried out using the statistical package SPSS 21.0 (SPSS 2010). Normality of parameters distribution was verified with the Kolmogorow-Smirnow test (of all parameters examined only egg laying had normal distribution). The effect of cage type, hens age and the effect of cage change on laying performance was examined with one-way analysis of variance. Differences in egg laying between groups in particular weeks were determined with the T-test. The impact of cage type on egg weight was estimated with the Mann-Whitney test, and the impact of cage change – with the Kruskal-Wallis test. The contribution of eggs in particular egg weight classes was compared with the Chi-square test. The differences were considered significant at $P \leq 0.01$ and $P \leq 0.05$. The variability of the investigated traits was expressed by the standard error of the mean (\pm SE).

RESULTS AND DISCUSSION

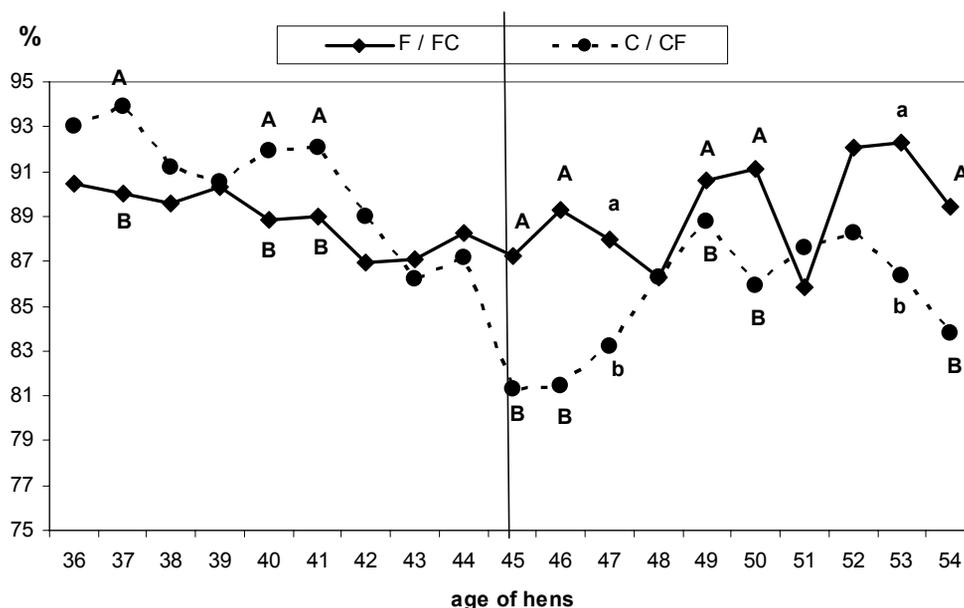
Laying performance

The laying performance of ISA Brown hens kept in both types of cages before the change of the housing system (36–44 week of life) was at a similar level of ca. 89% (Table 2) and slightly lower than the standard values of 91–94% (www.hendrix-genetics.com 2008). A significantly higher ($P \leq 0.01$) laying performance in group C was determined only in week 2, 5 and 6 of observations (Fig. 3), which was however insignificant to the total result from this period. The higher laying performance of hens kept in conventional cages compared to furnished cages was confirmed by Glatz and Barnett (1996), whereas Appleby et al. (2002) as well as Guesdon and Faure (2004) achieved a similar laying percentage in both types of cages. Egg laying was found to depend to the greatest extent on the rapid transfer to a different type of cage. In both groups analyses demonstrated a decrease in laying performance between week 44 and 46 of hens life: by 8.0% in hens moved from group C to group CF, and by 1.0%

TABLE 2. Least squares means (LSM) and SE of the ISA Brown laying hens production (%) depending on cage type

Hens age (weeks)	Laying production (%)				Effect of cage type
	Conventional cage (C)		Furnished cage (F)		
36–44	LSM	\pm SE	LSM	\pm SE	NS
	89.5	0.4	88.9	0.3	
	Furnished cage (CF)		Conventional cage (FC)		
46–54	LSM	\pm SE	LSM	\pm SE	**
	85.7	0.4	90.4	0.5	
	**		**		
Effect of cage type conversion	**		**		×

**difference significant at $P \leq 0.01$.



a, b – difference significant at $P \leq 0.05$; A, B – difference significant at $P \leq 0.01$.
 FIGURE 3. Weekly laying production (%) of ISA Brown hens during experimental period. F/FC – hens kept in furnished cage and transferred to conventional ones; C/CF – hens kept in furnished conventional cage and transferred to furnished ones

in hens moved from group F to group FC (Fig. 3). The statistical analysis of the entire period after cage shifting demonstrated significantly higher ($P \leq 0.01$) laying performance in FC group compared to CF group. Also in the successive weeks of the experiment the higher laying performance was observed in the hens from conventional cages – except for the 4th and 7th week after the change of cages (Fig. 1).

The laying performance of hens moved from group C to group CF decreased significantly ($P \leq 0.01$) reaching the maximum value of barely 88.8% in week 50 of hens life and the minimal value of 81.3% in week 46 of hens life (Fig. 3). Undoubtedly the significant difference in the laying performance of hens after movement to a different type of

cage was due to the number of birds having both direct and visual contact. Hens kept individually in groups C and FC had only visual contact with two neighboring hens, whereas layers kept in group cages (F and FC) had a direct contact with 9 hens housed in the same cage and visual contact with 20 hens from neighboring cages. It may be speculated that the rapid decrease in laying performance directly after hens transfer from C to CF resulted from a hierarchy being established in a group of hens that have so far been kept individually (Fig. 3). Already after 4 weeks the performance returned to the level determined before cage change. The mean egg production in this period was lower by 3.8% ($P \leq 0.01$) compared to the first stage of the study (Table 2). In contrast, hens moved to cages with

a significantly lesser area adapted to new housing conditions as early as after a week. It may be speculated that the diminished egg production was caused rather by hens taking out of the cages and their transfer than by the change of rearing conditions. As reported by Lay et al. (2011), the DEFRE (Department for Environment, Food and Rural Affairs 2006) study shows that the method of birds catching and taking out of cages affects their blood level of corticosterone. In addition, in the successive weeks of the experiment analyses showed in this group higher laying performance compared to the period when these birds were housed in the group furnished cages. The laying performance of hens moved from group F to group FC was higher after cage shifting by 1.5% ($P \leq 0.01$; Table 2), reaching the maximum value of 92.3% in week 52 (Fig. 3). It suggests that the direct contact with other hens may be a more stressful factor than the reduction of living space. Investigations by Douglis (1948) dem-

onstrate that hens are capable of recognizing up to 27 other hens and treating them as members of their flock. A higher number of hens in a group induces stress and predisposes to continuous fights for dominance. It seems, therefore, that in our experiment the number of hens and behavioral interactions between them had a greater impact on changes in their laying performance than the size and equipment of cages they were kept in.

Egg weight and egg weight classes

Egg weight in the first and second stage of the experiment was significantly higher ($P \leq 0.01$) in the groups kept in furnished cages (by 0.3 g and 0.4 g, respectively, Table 3). The egg weight was also found to be significantly ($P \leq 0.01$) affected by the change of cage type. In both variants of the change, higher egg weight was determined in hens from the second stage of the study (Table 3). When comparing laying performance of the investigated hens (Fig. 3) and

TABLE 3. Least squares means (LSM) and SE of the ISA Brown hens' eggs weight (g) depending on cage type

Hens age (weeks)	Eggs weight (g)				Effect of cage type
	Conventional cage (C) (<i>n</i> = 4 738)		Furnished cage (F) (<i>n</i> = 4 608)		
36–44	LSM	±SE	LSM	±SE	**
	61.0	0.07	61.3	0.07	
	Furnished cage (CF) (<i>n</i> = 4 109)		Conventional cage (FC) (<i>n</i> = 4 669)		
46–54	LSM	±SE	LSM	±SE	**
	62.5	0.08	62.1	0.07	
	**		**		
Effect of cage type conversion	**		**		
Effect of hens age	** (<i>F</i> = 37.86)		** (<i>F</i> = 15.76)		

**difference significant at $P \leq 0.01$; *F* – Fischer test.

egg weight it may be concluded that the higher level of laying performance corresponded to lower weight of eggs, and that lower egg production corresponded to higher egg weight. A similar tendency was observed in the contribution of eggs in particular weight classes (Table 4). Both in the first ($P \leq 0.01$) and in the second ($P \leq 0.05$) stage of the experiment a significant effect of cage type was

and a lower number of small eggs (S, M) were determined after cage change.

The tendency for a lesser egg weight along with a higher egg production has been known for years. Even the study of Jully (1930) demonstrates that hens laying eggs with the weight higher than the average for the flock were characterized by lower laying performance than the hens laying lighter eggs than the average

TABLE 4. Share (%) of ISA Brown hens' eggs in different weight classes (S, M, L, XL)

Hens age (weeks)	Share of eggs in egg weight classes								Effect of cage type
36-44	Conventional cage (C) (n = 4 738)				Furnished cage (F) (n = 4 608)				**
	S	M	L	XL	S	M	L	XL	
	3.4%	64.5%	31.0%	1.1%	2.0%	63.3%	33.6%	1.1%	
46-54	Furnished cage (CF) (n = 4 109)				Conventional cage (FC) (n = 4 669)				*
	S	M	L	XL	S	M	L	XL	
	1.6%	59.7%	37.1%	1.6%	1.5%	63.3%	34.0%	1.2%	
Effect of cage type conversion	**				NS				×

*difference significant at $P \leq 0.05$; **difference significant at $P \leq 0.01$; NS – difference not significant.

Egg weight classes: S (48–53 g); M (54–63 g); L (64–73 g); XL (>74 g).

demonstrated on this parameter. Better results were achieved in the case of hens kept in furnished cages: in the first stage (F) they were characterized by a higher number of large eggs (L) and a fewer number of small eggs (S), whereas in the second stage (CF) – also by a higher number of large eggs (L) but also of extra large eggs (XL). In turn, the effect of cage change turned out to be significant only in groups C–CF ($P \leq 0.01$), where a higher number of large eggs (L, XL)

for the flock. It is difficult to conclude explicitly whether the decline of laying performance induced by stress after hens transfer to a different type of cage was due to disorders in the ovulation process or to simply elongation of egg formation period, which normally spans for ca. 24 h (Hiramoto et al. 1990). The longer period of egg formation is usually linked with its longer retention in the shell gland and formation of a thicker shell that may affect its weight (Berg 1945).

The second factor that could contribute to differences in egg weight in the first and second stage of the study was the age of laying hens (Table 3). Baumgartner et al. (2007) and Zita et al. (2009) demonstrated a significant impact of layers age on the weight of laid eggs. According to the ISA Brown Management Guide, the mean egg weight in weeks 36–44 of hen life reaches 64.6 g, whereas in weeks 46–54 it accounts for 65.1 g (www.hendrix-genetics.com 2008). Though the weight of eggs determined in our experiment was lower than the standard values, the differences in egg weight between the subsequent stages of the study could therefore result from various age of the laying hens.

CONCLUSIONS

In summary, the type of cage the laying hens were kept in (conventional cages and furnished cages) had no significant effect on their laying performance in the first stage of the experiment. Differences in egg production after birds transfer suggest that the layers were more easily and faster adapting to conditions of the conventional cages, despite a smaller living space. The weight of laid eggs was, probably, more dependent on the laying performance and age of hens than on cage type. No sound evidence was achieved from results of the analysis of two experimental factors (laying performance and egg weight) to declare the furnished cages as better from the viewpoint of hens laying performance and thus cost-effectiveness of egg production.

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Streszczenie: Porównanie nieśności i masy jaj kur nieśnych utrzymywanych w dwóch typach klatek. W badaniach porównywano wyniki produkcyjne kur niosek utrzymywanych w dwóch typach klatek: klatkach wzbogaconych i klatkach konwencjonalnych. Badaniem objęto okres od 36. do 54. tygodnia życia kur podzielony na dwa etapy: I – od 36. do 44. tygodnia, II – od 46. do 54. tygodnia. Do 44. tygodnia 190 niosek utrzymywano grupowo (po 10 kur) w klatkach wzbogaconych (F) i 190 niosek utrzymywano pojedynczo w klatkach konwencjonalnych (C). W 45. tygodniu noski z klatek wzbogaconych (F) przeniesiono do klatek konwencjonalnych (FC), a ptaki z klatek konwencjonalnych (C) połączono losowo po 10 i wprowadzono do klatek wzbogaconych (CF). W obu etapach badań kontrolowano nieśność (%) i masę jaj (g), określono też procentowy udział jaj w standardowych klasach wagowych. Wykazano istotny wpływ ($P \leq 0,01$) typu klatki na nieśność kur, ale tylko w drugim etapie badań, oraz istotny wpływ ($P \leq 0,01$) przeniesienia kur. W obu przypadkach większą nieśność wykazywały kury w klatkach konwencjonalnych. Masa

jaj w pierwszym i drugim etapie doświadczenia była istotnie większa ($P \leq 0,01$) w grupach utrzymywanych w klatkach wzbogaconych. Większą masę jaja ($P \leq 0,01$) stwierdzono u kur w drugim etapie badań. Zarówno w pierwszym ($P \leq 0,01$), jak i w drugim ($P \leq 0,05$) etapie badań wykazano istotny wpływ typu klatek na udział (%) jaj w poszczególnych klasach wagowych. Więcej jaj w klasie L uzyskano u kur utrzymywanych w klatkach wzbogaconych. Różnice w nieśności kur po przeniesieniu sugerują, że nioski o wiele łatwiej i szybciej adaptują się do warunków klatek C. Masa uzyskanych jaj była bardziej zależna od poziomu nieśności i wieku kur niż rodzaju klatki.

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The effect of dietary fat source on feed digestibility in chinchillas (*Chinchilla lanigera*)

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Abstract: *The effect of dietary fat source on feed digestibility in chinchillas (Chinchilla lanigera).* The objective of this study was to determine the effects of the inclusion of the vegetal and animal fat to the diet on the apparent digestibility in chinchillas. 18 young chinchillas were assigned to three groups and fed control diet or with the addition of either linseed (VF) or lard (AF). The apparent digestibility coefficient (ADC) was calculated for dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), nitrogen free extract (NFE) and ether extract (EE). The results showed that there was no significant effects of fat addition on most of the studied constituents except for increased digestibility of EE.

Key words: chinchilla, digestion, fat, palatability

INTRODUCTION

Among companion mammals, small herbivores constitute a substantial majority. One of them, chinchilla (*Chinchilla lanigera*), originating from South America, can be regarded popular, yet scarcely described in scientific literature. The basic specifics of its nutrition were reported by Wolf et al. (2008), who suggested that the crude fibre level in the chinchilla diet should not exceed 15%. Alike in guinea-pigs, crude fibre is digested more efficiently by chinchillas than by rabbits or rats (Sakaguchi 2003).

The ability to utilize fibrous feed in chinchillas is attributed to their volumi-

nous colon and caecum as it was showed also in other rodents (Langer 2002, Pérez 2011). The colonic separation mechanism, leading to the accumulation of microorganisms in caecum, results in the formation of re-ingested caecotrophes (Holténus and Björnhag 1985), rich in microbial derived protein that contribute to overall nutritional balance (van Zyl and Delpont 2010).

Fat supplementation in animal diets is usually performed either to increase the energy value of the feed or to improve the nutritional quality of products derived from animals (Doreau and Chilliard 1997). Interesting aspects of rabbit dietary fat supplementation were discussed by Casado et al. (2012), who claimed that (first) it does not decrease the fibre content, resulting in a reduction of production costs and (second) improves feed palatability. The latter is a matter of great concern for chinchilla housing in captivity due to their rather refined preferences (unpublished observations).

Little is known about the digestion of supranutritional doses of fat in companion rodents on the contrary to rats, probably the most common laboratory species (Wang et al. 2011). In the case of chinchillas and guinea-pigs, despite major resemblances, the considerable

differences were reported in their metabolic patterns (Holtenius and Björnhag 1985). Moreover, the nutrients digestion in guinea-pigs, as compared to rabbits, also showed substantial differences (Franz et al. 2011). Therefore, regarding the above we found justifiable to study the possible effects of dietary addition of animal and vegetal fats on the feed consumption and digestion in chinchillas.

MATERIALS AND METHODS

Animal, diets and management

A total of 18 young chinchillas (± 74 days old) were assigned to three groups ($n = 6$) and placed in metabolic cages for 2 weeks. The environmental conditions were as follows: temperature 18–19°C and humidity 30–35%. Chinchillas in control group (C) were fed commercial pelleted chinchilla feed (Table 1), and with the 3% (of feed DM) addition of either vegetable (linseed oil – VF group) or animal fats (lard – AF group). Deliberately measured amounts of pellets

(35 g per 1 chinchilla) were thoroughly mixed with the appropriate amount of linseed oil. Similar procedure was performed for lard, but before mixing it was slightly heated in the water bath (Table 2). Drinking water was constantly available.

TABLE 2. The chemical composition of diets offered to control and experimental animals (%)

Item	C	VF	AF
DM	88.58	89.52	89.96
OM	81.50	82.63	82.92
CP	19.35	18.90	18.77
EE	3.58	6.61	6.22
CF	8.73	8.13	8.16
NFE	49.84	48.99	49.77

C – control group; VF – vegetable fat group; AF – animal fat group; DM – dry matter; OM – organic matter; CP – crude protein; EE – ether extract; CF – crude fibre; NFE – nitrogen-free extract.

Sample collection

After 7 days of adaptation period, feces and urine were collected daily for next week. The feed and water intake were also recorded.

Sample analytical determinations

Content analyses in collected material were performed by Weenden's method, a conventional laboratory procedure.

The apparent digestibility coefficients (ADC) of nutrients were calculated as:

$$\text{ADC} = \frac{\text{intake} - \text{excretion (g)}}{\text{intake (g)}} \times 100 (\%)$$

Statistical analysis

One-way ANOVA analysis of variance was performed using Statistica 9 software (StatSoft Poland, Cracov). Dif-

TABLE 1. Composition of the basal diet

Ingredients	Content (per 1 kg of feed)	
Protein	175	g
Fibre	120	g
Fat	40	g
Ash	65	g
Lysine	12	g
Methionine + Cysteine	10	g
Metabolizable energy	9.8	MJ
Ca	8.5	g
P	6.5	g
Na	2	g
Vitamin A	12 000	i.u.
Vitamin D ₃	1 200	i.u.
Vitamin E	72	mg
Cu	10	mg

ferences were considered significant at $p < 0.05$.

Data are presented in tables as means \pm standard deviation.

RESULTS AND DISCUSSION

The addition of fat had an effect of the feed intake in chinchillas (Table 3). Compared to control, animals offered diet supplemented with linseed oil consumed lower amounts of feed and animal fat supplementation caused higher intake in chinchillas.

Interestingly, inverse effects were noted for water intake. Animals receiving pellets with linseed oil drank significantly more water than those, who consumed lard enriched diet. However it should be noted, that the water intake in VF group was substantially higher also than that in control group, which possibly indicates that the dietary addition of linseed oil had stronger effect on animals than that of lard.

Wolf et al. (2003) reported higher amounts of feed and water intake for chinchillas fed complete (pelleted) diet, but it has to be noted that the feed composition in our study was apparently different. Thus, neither the feed's quality nor palatability effects on the intake cannot be dismissed. In captive chinchillas nutrition, the quality of the complete dry feed is essential. Poor quality of pelleted feed often implicates digestive disorders and low palatability may cause starvation in chinchillas (unpublished observations).

Early studies on rats showed that the preference for diets with high level of beef tallow (34%) was similar to that for

a diet with quite low content of saturated animal fat (5%) and significantly lower than that for a corn oil (Mullen and Martin 1990). These results were attributed to the chemical composition of fats added, suggesting differential alterations in membrane composition and cellular function possibly occurring in central nervous system (e.g. brain).

One plausible explanation of the reversed relation between feed and water consumption in both experimental groups can be of a behavioral type. Possibly, increased water drinking compensated the decrease in feed ingestion as it was proposed by Wolf et al. (2008). Considering numerous reports on strong preference for diets supplemented with saturated/solid fats in rats, the claim that the addition of linseed oil decreased the palatability of feed in chinchillas is justifiable (Mullen and Martin 1990, Wang et al. 2011).

The addition of linseed oil significantly increased water intake in chinchillas, compared to C and AF groups. However, our results are more similar to those recorded for animals fed diet mixed of native components (Wolf et al. 2003). Interestingly, chinchillas fed hay-only and complete feed diets showed substantially higher water intake. The most striking difference was an enormously high water consumption reported for chinchillas fed fresh grass. To our knowledge it is extremely unusual to offer fresh forage (grass) for captive chinchillas. The extensive feeding of greens and fresh fruits to chinchillas was reported a cause of bloat, serious digestive system disease (Richardson 2003). Therefore there is a need for a gradual and sparingly serving of fresh feedstuffs in chinchillas.

The rearing conditions like temperature and humidity are crucial not only for the well-being but also for the reproduction of chinchillas (Richardson 2003, Busso et al. 2012). Since such data are missing in Wolf et al. (2003) paper, the conception that above mentioned discrepancies occurred due to the effect of environmental factors cannot be dismissed.

There were no significant differences in the amount of excreted feces observed in our study but the volume of urea in VF group was significantly higher than that in control, which likely reflects the increased intake of water (Table 3).

The DM digestibility coefficients of all diets were similar (Table 4). Our results confirmed that feeds with high DM content are relatively well digested by chinchillas. Compared to other small rodent species, only guinea-pigs show similarly high rate of DM digestion as

chinchillas – 70.9 vs. 71.13 respectively (Sakaguchi et al. 1987). However, higher ADC values were recently reported for greater cane rat, related to chinchillas as well as to guinea-pigs (van Zyl and Delpont 2010). It was suggested that the coprophagy significantly contributed to cane rats ability to utilize high fibrous food. Although in our study we did not measure coprophagy, it's impact on ADC in chinchillas cannot be dismissed, regardless of dietary fat level or source.

In the present study we did not observe significant differences in OM digestibility between groups. Overall value of ADC for OM in chinchillas resembled that, reported for rabbits fed diets with the intermediate level of fibre (Gidenne et al. 2000). Interestingly, Sakaguchi et al. (1987) described lower OM digestibility in guinea-pigs, rabbits, rats and hamsters fed diet containing different levels of CP and CF than those used in

TABLE 3. Daily feed and water consumption with feces and urine excretion (g)

Item	C		VF		AF	
Feed intake	22.95	±1.95 ^a	21.35	±1.99 ^b	23.04	±3.65
Feces excretion	11.30	±3.70	10.81	±2.31	11.71	±2.86
Water intake	21.67	±4.50 ^a	26.90	±8.44 ^b	21.50	±6.62
Urea excretion	12.33	±4.48 ^a	17.57	±6.56 ^b	13.31	±5.51

a-b – difference significant at $p < 0.05$.

C – control group; VF – vegetable fat group; AF – animal fat group.

TABLE 4. ADC of nutrients (%)

Item	C		VF		AF	
DM	71.13	±4.87	71.02	±2.86	71.20	±2.65
OM	73.79	±4.60	74.01	±2.54	73.91	±2.38
CP	71.18	±9.87	70.85	±7.22	69.49	±6.59
CF	37.55	±9.58	32.87	±6.14	33.56	±5.80
NFE	80.38	±2.70	79.68	±1.45	80.08	±1.77
EE	84.51	±5.19 ^a	91.66	±1.96 ^b	90.80	±1.55 ^b

a-b – difference significant at $p < 0.05$.

C – control group; VF – vegetable fat group; AF – animal fat group; DM – dry matter; OM – organic matter; CP – crude protein; CF – crude fibre; NFE – nitrogen-free extract; EE – ether extract.

our study (196 and 126 vs. 175 and 120 g per 1 kg of CP/CF, respectively). On the other hand, in guinea-pigs and rabbits fed hay only diet – 72 g per 1 kg of CP (Franz et al. 2011), the digestibility of OM was even lower than observed in chinchillas. Therefore it seems reasonable to elucidate the differences in OM digestion in chinchillas with the effect of the dietary CP level, a dependency confirmed by Rogier (1971).

The dietary addition of fat did not alter the CP digestibility. Interestingly, the ADC for protein previously reported for other rodent species (rat, hamster) was substantially higher (Sakaguchi et al. 1987). However, our results are in accordance with those reported by Rogier (1971), suggesting that typical CP digestibility in chinchillas, regardless of coprophagy level, is about 70%.

The digestibility of CF was lower in experimental groups as compared to control. This effect can be most likely attributed to the detrimental effect of high fat diet on the number of cellulolytic bacteria, previously reported for ruminants as well as for non-ruminants (Doreau and Chilliard 1997).

Differences in NFE digestion, observed in our study, were negligible. The apparent digestion of NFE in chinchillas was similar to that, recorded for guinea-pigs and other non-ruminant species (horses, ponies and rabbits) fed alfalfa-grain diet in digestibility comparison trial (Slade and Hintz 1969).

The significant effect of dietary fat addition on digestibility was found in EE. Chinchillas in both experimental groups digested EE more effectively than those in control. Noticeably, the animals in VF and AF groups revealed similar EE di-

gestive efficiency, regardless of the type of added fat (Table 4). It seems likely that the heating of lard just before adding it to the feed, made it more accessible to the digestive enzymes, therefore improving its absorption (Wang et al. 2011).

CONCLUSIONS

The study showed that the addition of fat to chinchilla diet had a moderate effect on the apparent digestion of most of the constituents. Taken together with the feed intake results it may be suggested, that the differences in chemical composition of dietary fats may contribute to their effects on diet preference and consequently have an influence on the intake of protein and carbohydrates in chinchillas.

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Streszczenie: *Wpływ dodatku tłuszczu na strawność paszy u szynszyli.* Celem badań było określenie wpływu dodatku tłuszczu roślinnego i zwierzęcego na współczynnik strawności pozornej u szynszyli. 18 młodych osobników przyporzędkowano do trzech grup żywieniowych, które otrzymywały paszę podstawową (grupa kontrolna) bądź wzbogaconą o dodatek oleju lnianego (VF) lub łoju (AF). Poziom strawności pozornej (ADC) oznaczono dla suchej masy (DM), materii organicznej (OM), białka surowego (CP), włókna surowego (CF), związków bezazotowych wyciągowych (NFE) oraz ekstraktu eterowego (EE). Wykazano brak istotnych różnic w wynikach oceny strawności pomiędzy grupami, z wyjątkiem EE.

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Changes in glioblastoma multiforme ultrastructure after diamond nanoparticles treatment. Experimental model *in ovo*

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Abstract: *Changes in glioblastoma multiforme ultrastructure after diamond nanoparticles treatment. Experimental model in ovo.* Glioblastoma multiforme (GBM) is the most common primary malignancy in the brain and confers a uniformly poor prognosis. Despite decades of research on the topic, limited progress has been made to improve the poor survival associated with this disease, new therapeutic strategies are still needed. The application of nanotechnology to disease treatment, diagnosis, monitoring, drug delivery platform and to the control of biological systems is promising, also in cancer therapy. Diamond nanoparticles (DN) are bioactive substance toward glioma tumour cultured on the chicken embryo chorioallantoic membrane (CAM). DN reduce tumor mass and volume and inhibited new blood vessel development in glioma tumor. In the present experiment we additionally observed, that DN caused changes in the tumor ultrastructure testify to the ongoing process of cell death, probably carried out by autophagy.

Key words: autophagy, diamond nanoparticles, glioma, *in ovo* culture, transmission electron microscope

INTRODUCTION

Malignant glioma cells like glioblastoma multiforme (GBM) have rapid growth rate and an aggressive nature. This cancer is the one of the most common brain tumors and is known for the relatively high morbidity. The short time prognosis for patients with GBM is associated with intratumoral heterogeneity on the genomic and cytopathologic level and

general lack of the successful therapy. Therefore, it is important to identify potential drugs and explore more efficient therapeutic strategies for the treatment of malignant gliomas (Sathornsumetee and Rich 2008, Jain 2013). Animal models for cancer experiments, such as chick embryo chorioallantoic membrane, are successful and powerful tools to investigate therapeutic aspects of glioma (angiogenesis, metastasis) that cannot be studied in 2D cell culture systems (Strojnik et al. 2010).

Increasing usage of nanomaterials (also nanoparticles) in the past decades, for various biological and medical applications (such as imaging, diagnosis, therapy and drug delivery), shows that nanotechnology is the important tool for developing modern life sciences (Roco 2003). The unique potential of nanoparticles is due to their size in nanoscale and physicochemical properties that allow to overcome the limitation of using the bulk materials of the same composition as a traditional therapeutic and diagnostic agents (Zhang et al. 2008). Among various nanoparticles, diamond nanoparticles (DN) are the one with promising prospects in applications that require optical transparency, chemical inertness, hardness and low cytotoxicity toward living organism. Moreover, DN are the most bioactive nanoparticles among all

the allotrope forms of carbon, including C60, fullerenes, carbon black, and single and multi-walled carbon nanotubes (Huang et al. 2007, Liu et al. 2007) also towards gliomas. Diamond nanoparticles reduced tumor mass and volume and inhibited new blood vessel development in GBM tumors cultured *in ovo*. It was observed that DN significantly decreased expression of angiogenic factors: FGF-2 and VEGF (Grodzik et al. 2011). The effect of diamond nanoparticles on morphology of glioblastoma multiforme cells in tumor is still unclear. The objective of the investigation was to evaluate changes in glioma tumor cells ultrastructure after DN treatment being the effect of metabolic changes in the cell.

MATERIALS AND METHODS

Diamond nanoparticles

Diamond nanoparticles (DN) were obtained from SkySpring Nanomaterials (Houston, TX, USA). In the experiment, concentration 500 µg per 1 ml was used. Physicochemical characteristics of DN were performed. The shape and the size

of nanoparticles were determined by JEM-2000EX transmission electron microscope (TEM) at 200 kV (JEOL Ltd., Tokyo, Japan). Figure 1 shows the image of the diamond nanoparticles from TEM. The nanoparticles were 4–5 nm size and rounded shape. Zeta potential of nano-diamond hydrocolloid was examined with Zetasizer Nano-ZS90 (Malvern Instruments Ltd., Malvern, UK) and was measured as -39 mV. To prevent particles aggregation, sonification was performed before every application.

Cells and chicken embryos

U87MG glioblastoma multiforme (GBM) cells (HTB-14; American Type Culture Collection, Manassas, VA) were maintained in Dulbecco's modified Eagle medium (Sigma-Aldrich Corporation, St Louis, MO) with addition of 10% fetal bovine serum (Sigma-Aldrich) and 1% Antibiotic Antimycotic Solution (Sigma-Aldrich).

The fertilized eggs (*Gallus gallus*) were supplied by a commercial, local hatchery (Marylka, Poland). The strains used depending on availability, included various crosses among Ross, Cobb, and

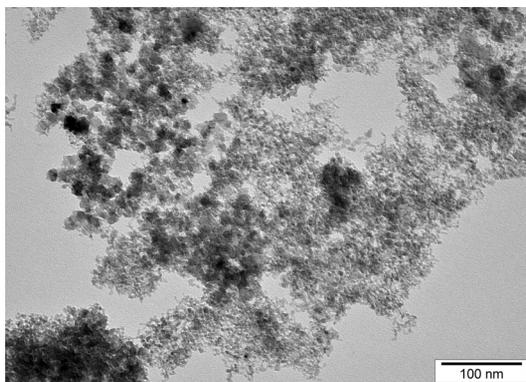


FIGURE 1. TEM image of diamond nanoparticles. Scale bar – 100 nm

Hubbard, kept for 4 days at 12°C. Chicken embryos were incubated at 37°C with 60–70% of relative humidity in incubator (PHU Walenski, Gostyn, Poland). Embryonic day 0 (E0) was designated as the day when the eggs were placed into the incubator.

Glioma tumor culture

The eggs were divided into two groups (2×40 eggs): group I (control) and group II (tumor treatment diamond nanoparticles). The GMB culture suspension (3–4 ·10⁶ cells in 30 µl cell culture medium) was injected inside the silicon ring placed onto the CAM at day E6. Diamond nanoparticles were injected into the tumor (form II group) after 7 days from start of the culture. The chicken embryos were killed by decapitation at day E18. The GBM tumors that grew inside or near the silicone ring were resected and passed for further investigations.

Transmission Electron Microscopy

The tumors were cut into pieces of about 1 mm³ and fixed in the 2.5% solution of glutaraldehyde (grade I, 25% in H₂O, purified for use as an electron microscopy fixative, Sigma-Aldrich) in 0.1 M phosphate buffer (pH 6.9). Then, the samples were rinsed in the same buffer and transferred to the 1% solution of osmium tetroxide (Sigma-Aldrich) in 0.1 M phosphate buffer (pH 6.9) for 1 h. Subsequently, the samples were rinsed in distilled water, dehydrated in the ethanol gradient and impregnated with epoxy embedding resin (Epoxy – Embedding Kit, Fluka, Sigma-Aldrich). The next day, the samples were embedded in the same resin and baked for 24 h at 36°C. Then, the blocks were transferred to 60°C and

baked for another 24 h. The blocks were cut into ultrathin sections (50–80 nm) using an ultramicrotome (LKB Ultratome III, Sweden) and transferred onto copper grids, 200 mesh (Agar Scientific Ltd. GB). Subsequently, the sections were contrasted using uranyl acetate (uranyl acetate dehydrate, puriss. p.a., ACS reagent, ≥98.0% (T) Fluka, Sigma-Aldrich) and lead citrate (lead (II) citrate tribasic trihydrate – purum, for electron microscopy, Sigma-Aldrich). The morphological structure of each GBM was inspected using a JEM-1220 transmission electron microscope (TEM) at 80 keV (JOEL, Japan) coupled with a digital camera (Morada) and Olympus Soft Imaging Solutions software (Olympus, Germany).

RESULTS AND DISCUSSION

Diamond nanoparticles reduce mass, volume and number of vessels in glioblastoma multiforme tumor cultured on chicken's embryo CAM (Grodzick et al. 2011). In the present experiment we additionally observed, that DN caused changes in the tumor ultrastructure. The electron microscopy images from both groups (control group and group treated with diamond nanoparticles) showed a typical ultrastructure of the glioma tumors with glioblastoma multiforme cells, epithelium cells and erythrocytes (Fig. 2). In the control group (Fig. 2 A, B, C; Fig. 3 A, B, C) glioblastoma multiforme cells with cell structures (nucleus, mitochondria, Golgi apparatus, rough endoplasmic reticulum with ribosomes, transport vesicles) were visible. Cancer cells have characteristic and unique metabolism, that is an adaptation to their microenvironment (low pH,

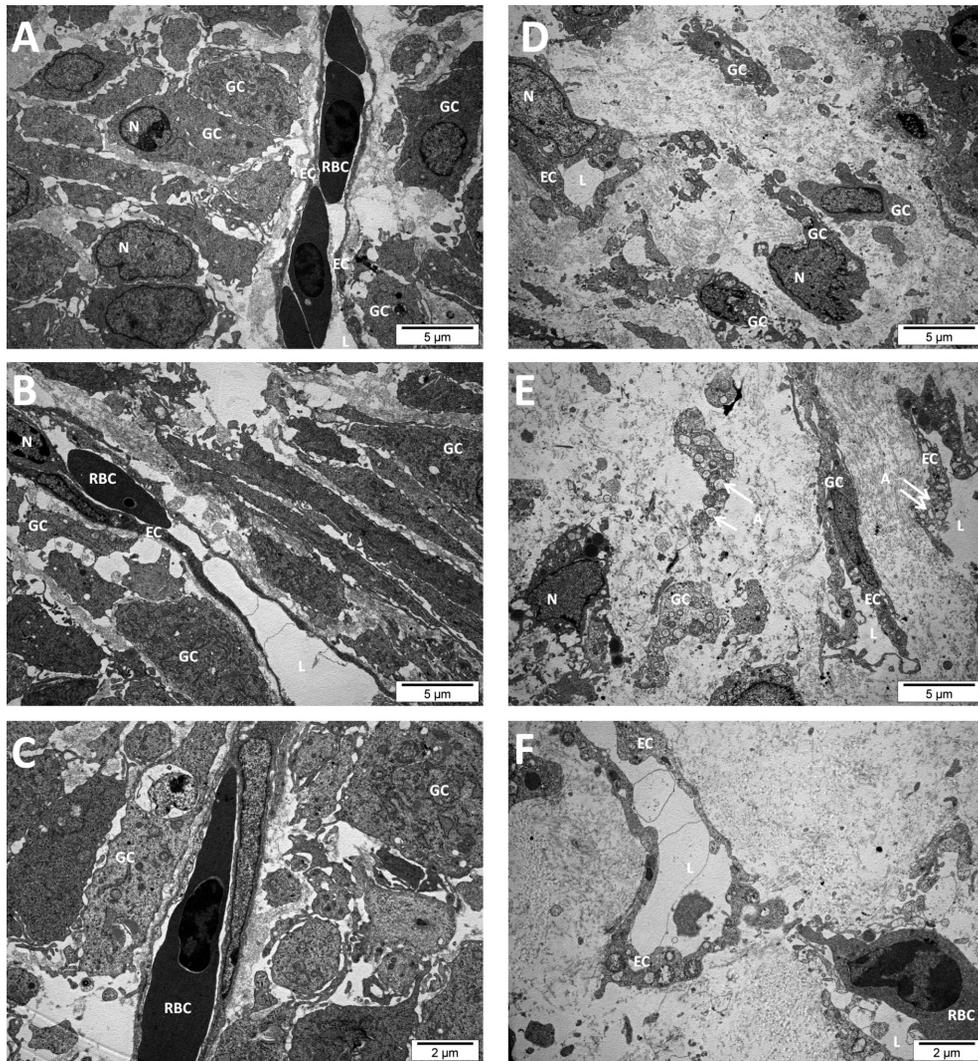


FIGURE 2. Glioblastoma multiforme ultrastructure from control group (A, B, C) and after diamond nanoparticles treatment (D, E, F). Scale bar: A, B, D, E – 5 μm, C, F – 2 μm; GC – glioblastoma multiforme cell, RBC – red blood cells, L – lumen of vessel, EC – endothelial cell, N – nucleus, A – autophagosome

hypoxia, anaerobic glycolysis, intensive cell divisions). Well-developed rough endoplasmic reticulum (ER) and numerous secretory and endocytotic vesicles prove high secretory activity of GBM cells. Endoplasmic reticulum (ER) is the

structure of eukaryotic cells, where lipid synthesis, protein folding, and protein maturation take place. ER is the major signal-transducing organelle that senses and responds to changes of homeostasis (Baumann and Walz 2001). In general,

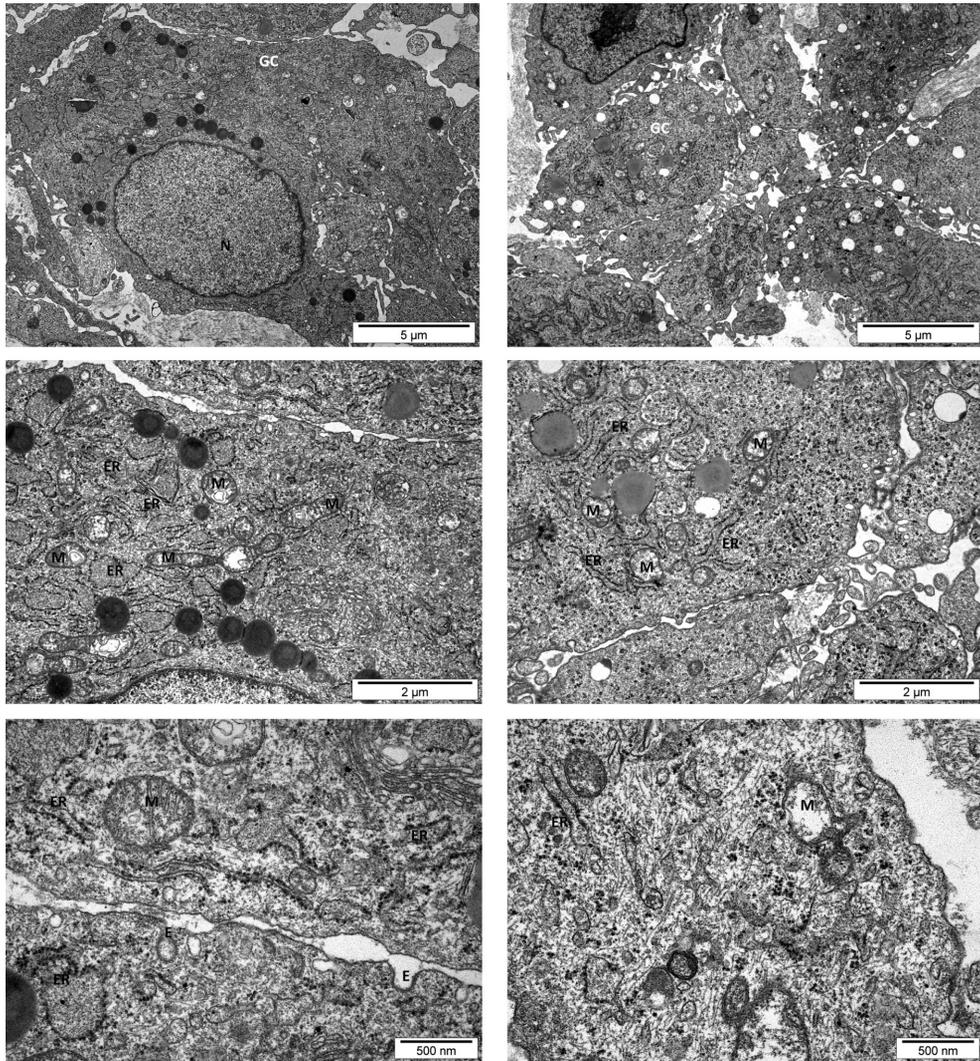


FIGURE 3. Glioblastoma multiforme ultrastructure from control group (A, B, C) and after diamond nanoparticles treatment (D, E, F). Scale bar: A, D – 5 μm , B, E – 2 μm , C, F – 500 nm; GC – glioblastoma multiforme cell, RBC – red blood cells, L – lumen of vessel, EC – endothelial cell, N – nucleus, M – mitochondrion, ER – rough endoplasmic reticulum, E – endocytosis

intense or persistent ER stress induces apoptosis or autophagy, resulting in cell death. Also the structure of glioma cells proves intensive cellular metabolism. Structures characteristic for endocytosis can be observed (membrane cavities of

varying width and depth, transport vesicles). Endocytosis is essential for all the cells to internalize nutrients, antigens, pathogens and cell surface receptors, from the plasma membrane into membrane-bounded, endocytic vesicles, to

regulate cell homeostasis, cell signaling and development. There are multiple pathways for endocytic uptake into cell, depending on the size and kind of the transported compound or substance, they can be divided into 4 classes: clathrin-mediated endocytosis, caveolae, macropinocytosis, and phagocytosis (Doherty and McMahon 2009). Very important and well visible cell structures are the mitochondria, which key role is the energy metabolism and regulation of cell death (Wen et al. 2013). The mitochondria of glioblastoma multiforme cells in control group have clearly defined folds in the inner membrane, named cristae.

In the group treated with diamond nanoparticles, despite the cells that can be identified are the same as in the control group, the morphology of these cells is different (Fig. 2 D, E, F; Fig. 3 D, E, F). First of all, the large spaces between the few deformed cells of glioblastoma multiforme are visible. Primary tumor cells that previously existed in the extracellular matrix probably influenced by ND underwent cell death. Glioma cells have irregular shapes; look like they lost their elasticity and rigidity. Inside the cell, cell structures have also a different morphology comparing to the control group. The number of organelles essential for proper metabolism is decreased. Additionally, in the mitochondria crests were degenerated, endoplasmic reticulum is less visible (smaller network of cisterns and ribosomes), endocytosis is practically stopped. In the ultrastructural image of these cells appeared vesicles characteristic for autophagy – highly conserved cellular homeostatic process. Changes can be observed in the structure of blood vessels as well. Their lumen is irregular

and collapsed, vascular endothelial cells have lost their shape and elasticity, inside degenerated mitochondria, deprived of cristae, and numerous autophagocytic vesicles (autophagosomes) can be seen.

Changes visualized in glioblastoma multiforme ultrastructure testify to the ongoing process of cell death, probably carried out by autophagy. Autophagy, also known as programmed cell death type II, can be induced by various cellular stress mediated signaling pathways involved in nutrient signaling, growth factor status, energy sensing, hypoxia, oxidative and ER stress. The role of autophagy is complicated and may have diametrically opposite consequences for the tumor cell, that is important for the regulation of cancer development and maintain, as well as for the response of tumor cells to anticancer therapy (Liu et al. 2013). Reduction of the number of GBM cells, and a strong degeneration of those which remained alive suggest that autophagy activated in these cells leads to their death.

CONCLUSIONS

Diamond nanoparticles administration to glioblastoma multiforme tumor grown on the CAM, not only inhibit the development of the blood vessels but also affect the metabolism of cancer cells. Ultrastructure of the glioma cells after treatment with ND clearly suggests autophagy leading to the cell death. However, the mechanism of initiation of this process is still unclear. The results obtained encourage further research aimed at the anti-cancer application of diamond nanoparticles.

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Streszczenie: *Zmiany w ultrastrukturze glioblastoma multiforme po zastosowaniu nanocząstek diamentu. Badania modelowe in ovo.* Glioblastoma multiforme (GBM) jest najczęściej występującym złośliwym nowotworem pierwotnym mózgu o bardzo złych rokowaniach. Pomimo dekad lat badań na tym problemem, niewielki postęp został uczyniony aby wydłużyć życie chorym, nowe strategie terapeutyczne są nadal poszukiwane. Zastosowanie nanotechnologii w leczeniu chorób, diagnostyce, monitoringu, platformach dostarczania substancji aktywnych i kontroli systemów biologicznych daje nadzieję na poprawę aktualnej sytuacji, również w terapii nowotworów. Nanocząstki diamentu (DN) są bioaktywnymi substancjami w stosunku do guza mózgu hodowanego na błonie kosmówkowo-omoczniowej zarodka kury. DN redukuje masę i objętość guza oraz hamuje rozwój nowych naczyń krwionośnych (angiogenezę). W prezentowanym doświadczeniu dodatkowo zaobserwowano zmiany w ultrastrukturze komórek guza pod wpływem działania nanocząstek diamentu, które świadczą o zachodzących procesach śmierci komórkowej, prawdopodobnie na drodze autofagii.

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The occurrence of entomopathogenic fungi in the Chojnowski Landscape Park in Poland

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Abstract: *The occurrence of entomopathogenic fungi in the Chojnowski Landscape Park in Poland.* The study was aimed at estimating species composition and abundance of entomopathogenic fungi in the Chojnowski Landscape park. The effect of site, season (spring, summer, autumn) and temperature on the frequency of isolation of entomopathogenic fungi was also analysed. The effect of the first two factors was estimated based on the analysis of soil samples taken from meadow 1, forest 1 and orchard in spring, summer and autumn 2010. Three species of entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea*) were isolated in the study area. Site and temperature affected the frequency of isolation of particular species. On meadow 1 and in orchard *M. anisopliae* appeared to be the dominating species while forest 1 was dominated by *B. bassiana*. From among meadow and forest sites sampled in the autumn, forest 3 (nature reserve) was characterised by the highest density of entomopathogenic fungi. *M. anisopliae* and *B. bassiana* were most often isolated from meadow sites while *B. bassiana* and *I. fumosorosea* – from forest sites. *B. bassiana* and *I. fumosorosea* infected insects with significantly higher frequency at 20°C than at 25°C.

Key words: entomopathogenic fungi, biology, bio-insecticides, *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea*, the Chojnowski Landscape Park

INTRODUCTION

Entomopathogenic fungi focussed researchers' attention already in the 19th century. Now, they are used in a small scale in organic farming in Western Europe, USA, in the counties of Latin America and in Australia. The fungi proved useful in replacing chemical insecticides, particularly when controlling pests which move vertically in soil according to ground water changes and the range of the root zone. Most promoted are the bio-preparations based on local strains of microorganisms. The use of imported ecotypes developed under different selective factors (e.g. temperature ranges) does not often bring expected results (Mierzejewska 2001).

Entomopathogenic fungi are an environmental-friendly alternative to plant protection chemicals. They are used to control harmful insects and arachnids (mainly mites, Acarina) which make losses in agricultural and forest crops and transfer microorganisms that are pathogenic to humans, animals and plants (Bałazy 2004). Biological control of pests does not result in the acquired resistance in contrast with the use of chemical pesticides (Paruch and

Janowicz 2004). Moreover, fungal bio-preparations do not need obeying the waiting periods or special protective measures (Mierzejewska 2001).

It is extremely important to create appropriate habitat conditions (e.g. refuges) to enable survival of species susceptible to man-made changes in the landscape. Such approach would allow for maintaining possibly richest gene pool of potential entomopathogenic organisms (Bałazy 2004, Quesada et al. 2007).

The Chojnowski Landscape Park has great landscape and natural values. It is part of the so-called green ring surrounding the city agglomeration of Warsaw. The park is localised on light sandy or sandy-loamy soils of relatively high fertility which supports many forest and bog-meadow communities. Apart from rich flora, the park provides also favourable conditions for the development of abundant fauna (Walczak et al. 2001). Practically no chemical control measures are applied to protect tree stands in forest areas of the park. This is favourable for the development of soil mesofauna including insects and their natural enemies like entomopathogenic nematodes and fungi. Forest entomopathogenic fungi find convenient conditions for the growth and development in forest litter and on the soil surface. For the year round they have a chance to contact their hosts permanently dwelling forest bottom or penetrating soil for pupation or wintering (Tkaczuk 2008).

MATERIAL AND METHODS

Entomopathogenic fungi were isolated from soil with the method of trap insects (Zimmerman, 1986 in Górny and Grüm

1993) used also to isolated entomopathogenic nematodes. The method consists in placing in soil some live insects e.g. caterpillars of various butterfly species which are attacked by isolated pathogens. Soil with trap insects is incubated in the lab at appropriate temperature (Górny and Grüm 1993).

Caterpillars of the greater wax moth (*Galleria mellonella* L.) from own culture of the Department of Zoology, Warsaw University of Life Sciences were used as trap insects in this study. Soil samples were collected in 2010 from various meadow and forest habitats and from an orchard situated in the Chojnowski Landscape Park (Masovian Province). Sampling sites were characterised by the following properties (names of sites are further used throughout the text):

- meadow 1 – situated in the village Łoś near the Jeziorka River on peat soils and bordering forest 1,
- meadow 2 – wet meadow growing on alluvial and peat soils situated by the Jeziorka River in the village Gołków,
- meadow 3 – several hundred metres SW of Wólka Pęcherska. Single young pine, birch and oak trees grow on this untypical meadow situated in the close vicinity of forest 3,
- forest 1 – mixed forest (pine, oak, birch, small-leaved linden and other tree species) situated in the village Prace Duże. Soil samples were taken from sites covered by brown soils,
- forest 2 – mixed forest (pine, oak, birch, small-leaved linden and other tree species) situated in the village Bogatki. Soil samples were taken from sites covered by brown and rusty soils,

- forest 3 – nature reserve Biele Chojnowskie, soil samples were taken from sited overgrown by mixed coniferous forest *Quercus robur*-*Pinetum* (old-growth forest of Scotch pine with a small admixture of the common oak and the common birch). Soil samples were taken from the forest outskirts,
- orchard – a young apple orchard situated on brown soils in a village.

Mixed soil samples were taken from studied habitats with the Egner's cane. Soil from each site was placed in a plastic container ($V = 250 \text{ cm}^3$) and 10 larvae of the greater wax moth (*G. mellonella* L.) were added to each container. Caterpillars with soil were incubated at 20 and 25°C. Experiments were made in triplicate. In total, entomopathogenic fungi infected 508 trap larvae out of 1,753 isolated dead insects during the whole experiment.

The first observation of larval mortality was made after 4 days of contact of caterpillars with soil, the next were made every 3 days until the 40th day of experiment. Dead insects were removed and containers with soil were supplemented with live caterpillars. Dead insects when soft suggested infection by nematodes while tough insects with the symptoms of mummification were superficially sterilised with disinfectant 1% sodium hydroxide solution and rinsed in distilled water. Individuals with a visible mycelium did not undergo sterilisation. Then, the caterpillars were transferred to Petri dishes lined with filter paper to achieve sporification of fungi necessary for their determination.

The effect of habitat was determined for 6 sites (meadow 1, meadow 2, meadow 3, forest 1, forest 2, forest 3), from

which soil samples were taken in autumn 2010. Results obtained from the analysis of soil samples taken in spring and summer (forest 1, meadow 1 and orchard) and in autumn (meadow 1, meadow 2, meadow 3, forest 1, forest 2, forest 3 and orchard) served for testing the effect of temperature on the frequency of isolation of particular species of entomopathogenic fungi. Obtained results were statistically processed with the Statgraphics Plus 4.1 software using simple and multi-parametric ANOVA. Tukey's test was used to compare the means at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Polish soils are characterised by a great abundance of entomopathogenic fungi and, as shown in studies by Tkaczuk (2008), by rather diverse species composition. This author found at least two species of entomopathogenic fungi in more than 80% of soil and litter samples from various habitats in Poland (one species in 18.2% samples, two species in almost 40% samples, three species in 34.3% samples and four or more species in 7.9% samples). For comparison, in more than 40% of soil samples from abroad the author found the presence of only one species of entomopathogenic fungi, in more than 30% samples – two species and in 22.5% and 5.6% samples – the presence of three and four or more species, respectively. In each of these examples the respective figures are smaller than those from Polish soils.

Despite the fact that entomopathogenic mitosporic fungi are widespread, especially in the soil habitat, the

knowledge of the factors affecting their occurrence, population structure, mechanisms of their persistence and virulence against potential hosts is still scarce. Definitely better studied in this aspect are fungi of the order Entomophthorales (Tkaczuk 2008).

Three species of entomopathogenic fungi: *B. bassiana*, *M. anisopliae* and *I. fumosorosea* were found in studied sites. They were isolated from 96, 77 and 73% of collected soil samples, respectively. *B. bassiana* was the most often isolated species. It was present in every site in a given season. *M. anisopliae* was not found in summer in forest 1 and *I. fumosorosea* was absent from meadow 1 in spring and from meadow 1 and meadow 2 in autumn (Table 1).

Common occurrence of these fungi in soils from various country habitats was confirmed in many studies (Miętkiewski et al. 1991, 1991–1992 and 1998, Miętkiewski and Kloczarek 1995, Bajan and Kmitowa 1997, Marjańska-Cichoń et al. 2005, Sapięha-Waszkiwicz et al. 2006). Clear dominance of mentioned species in Polish soils, without the distinction of particular habitats, was also noted by Tkaczuk (2008). According to this author, the species less frequently recorded in Polish soils are *I. farinosa*, *L. lecanii* and *M. flavoviride* which were not found in the study sites.

Entomopathogenic fungi caused death of 15 to 53% (mean 29%) of trap caterpillars depending on site and season. The lowest mortality was found in soil taken from forest 1 in summer and the highest – in that taken from forest 3 in autumn. A high fungal activity was also observed in soil sampled from

TABLE 1. Mortality of trap insects placed in soil in relation to season and site (%)

Mortality factor	Spring									
	Orchard			Meadow 1			Forest 1			mean
	20°C	25°C	mean	20°C	25°C	mean	20°C	25°C	mean	
<i>B. bassiana</i>	3.03	8.33	5.68	20.69	5.56	13.12	12.90	18.18	15.54	
<i>M. anisopliae</i>	3.03	11.11	7.07*	3.45	50.00	26.72	0.00	6.82	3.41*	
<i>I. fumosorosea</i>	12.12	0.00	6.06	0.00	0.00	0.00	12.90	9.09	11.00	
Total fungi	18.18	19.44	18.81	24.14	55.56	39.85	25.81	34.09	29.95	
Other factors	6.06	13.89	9.97	6.90	2.78	4.84	1.61	9.09	5.35	
Mortality factor	Summer									
	Orchard			Meadow 1			Forest 1			mean
	20°C	25°C	mean	20°C	25°C	mean	20°C	25°C	mean	

<i>B. bassiana</i>	7.14	2.63	4.89	3.80	3.45	3.62	15.89	6.90	11.39			
<i>M. anisopliae</i>	11.22	6.58	8.90	31.65	29.31	30.48*	0.00	0.00	0.00			
<i>I. fumosorosea</i>	9.18	2.63	5.91	10.13	1.72	5.93	3.74	3.45	3.59			
Total fungi	27.55	11.84	19.70	45.57	34.48	40.03	19.63	10.34	14.99			
Other factors	5.10	11.84	8.47	10.13	18.97	14.55	3.74	8.62	6.18			
Autumn												
Mortality factor	Orchard			Meadow 1			Forest 1					
	20°C	25°C	mean	20°C	25°C	mean	20°C	25°C	mean			
<i>B. bassiana</i>	4.82	0.00	2.41	18.68	2.94	10.81	7.59	21.74	14.67			
<i>M. anisopliae</i>	19.28	14.44	16.86*	13.19	11.76	12.48	0.00	6.52	3.26			
<i>I. fumosorosea</i>	13.25	2.22	7.74	0.00	0.00	0.00	12.66	26.09	19.37*			
Total fungi	37.35	16.67	27.01	31.87	14.71	23.29	20.25	54.35	37.30			
Other factors	3.61	2.22	2.92	2.20	1.47	1.83	2.53	2.17	2.35			
Autumn												
Mortality factor	Meadow 2			Meadow 3			Forest 2			Forest 3		
	20°C	25°C	mean									
<i>B. bassiana</i>	47.92	1.59	24.75*	25.00	21.05	23.03	6.36	6.52	6.44	20.00	44.59	32.30*
<i>M. anisopliae</i>	6.25	3.17	4.71	4.69	7.89	6.29	0.00	6.52	3.26	0.00	4.05	2.03
<i>I. fumosorosea</i>	0.00	0.00	0.00	10.94	26.32	18.63*	7.27	6.52	6.90	30.67	6.76	18.71
Total fungi	54.17	4.76	29.46	40.63	55.26	47.94	13.64	19.57	16.60	50.67	55.41	53.04
Other factors	4.17	11.11	7.64	3.13	2.63	2.88	0.00	2.17	1.09	1.33	8.11	4.72

*Tukey's test $\alpha < 0.05$.

meadow 1 in summer and in soil from meadow 3 taken in autumn (Table 1).

Most trap larvae infected by entomopathogenic nematodes, which caused death of from 45 to 79% of the larvae of *G. mellonella*, were found in soil taken from meadow 1, forest 1 and orchard in spring, summer and autumn. In soil taken from meadow 2, meadow 3 and forest 2 entomopathogenic nematodes were also the reason of the highest mortality of trap larvae (from 49 to 82%). Only in soil samples taken from forest 3 the number of *G. mellonella* larvae infected by nematodes (42%) was lower than those infected by entomopathogenic fungi (53% – Table 1). The death of caterpillars was also caused by other factors like saprophytic fungi, mites, non-fruiting mycelium, bacteria or other unidentified factors.

Not all species of entomopathogenic fungi are equally characteristic for various ecosystems – forests, meadows, croplands and other (Bajan and Kmitowa 1997, Miętkiewski et al. 1998, Tkaczuk 2008). As shown by Tkaczuk (2008), *M. anisopliae* is the dominating species in the country meadow and pasture soils. According to other studies (Miętkiewski et al. 1991–1992 and 1998), *M. anisopliae* predominated over other species of fungi in meadow soil. This species appeared also characteristic for such type of soils in the Chojnowski Landscape Park, especially for meadow 1, where it dominated in every season and was the reason of remarkable mortality of trap insects.

B. bassiana definitely dominated in meadow 2, from where soil samples were taken only in autumn. *M. anisopliae* caused higher mortality of trap caterpil-

lars in meadow 2 than in forest habitats but showed much smaller activity there as compared with that in meadow 1. Miętkiewski et al. (1991–1992 and 1998) also reported abundant occurrence of *B. bassiana* in meadow soil but *M. anisopliae* was the dominating species in their studies. *B. bassiana* is known to grow better in habitats rich in organic substances which is probably associated with its ability to develop in the saprophytic phase. Maybe this was the reason of the dominating role of *B. bassiana* in infecting trap insects from soil of meadow 2 since the meadow was situated on river alluvia and peat soils.

M. anisopliae showed relatively high activity in soil sampled from orchard being the dominating species there. The prevalence of *M. anisopliae* compared with other species could result from its resistance to agricultural factors like plant protection chemicals, mineral fertilisation, intensive soil cultivation (Tkaczuk 2008). Some studies (Janowicz et al. 2004, Paruch et al. 2004) indicate, however, quite opposite that *M. anisopliae* is a species particularly sensitive to plant protection chemicals. The species can best stand the absence of potential hosts from among other species of entomopathogenic fungi. Its conidia are able to survive on a primary host for a long period of time (Miętkiewski 1992).

Three species of fungi: *M. anisopliae*, *I. fumosorosea* and *B. bassiana* were isolated from the soil from orchard. The presence of the same species of entomopathogenic fungi in orchard soils was noted by many authors (Marjańska-Cichoń et al. 2003, 2005, Janowicz et al. 2004, Paruch et al. 2004). Paruch et al. (2004) reported higher mortality of cat-

erpillars caused by fungi from orchards without chemical protection measures compared with the soil from a control orchard that was subjected to chemical protection and nursing practices. As seen from their studies, frequent application of plant protection chemicals reduced the populations of beneficial, entomopathogenic soil fungi.

Pesticides were used moderately in the studied orchard. Observed occurrence of entomopathogenic fungi, mainly of *M. anisopliae* and *I. fumosorosea*, differed from that presented by Paruch et al. (2004). In the studied orchard *M. anisopliae* was dominating and its activity was highest in autumn. In the control orchard of Paruch et al. (2004), *M. anisopliae* was not found while in orchards, where pesticides were not used, the species was most active only in spring. *I. fumosorosea* dominated in orchards studied by Paruch et al. (2004).

Statistical analysis showed significant site-specific occurrence of *I. fumosorosea*. This fungus was isolated more frequently from forest than from meadow habitats. The least frequent in studied sites was *M. anisopliae*. This species was isolated more often from meadow and orchard than from forest habitats. In autumn it dominated in meadow 1 and in spring and summer – in orchard (Table 1). In the soil from meadow 1, the mean contribution of *M. anisopliae* to all entomopathogenic fungi isolated there was 66% while in the soil from orchard it amounted 50%. The frequency of isolation of *M. anisopliae* in forest 1 was significantly lower than that in meadow and orchard.

Numerous occurrence of *M. anisopliae* in orchard soil as compared with

other species of fungi was noted by Miętkiewski et al. (1992). The authors found also an abundant presence of *B. bassiana*. In my study *B. bassiana* was much less frequent but the second numerous species after *M. anisopliae* was *I. fumosorosea*, the species not recorded by Miętkiewski et al. (1992). Studies carried out by Miętkiewski et al. (1992) differed from mine in that they compared the occurrence of entomopathogenic fungi in soils from herbicide fallow and orchard sward. They found that *M. anisopliae* infected larvae more often in soils from herbicide fallow than in soils from orchard sward, *B. bassiana* showed the reverse pattern.

Two species of entomopathogenic fungi: *B. bassiana* and *I. fumosorosea* dominated in studied forest habitats. In each of the three studied forests, *M. anisopliae* was less frequent. According to Tkaczuk (2008), the dominating species in Poland, in both forest soil and litter, is *B. bassiana*. Similarly Głowacka and Świeżyńska (1993) and Bajan et al. (1995) pointed to *B. bassiana* as the most frequent species in forest habitats. The species dominated in studied forest 1 and forest 3, from which soil samples were taken only in autumn. *I. fumosorosea* was also numerous in studied forests being the most frequently isolated species in forest 1 and 2 in autumn. As shown by Tkaczuk (2008) *I. farinosa*, *I. fumosorosea* and *M. anisopliae* are almost equally frequent in soil-litter samples from various forests in Poland and their abundance is nearly equal. In my studies *I. farinosa* was not found at all and *I. fumosorosea* was isolated more often than *M. anisopliae*.

Studied habitats were characterised by different abundance of entomopathogenic fungi. More of these microorganisms were found in meadow 1 than in orchard and forest 1. Various factors like favourable location (close to the river and forest) that provided appropriate soil moisture and enhanced the development of diverse flora and fauna could be the reason of observed pattern. Greatest richness of meadow soil in entomopathogenic fungi compared with orchard soil might be also associated with sporadic application of pesticides and less intensive cultivation.

From among meadow and forest habitats sampled in autumn particularly great abundance of entomopathogenic fungi was noted in the nature reserve – forest 3. This confirms earlier findings (Miętkiewski et al. 1991–1992, Bałazy 2006, Tkaczuk 2008) that more diverse ecosystems with richer flora and fauna (including entomofauna) are more attractive for entomopathogenic fungi, which may find their potential hosts there.

In general, a greater activity of entomopathogenic fungi is observed in autumn due to the translocation of insects to soil for wintering. Insects dying of mycosis substantially enrich the soil in the infection material (Tkaczuk 2008). The number of conidia produced by *B. bassiana* on insects in the forest litter in autumn varies from 109 to 1,010 m² while in late spring and summer it is 107–108 m² (Bałazy 2006).

Temperature at which trap insects contacted the soil significantly affected the activity of *B. bassiana* and *I. fumosorosea*. Both species infected more larvae at 20°C than at 25°C. No significant differences were noted in the infection

of trap larvae by *M. anisopliae* at different values of temperature. The species was slightly more frequent at 25°C. Miętkiewski et al. (1994) obtained different results in this aspect. They confirmed lower thermal requirements of *I. fumosorosea* but found that *B. bassiana* infected most often at 25°C. However, in the study by Sapięha-Waszkiewicz et al. (2006) *B. bassiana* and *I. fumosorosea* infected trap larvae more frequently at 20°C than at 25°C, similarly as they did in my experiments. Both species dominated also at lower (18°C) but not at higher (28°C) temperature in the study of Tkaczuk and Miętkiewski (1996). This phenomenon may be explained by a marked differentiation of features of the strains originating from various habitats.

M. anisopliae infected more trap larvae at 25°C but the difference was not statistically significant. High thermal requirements of this species were also observed by various authors (Miętkiewski et al. 1993 and 1994, Tkaczuk and Miętkiewski 1996, Sapięha-Waszkiewicz et al. 2006).

Entomopathogenic fungi have a great biocoenotic value being a factor controlling population density of insects. Therefore, some species may be used for biological control of plant pests. That is why the understanding of species composition and ecology of this group of fungi and protection of their habitats is so important. Protected forests, nature reserves and national parks should play a role of shelters and refuges for these fungi (Bałazy 1981). Semi-natural habitats play similar role for the maintenance and species diversity of entomopathogenic fungi in agricultural landscapes (Tkaczuk 2008).

CONCLUSIONS

1. The following species of entomopathogenic fungi were isolated in the study area:
 - *Beauveria bassiana*,
 - *Metarhizium anisopliae*,
 - *Isaria fumosorosea*.
2. Habitat and temperature affected the frequency of isolation of particular species of entomopathogenic fungi. Such a relationship was not found for season.
3. *B. bassiana* and *I. fumosorosea* infected insects significantly more often at 20°C than at 25°C.
4. The species most often isolated in autumn were: *B. bassiana* and *M. anisopliae* in meadow habitats and *B. bassiana* and *I. fumosorosea* in forest habitats.

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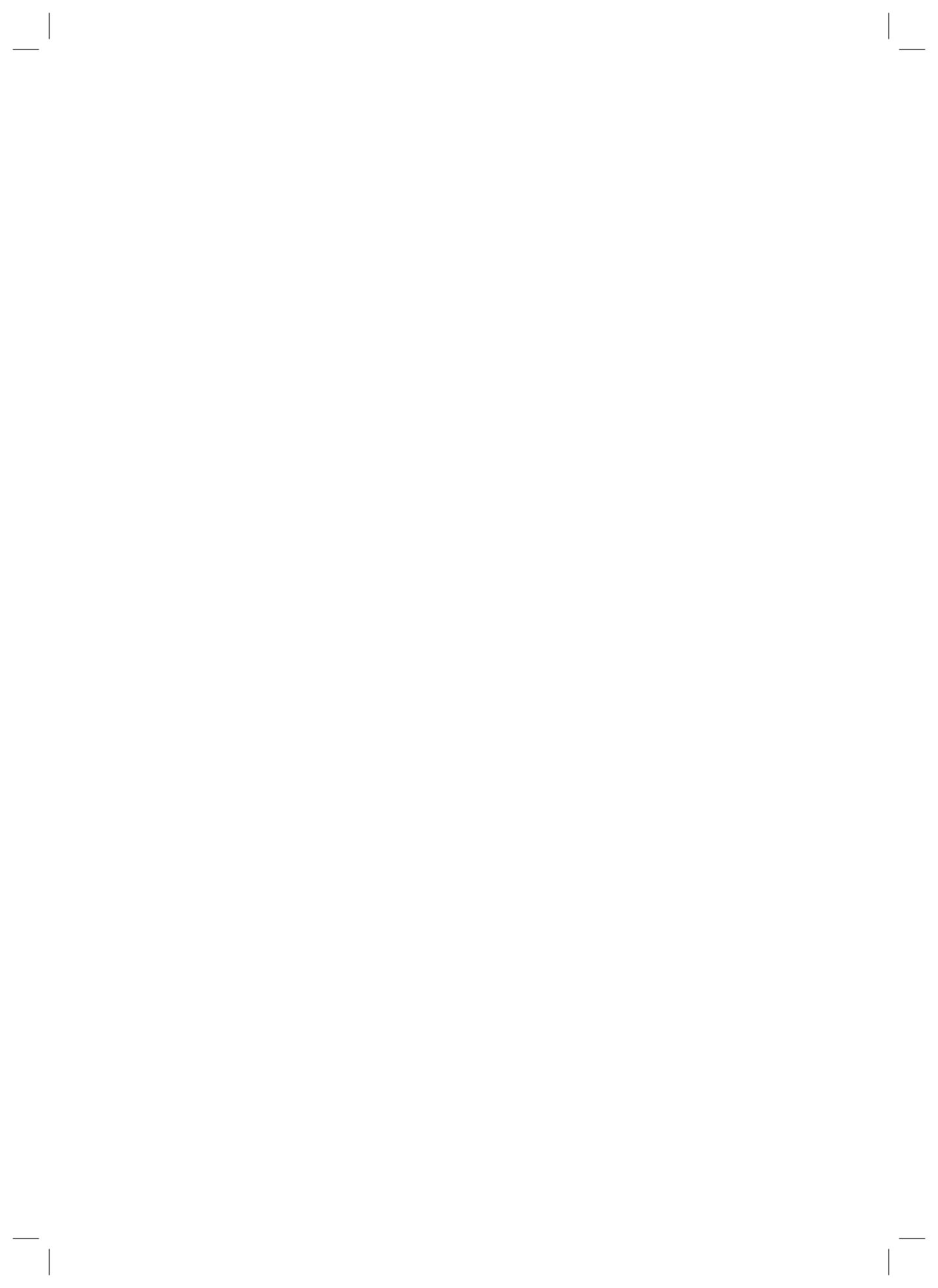
Streszczenie: *Występowanie grzybów entomopatogennych na terenie Chojnowskiego Parku Krajobrazowego.* Celem podjętych badań było określenie składu gatunkowego i nasilenia występowania grzybów entomopatogennych na wybranych siedliskach w Chojnowskim Parku Krajobrazowym. Zbadano także, czy na częstość izolowania grzybów entomopatogennych wywierają wpływ następujące czynniki: siedlisko, pora roku (wiosna, lato, jesień) oraz temperatura. Na podstawie badań prób glebowych pobranych z łąki 1, lasu 1 i sadu w okresie wiosny, lata i jesieni 2010 roku został oceniony wpływ siedliska i pory roku na występowanie grzybów entomopatogennych. Na badanym terenie wyizolowano trzy gatunki grzybów entomopatogennych

(*Beauveria bassiana*, *Metarhizium anisopliae* i *Isaria fumosorosea*). Stwierdzono wpływ siedliska i temperatury na częstość izolacji poszczególnych gatunków. Na łące 1 i w sadzie gatunkiem dominującym okazał się *M. anisopliae*, zaś w lesie 1 *B. bassiana*. Z siedlisk łąkowych i leśnych, z których glebę pobrano jesienią, największe nasilenie grzybów entomopatogenicznych obserwowano w lesie 3 (rezerwacie). Na siedliskach łąkowych najczęściej izolowanymi gatunkami były *M. anisopliae* i *B. bassiana*, na siedliskach leśnych *B. bassiana* i *I. fumosorosea*. *B. bassiana* i *I. fumosorosea* istotnie częściej infekowały owady w 20°C niż 25°C.

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The effect of feeding on aminopeptidase and non-specific esterase activity in the digestive system of pike-perch (*Sander lucioperca* L.)

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Abstract: *The effect of feeding on aminopeptidase and non-specific esterase activity in the digestive system of the pike-perch (*Sander lucioperca* L.).* The pike-perch (*Sander lucioperca* L.) at the age of 18 days were fed for 21 days using three different diets: Aglo Norse (An), casein-gelatin (Cas), cod meal with gelatin (Mac) and nauplius *Artemia salina* (Art – control diet). On the last day of the experiment, fish fed Art and An diets had the statistically significant highest body mass, length, and survival. On the last day, the highest aminopeptidase activity in the anterior intestine and posterior intestine was registered in fish fed nauplius *Artemia salina*. The lowest activity of this enzyme in the anterior intestine was to be found in fish fed with Cas diet, while there was no difference between among groups in the posterior intestine. The non-specific esterase activity was registered in the stomach, liver, anterior intestine as well as in the posterior intestine. The lowest activity of this enzyme in the stomach was observed in the pike-perch fed with the Cas diet. In the anterior intestine, the highest activity was registered in fish fed with Art, and the lowest – on the Cas and Mac diet. The results of the current research prove that feeding the pike-perch An diet has a positive effect of the survival of the pike-perch, their growth rate and the activity of the enzymes. By contrast, feeding pike-perch Cas and Mac diets did not satisfy nutritional needs of fish, resulting in their low survival, growth rate and low activity of the enzymes examined.

Key words: pike-perch, *Sander lucioperca*, aminopeptidase, non-specific esterase, histochemistry

INTRODUCTION

Considering a dynamic development of aquaculture in the world, it becomes necessary to increase the production of fish fodder, to improve its quality and to look for cheaper, alternative protein sources (Hardy 1996). At present, the most common and the best source of protein for fodder production is fish meal. However, the prices getting higher and interest growing bigger makes us search for alternative protein sources to substitute fish meal in fodder (Hardy 1996). The most frequent sources are vegetable products and various food industry by-products. This is why research is done on introducing different protein sources to fodder, sources like soybean meal, casein, gluten and others (Ostaszewska et al. 2005a, Kamaszewski et al. 2010, Kamaszewski et al. 2013). However, alternative protein sources often have an unbalanced amino acid pattern and have endogenous dietary factors, which may lead to a decrease in growth rates as well as lesions in the digestive system of fish (Ostaszewska et al. 2005a, Ostaszewska et al. 2010).

The Percidae, among them the pike-perch (*Sander lucioperca*) are popular in aquaculture. Notwithstanding this, cannibalism, high death rate or poor uptake of artificial diets constitute a serious problem in breeding the pike-perch in recirculating aquafarming systems (Ostaszewska et al. 2005b, Szczepkowski et al. 2011). Therefore research is done to optimize feeding especially the earliest life stages of the pike-perch and to examine the physiology of the fish digestive system. To this end, studies are ongoing where digestive enzymes during ontogeny are observed, their occurrence, location and activity. This information can be helpful when time indications are made when to launch a diet feeding the species and whether the diet meets nutritional requirements of the species given (Kamaszewski et al. 2010).

The aim of this research was to define the influence of various diets on the survival, growth rate and distribution as well as the activity of aminopeptidase M, exopeptidase from intestine, and non-specific esterase, enzyme related in lipid metabolism, in the digestive system of juvenile pike-perch.

MATERIAL AND METHODS

The experiment was conducted in the Department of Ichthyobiology and Fisheries, Warsaw University of Life Sciences. Juvenile stages of pike-perch, at the age of 18-day post hatching (total body length 18.59 ± 1.68 mm; body weight 0.05 ± 0.01 g) were breeding for three weeks (from 18th to 39th day after hatching). Fish were stocked at a density of 5 individuals per litre in water recircu-

lation system equipped with a biological filter and UV lamps. The water temperature was $20.6 \pm 0.9^\circ\text{C}$, pH 7.5–8.1, and the content of total ammonia nitrogen did not exceed the level of 0.1 mg/L, and the nitrite – 0.01 mg/L. The 14 h light: 10 h dark photoperiod was applied. Aquariums were illuminated with poor light (100 lx). The experiment was conducted in four nutritional groups, five repetitions each. Fish were fed every two hours using following diets: commercial diet Aglo Norse – An (Larvae Feed Ewos – Bergen, Norway), two experimental Casein-gelatin diets – Cas (Ostaszewska et al. 2005a) and cod meal-gelatin diet – Mac (Kamaszewski and Ostaszewska 2013). The control group was fed *Artemia salina* nauplii *ad libitum*. The protein and lipid content is given in the Table 1. In the first week of the experiment, fish were given a feed ration representing 50% of their biomass, in the second week 15% and in the third – 10%.

TABLE 1. The content of protein (%) and lipids (%) in diets used in the experiment (according to the manufacturer's data)

Diet	Protein (%)	Lipid (%)
<i>Artemia salina</i> (Art)	50	10
Aglo Norse (An)	59	21
Casein-gelatin (Cas)	48	11
Cod meal-gelatin (Mac)	64	8

To conduct histochemical examinations, 10 fish were sampled from each group on the first and on the last day of the examination. Fish were anesthetized using MS-222 preparation (tricaine methanesulphonate, Sigma-Aldrich, Munich, Germany), weighed to the nearest 0.01 g (body weight, BW), measured

in total length (LT) to 0.02 cm and frozen with liquid nitrogen. The research material was stored at -80°C until it was analyzed. Samples were cut to a thickness of $10\ \mu\text{m}$ using a cryostat (Leica CH 1900, Leica Microsystems, Nussloch, Germany). The activity of aminopeptidase M (membrane alanyl aminopeptidase, EC 3.4.11.2) was detected using a method according to Nachlas et al. using a substrate L-Leucine β Naphthyl-amide (Sigma) in 0.1 M phosphate buffer of pH 7.0 (Lojda et al. 1979). The activity of non-specific esterase (EC 3.1.1) was detected using a method according to Gomori using a substrate 1-Naphthyl acetate (Sigma) in 0.1 M phosphate buffer of pH 7.4 (Lojda et al. 1979). The activity of enzymes on histochemical preparations was marked on the grounds of the intensity of coloration. In research was using scale, where (++++) means a very strong histochemical reaction (90–100% area of cell with a positive reaction), (++) means a strong histochemical reaction (60–90% area of cell with a positive reaction), (+) means a moderate histochemical reaction (40–60% area of cell with a positive reaction), (+/-) means a weak reaction (0–40% area of cell with a positive reaction) and (-) means no reaction on preparations examined. Microscopic examinations and photographs were made using

a microscope Nikon Eclipse 90i and a co-operating camera Nikon Digital Sight DS-U1 (Nikon Corporation, Tokyo, Japan).

The average as well as the standard deviation for the survival, total length and weight of the fish were calculated using a program Statistica ver. 10.0. Differences between the groups were tested using one-way ANOVA and Tukey's (HSD) post hoc test ($P \leq 0.05$).

RESULTS AND DISCUSSION

The highest survival rate in the experiment was observed in fish fed with nauplii *Artemia salina* ($65.5 \pm 14.8\%$), while the lowest rate was shown in the fish fed with Mac ($35.4 \pm 16.4\%$), and the differences were statistically significant (Table 2). On the twenty first day of the experiment fish fed with *Artemia salina* and An diet had a statistically significant higher total body length and body weight in comparison to fish fed experimental diets Cas and Mac (Table 2).

When breeding a pike-perch in aquaculture, it is a common practice to feed the earliest stages of this species with nauplii *Artemia salina*, and only 17–19 days after hatching feed them artificial diets. It is a period when the digestive system of a pike-perch is fully

TABLE 2. Survival (%), total length, and body weight of pike-perch fed with experimental diets on twenty first day of the experiment (mean \pm SD, $n = 10$)

Growth parameters	Diets			
	Art	An	Cas	Mac
Survival (%)	65.5 ± 14.8^a	62.3 ± 7.7^a	43.9 ± 18.1^{ab}	35.4 ± 16.4^b
Total body length (mm)	43.2 ± 1.9^a	42.6 ± 2.7^a	26.2 ± 2.5^b	28.5 ± 2.6^b
Body weight (g)	0.61 ± 0.08^a	0.58 ± 0.11^a	0.11 ± 0.03^b	0.14 ± 0.04^b

Means with different letter superscripts in the same row are significantly different ($P \leq 0.05$).

differentiated and becomes capable of digesting and absorbing nutrient contents from diets (Ostaszewska 2005). According to Ljunggren et al. (2003) and Ostaszewska et al. (2005b), a fodder that meets the nutritional requirements of juvenile pike-perch stages is the Aglo Norse diet (An). It was also proven in the current research that fish fed the An diet had the quickest growth rate as well as the greatest survival rate among fish fed on experimental diets.

Histochemical analysis revealed the presence of aminopeptidase M in the brush border and in the supranuclear enterocyte area of the anterior and posterior intestine in the pike-perch of all nutritional groups on the first and the last day of the experiment (Fig. 1). As with the examined fish, the presence of aminopeptidase in the anterior and posterior intestine was observed in other species of fish (Segner et al. 1989, Tengjaroenkul et al. 2000). On the last day of the experiment an elevated aminopeptidase M grade was reported in all the nutritional groups in comparison to the first day of the experiment (Table 3), as in Segner et al. (1989) and Gisbert et al. (1999). On the last day of the experiment there was a very strong reaction of aminopeptidase M in the brush border of the anterior intestine in fish fed with nauplii *Artemia salina*, while the weakest reaction was registered in fish fed Cas diet (Table 3).

In the posterior intestine the activity of aminopeptidase M was weaker comparing to the anterior intestine (Table 3). On the last day of the experiment a strong reaction of aminopeptidase M in the brush border of the anterior intestine in fish fed with nauplii *Artemia salina* was observed, while in other groups the

activity of the enzyme experienced no difference and showed the same level as on day one (Table 3). Influence the feeding had on the aminopeptidase M activity in juvenile pike-perch stages was observed in the brush border of the anterior intestine. High activity of this enzyme was present in specimen fed with nauplii *Artemia salina*. As in Segner et al. (1989) it was proven that the larvae of European whitefish (*Coregonus lavaretus*) fed with zooplankton showed higher aminopeptidase M activity than fish fed with commercial fodder. The lowest activity of aminopeptidase M in the brush border of the anterior intestine was to be observed in the pike-perch fed with Cas diet. The low activity of the enzyme can be a proof that the Cas diet does not meet nutritional requirements of the developing pike-perch (Kamaszewski and Ostaszewska 2013).

The presence of non-specific esterase was reported in the gastric epithelium, gastric glands, anterior intestine, posterior intestine and liver of the pike-perch. A similar location of this enzyme in various fish species was described by many authors (Hirji and Courtney 1983, Gawlicka et al. 1995, Gisbert et al. 1999, Kozarić et al. 2006).

On the first day of the experiment the gastric epithelium was reported to show a non-specific esterase activity on a modest level, and the gastric glands – on a weak level (Fig. 1; Table 3). The activity expression of non-specific esterase in gastric epithelium maintained a steady level in all the nutritional groups excluding the Cas group, where the activity of the enzyme declined on the last day of the experiment (Table 3). On the last day, the activity level of the enzyme

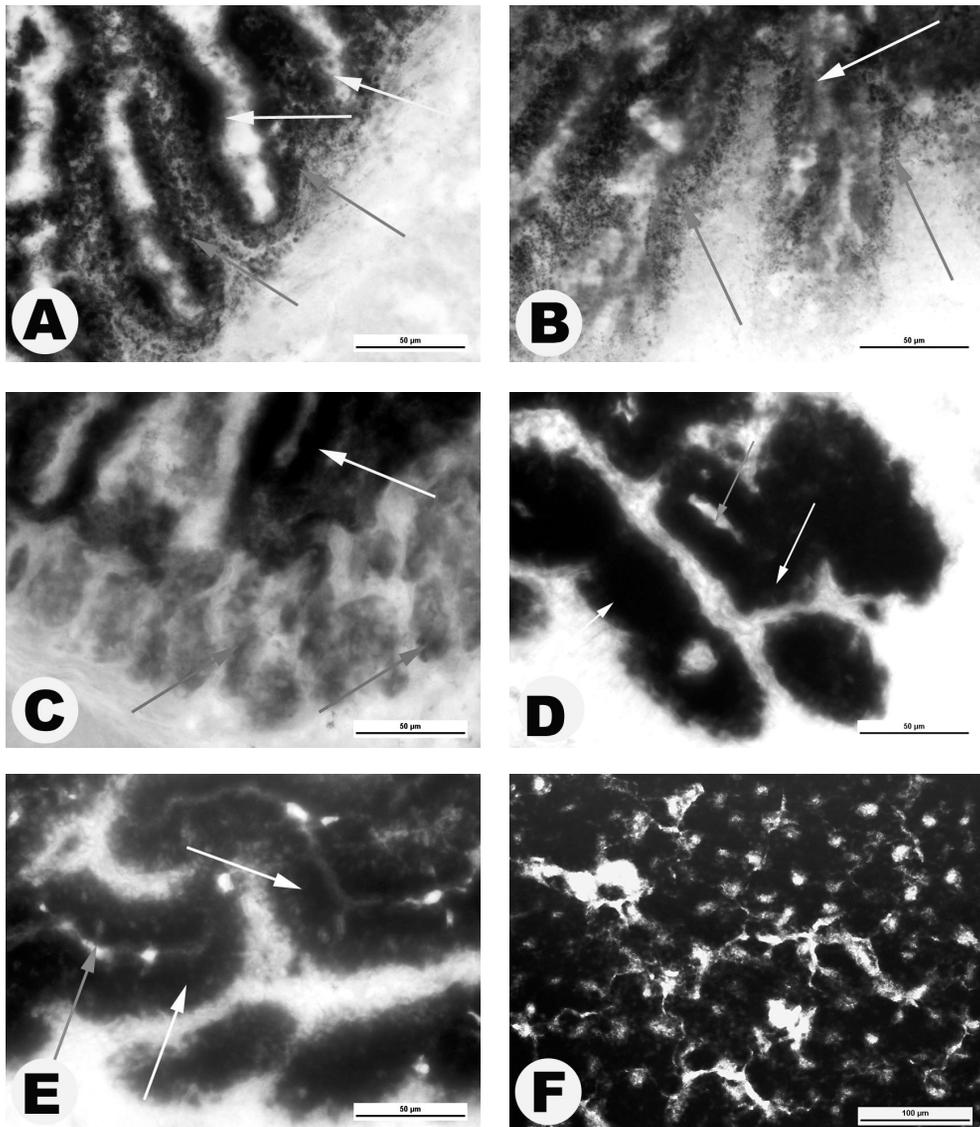


FIGURE 1. Localization of the digestive enzymes in the cross-section of pike-perch gastrointestinal tract. Aminopeptidase M location: in the striated border (white arrows) and in the supranuclear area enterocyte (grey arrows) of the anterior intestine (A) and posterior intestine (B). Non-specific esterase location: in the gastric epithelium (white arrows) and in gastric glands (grey arrows) (C), in the enterocyte cytoplasm (white arrows) and in the striated border (grey arrows) of the anterior intestine (D) and posterior intestine (E). Location of non-specific esterase in hepatocyte cytoplasm (F)

TABLE 3. Location and activity of aminopeptidase M and non-specific esterase in gastric epithelium and gastric glands, anterior and posterior intestines and liver hepatocytes of the pike-perch fed with: nauplii *Artemia salina* (Art), Aglo Norse (An), casein-gelatin (Cas), cod meal with gelatin (Mac) on the first and the last day of the experiment

Enzyme	Distribution of enzymes		1 st day of experi- ment	21 st day of experiment			
				Art	An	Cas	Mac
Aminopeptidase M	Anterior intestine	Brush border	+	+++	++	+	++
		Supranuclear area of enterocyte	+/-	++	+	+	+
	Posterior intestine	Brush border	+/-	++	+/-	+/-	+/-
		Supranuclear area of enterocyte	+/-	+	+	+	+
Non-specific esterase	Stomach	Epithelium	+	+	+	+/-	+
		Gastric glands	+/-	+	+	+	+
	Anterior intestine	Brush border	++	++	+	+/-	+/-
		Supranuclear area of enterocyte	+++	++	++	++	++
	Posterior intestine	Brush border	+	+	++	+	+
		Supranuclear area of enterocyte	++	++	++	++	++
	Liver		+++	+++	+++	+++	+++

was higher in gastric glands in all the nutritional groups in comparison to the first day (Table 3). The non-specific esterase activity increase in gastric glands can be a sign of the stomach being properly developed and carrying out all physiological functions (Gawlicka et al. 1995).

In the anterior and posterior intestine, the non-specific esterase was located in the brush border of the anterior intestine and in the supranuclear area of enterocyte (Fig. 1). On the first day of the experiment a weaker reaction of non-specific esterase in the brush border was reported, while the activity was higher in the supranuclear area of enterocyte of the anterior and posterior intestine (Table 3). In comparison to the first day of the experiment, there was a moderate activity of the enzyme in the brush border

in the pike-perch fed with An diet, and a low activity in the specimen fed with Cas and Mac (Table 3). On the last day of the experiment, the non-specific esterase activity in the supranuclear area of enterocyte declined slightly in all nutritional groups (Table 3). The non-specific esterase activity in the enterocyte brush border of the anterior intestine in fish fed with nauplii *Artemia salina* stayed high from the first to the last day of the experiment (Table 3). In all the other nutritional groups the activity decreased (Table 3). In the brush border of the posterior intestine, the non-specific esterase activity on the last day of the experiment was low, as it was on the first day except for fish fed with An diet, where the activity was slightly higher (Table 3). On the last day of the experiment the

non-specific esterase activity in the supranuclear area of enterocyte of the posterior intestine was on a similar level in all the nutritional groups and did not change compared to the first day of the experiment (Table 3). The non-specific esterase in the pike-perch showed a higher activity in the supranuclear area of enterocyte compared to other fish species (Segner et al. 1989, Baglole et al. 1998, Tengjaroenkul et al. 2000, Kozarić et al. 2006). The non-specific esterase induces and supports the process of pinocytosis (Ribeiro et al. 1999), a mechanism of digesting and absorbing nutrients in the intestine. The non-specific esterase showed signs of lower activity in the anterior and midgut intestine of starving fish (Baglole et al. 1998). The pike-perch fed with Cas and Mac diets were reported to experience a lower non-specific esterase activity in the brush border of the anterior intestine compared to specimen fed with An and Art. The decrease in the activity was probably due to the fact that Cas and Mac diets do not meet the nutritional requirements of the developing fish.

The non-specific esterase activity in liver was on a similar level on the first and on the last day of the experiment (Table 3). High enzyme expression occurred in hepatocyte cytoplasm (Fig. 1). The high activity of the enzyme is associated with intensive lipid and carbohydrate metabolism ongoing in the hepatocyte cytoplasm (Gawlicka et al. 1995, Kozarić et al. 2006).

CONCLUSIONS

The results of present studies prove that feeding the pike-perch An diet has positive effects on their survival, growth rates

and enzyme activity. By contrast, casein-gelatin and cod meal-gelatin diets did not meet the nutritional requirements of the pike-perch, resulting in low survival rate, slow growth rates and low activity of the enzymes examined.

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Streszczenie: Wpływ żywienia na aktywność aminopeptydazy i niespecyficznego esterazy w układzie pokarmowym sandacza (*Sander lucioperca* L.). Sandacze (*Sander lucioperca* L.) w wieku 18 dni były żywione przez 21 dni trzema dietami: Aglo Norse (An), kazeina-żelatyna (Cas), mączka z dorsza z żelatyną (Mac) i naupliusami *Artemia salina* (Art – dieta kontrolna). Ostatniego dnia doświadczenia ryby żywione Art i An miały statystycznie istotnie większą masę i długość ciała oraz przeżywalność. Ostatniego dnia doświadczenia najwyższą aktywność aminopeptydazy w jelicie przednim i tylnym stwierdzono u ryb żywionych naupliusami *Artemia salina*. Najniższą aktywność tego enzymu w jelicie przednim stwierdzono u ryb żywionych dietą Cas, w jelicie tylnym zaś nie stwierdzono różnic między grupami doświadczalnymi. Aktywność niespecyficznego esterazy stwierdzono w żołądku, wątrobie, jelicie przednim i tylnym. Najniższą aktywność tego enzymu w żołądku obserwowano u sandaczy żywionych dietą Cas. W jelicie przednim najwyż-

szą aktywność stwierdzono u ryb żywionych Art, natomiast najniższą u ryb żywionych Cas i Mac. Wyniki obecnych badań potwierdzają, że żywienie sandaczy dietą An korzystnie wpływa na przeżywalność, tempo wzrostu ryb i aktywność enzymów. Żywienie sandaczy dietami Cas i Mac powodowało natomiast niską przeżywalność ryb oraz tempo wzrostu, a także niską aktywność badanych enzymów.

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Changes in the size of population of the European wild boar *Sus scrofa* L. in the selected voivodeships in Poland during the years 2000–2011

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Abstract. *Changes in the size of population of the European wild boar *Sus scrofa* L. in the selected voivodeships in Poland during the years 2000–2011.* The aim of the work was to analyze the changes in the population of *Sus scrofa* L. in the selected voivodeships in Poland in the years 2000–2011 and to determine their direction, with consideration of hunting and level of utilization. The data for analysis were obtained from Research Station of the Polish Hunting Association (PZŁ) in Czemiń. There was found the increase in population of wild boar in hunting season 2011/2012 vs. 2000/2011, including the highest one in the Świętokrzyskie Province (256%) and Małopolska Province (264%) with the simultaneous high rise in hunting (250% and 413%, respectively). The concentration of wild boar population in 6 examined voivodeships, irrespectively of basic size of the population (level of population in season 2000/2001 in the voivodeships from group I (>10 thousand heads) and group II (<1.5 thousand heads) was increased. A moderate level of population utilization occurred to be insufficient what caused a constant progression of the population number in the studies voivodeships in the years 2000–2011.

Key words: wild boar, population, changes

INTRODUCTION

The dynamics of changes in the population of wild boar is determined by environmental factors (Bieber and Ruf 2005, Geisser and Reyer 2005). On the territory of Europe, including also Poland, the increase of the number of the animals of

the discussed species is observed (Bombik et al. 2007). It results from its high adaptative capacities in relation to varying living conditions, change of broad-leaved and mixed forests into coniferous monocultures and from urbanization of natural habitats of wild boars (Podgórski et al. 2013). Wild boars play a positive role in forest environment. The animals of the mentioned species limit the number of harmful insects, which constitute a threat to particular tree species and when burrowing, they prepare a surface of earth to natural renewal of the forest. The wild boars remove dead and sick individuals what decreases the scale of incidence of new sources of diseases and a risk of their spread out (Haber 1969). They may also cause various damages. During digging of the land (burrowing), they dig out small tree seedlings and self-sown plants, destroy forest nursery due to digging out the seeds, they trample forest cultivations and burrow inner forest meadows. The sounders of wild boars cause the anxiety in hunting site and scare the animals of other species, destroy bird nests, built on the ground, eat their hatches and eggs. They consume also weak progeny of other game animals (Haber 1969). The wild boars constitute reservoir of various diseases what creates a threat to human and

animals health (Tropiło 1996, Lipowski 2003, Flis 2011). Sounders of wild boar destroy cultivated fields and grasslands and the urbanized ones on the peripheries of urban agglomerations (Sondej and Jaroszewicz 2010, Matysek 2012) what is connected with the necessity to pay the indemnities (GUS 2013). Environment protection, including population of many species of wild animals, also boars, is connected with the need of creating the passages for animals via quick traffic roads and combining of forest complexes with the aim to create ecological runs (Bobek et al. 2009).

The aim of the work was to analyze the changes in population of wild boar in the selected voivodeships in Poland in the years 2000–2011 and to determine their direction, including hunting and utilization level.

MATERIAL AND METHODS

Materials for research were obtained from the Research Station of PZŁ in Czempin. The results were gained from hunting regions of the Polish Hunting Association. The evaluation of the number of wild boars was carried out by the method of tracking and the whole-year observations (Błaszczuk 2006).

The basis for detailed analysis was constituted by the number of wild boars in six voivodeships. The first group included the voivodeships which possessed the greatest populations (in respect of number) of wild boars (>10,000 heads) in the hunting season of 2000/2001. There were the following voivodeships: West Pomerania, Warmia and Mazury, Wielkopolskie and Lubuskie. Group II covered two voivodeships where the

discussed populations were the smallest ones (in respect of number) in the scale of the country (<1,500 heads), i.e. Świętokrzyskie and Małopolskie. In the compared seasons, i.e. 2011/2012 and 2000/2001 for the mentioned above voivodeships, the analysis of the population size was performed and the results were referred to forest area (head per 1,000 ha) and the scale of hunting. Level of obtaining of wild boars was determined as a sum of animals, being shot during the hunting season, i.e. in the period since 1st April, until 31st March of the next year. Index of the level of utilization of wild boar population in a given season was calculated from the ratio of mean number of the obtained wild boars and the mean number of wild boar population according to the state on 31st March and was expressed in percentage.

RESULTS AND DISCUSSION

In hunting season of 2011/2012 vs. 2000/2001, there was recorded the increase of the number of wild boar population in Poland (Fig. 1). Progression concerned also the analyzed voivodeships (Fig. 2) where the population in seasons 2000/2001 was as follows: group I: West Pomerania – 18,255 heads; Warmia and Mazury – 10,729 heads; Wielkopolskie – 12,985 heads; Lubuskie – 10,030 heads; group II: Świętokrzyskie – 1,314 heads and Małopolskie – 1,314 heads. During the research period, a small decline in production was recorded only twice in the scale of the country. It occurred during hunting season of 2003/2004 and 2011/2012. In opinion of Haber (1969) it is a natural phenomenon for the

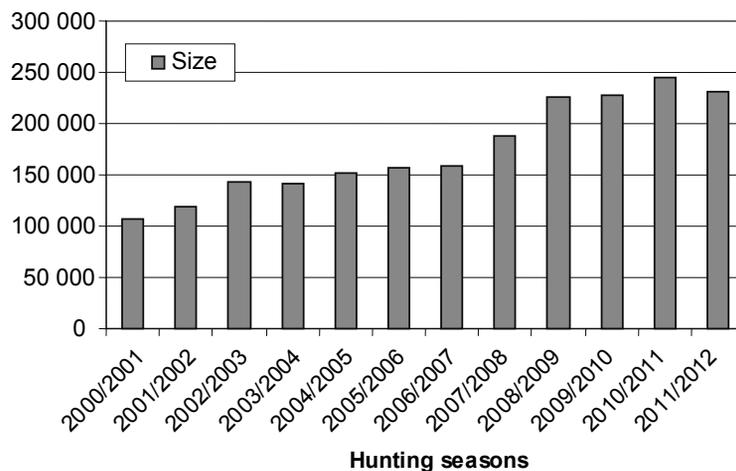


FIGURE 1. Size of wild boar *Sus scrofa* population in Poland in the years 2000–2011 (hunting seasons 2000/2001 and 2011/2012)

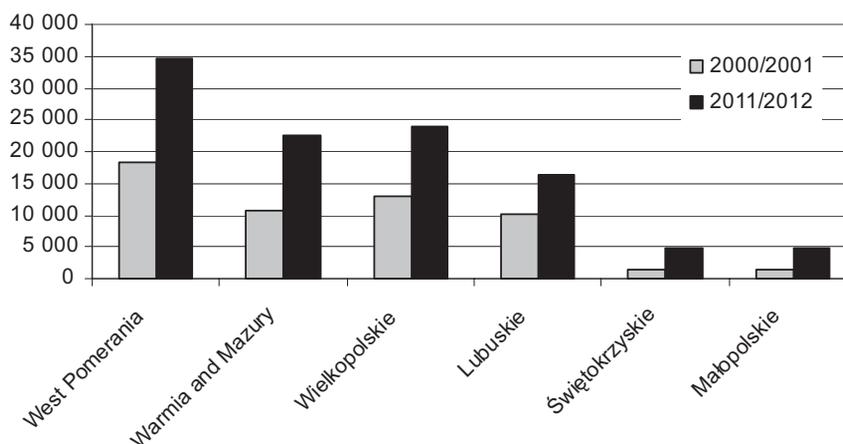


FIGURE 2. Size of wild boar *Sus scrofa* population in the voivodeships from group I and II in hunting seasons 2000/2001 and 2011/2012. Group I – hunting seasons of 2000/2001 – size of population >10,000: Voivodeships: West Pomerania, Warmia and Mazury, Wielkopolskie, Lubuskie; Group II – hunting season of 2000/2001 – size of population <1,500: Voivodeships Świętokrzyskie and Małopolskie

discussed species because during 1–3 years, the number of the population may decline twice or thrice or to become increased without inflow of individuals from other populations. It results from a degree of survivability of squeakers (piglets), intensity of shooting, level of

the annual rate of the population growth, changes in forest economy and climatic changes. The highest increase of the population in the scale of the country had place in the hunting seasons of 2008/2009 and amounted to 37,678 heads as compared to the previous season.

The progressive changes (heads) in the voivodeships from group I were as follows: West Pomerania – 5,077 (16.45%), Warmia and Mazury – 3,855 (19.99%), Wielkopolskie – 4,754 (26.42%), Lubuskie – 1,511 (10.23%) and from group II: Świętokrzyskie – 795 (27.49%) and Małopolskie – 1,137 (39.53%).

After 12 hunting seasons, the number of wild boars in the West Pomerania Voivodeship increased by 90% and in Warmia and Mazury – by 110%; however, the population stayed smaller than in the former voivodeship (i.e. West Pomerania). The population of the discussed animal species was also increased in the Lubuskie and Wielkopolskie voivodeships by 63 and 68%, respectively. In the voivodeships from group II, after 12 hunting seasons, the significant increases of the population size were recorded: Świętokrzyskie – by 256% and in Małopolska – by 264%, what indicates more dynamic changes than in the voivodeships from group I. Such quick increase of the population finds the source in accelerated sexual maturity, high fertility of the species and high natural birth rate (Haber 1969). The wild boars are characterized by a high adaptability to varying living conditions and specific reproduction predispositions (Bieber and Ruf 2005, Podgórski et al. 2013). Moment of obtaining sexual maturity by females is determined by threshold body weight amounting to 30 kg. When possessing abundant feed in their hunting place, the females may reach this state before completing the first year of life. Simultaneously, the better are the living conditions, the better is the survivability of young animals what causes the increase of the number

of population. When the population stays in poor conditions, the juvenile individuals do not receive the possibility of reproduction and the older animals do not loose it. Adult females constitute a reproduction reservoir for the population while the increase of the population is found on the normal level. When the conditions are very good, a considerable participation of the youngest group in the reproduction causes that the increase of the population may be doubled (Bieber and Ruf 2005).

The increasing big-area cultivations of cereals, potatoes and maize constitute the supplementation of natural but constantly decreasing feeding base being favourable for improvement of individual condition of animals. Additionally, the occurring climate changes, the effect of which brings mild winters with a small snow cover, make the survival of weaker individuals easier as their energy expense connected with seeking for feed is smaller (Haber 1969, Servanty et al. 2009). In the opinion of Chojnowski (2005), the mean temperature in winter period (December–February) amounting to ca. 0°C is favourable for increase of the population of wild boar.

Changes in agriculture alter the reproduction capacities of the species. Frequent penetration of maize cultivations and its high participation in diet increase the number of ovulating ovary cells in sows of wild boar. The average number of ripened cells per one ovary amounts to 4–5. When the diet is rich in insulinogenic maize, the number of the cells increases up to 6–7. Additionally, the reproduction potential is increased owing to a good abundance of the soils in bio-elements (Bieber and Ruf 2005, Chojnowski 2005).

During 12 seasons, hunting of the animals of the discussed species has been subject to changes (Fig. 3); in group I it increased as follows: West Pomerania – by 108%, Warmia and Mazury – by 83%, Wielkopolskie – by 93% and Lubuskie – by 68%. The increase of hunting material of wild boars by shooting in the voivodeships from group II was considerably higher: in the Świętokrzyskie – by 250% and in Małopolskie – as much as by 413%.

According to the Supreme Council of the Polish Hunting Association (Uchwała 1999), in the matter of the principles of management of wild boar population in Poland, the correct density of the animals of the discussed species is equal to 20 heads per 1,000 ha of forest area. In hunting season of 2000/2001, the degree of population density in three voivodeships may be recognized as correct – Warmia and Mazury, Wielkopolskie and Lubuskie (Table 1). After twelve

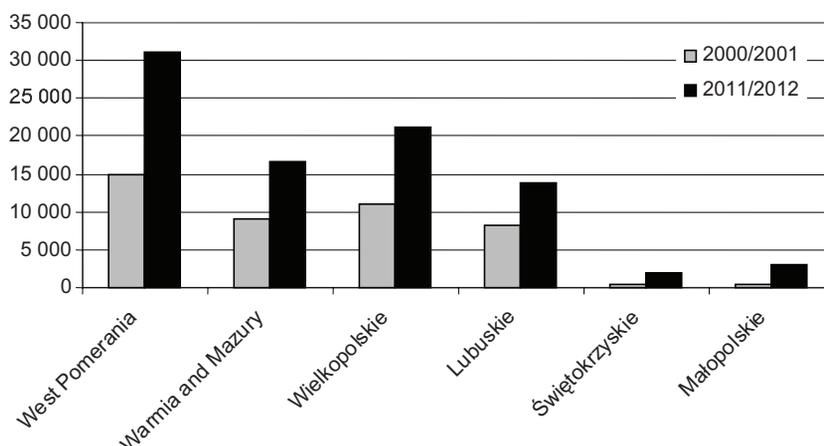


FIGURE 3. Obtaining hunting material of wild boar *Sus scrofa* in the voivodeships from group I and II in hunting seasons 2000/2001 and 2011/2012. Explanations the same as in Figure 2

TABLE 1. Mean density of wild boar population in the voivodeships from group I and II in hunting seasons of 2000/2001 and 2011/2012, in heads per 1,000 ha. Group I – hunting season 2000/2001 – size of population >10,000 heads in the voivodeship; Group II – hunting season 2000/2001 – size of population <1,500 heads in the voivodeship

Group	Voivodeship	Hunting season		Changes in season 2011/2012 vs. 2000/2001
		2000/2001	2011/2012	
I	West Pomerania	27.87	52.61	+88.77
	Warmia and Mazury	18.96	37.79	+99.31
	Wielkopolskie	20.54	36.30	+76.73
	Lubuskie	19.98	30.08	+50.55
II	Świętokrzyskie	4.64	14.77	+218.32
	Małopolskie	3.92	13.57	+246.17

seasons, the density in the voivodeships from group I was increased and was by 1.5–2 times higher than it results from the recommendations of SHC – Supreme Hunting Council (Uchwała 1999). The greatest differences in the size of forest area per one head in the compared seasons were recorded in the Małopolskie Voivodeship; it was by 3.5-fold decrease of forest area.

Burrowing of wild boar in field and forest cultivations increases the aeration of the soil what accelerates the degradation of humus particles and enriches the soil in nitrogen. It facilitates, *inter alia*, quicker natural renewal of forest cultures. On peat territories, burrowing causes, however, oxidation of unique substances present in the peat what disturbs incidence of natural species of plants and creates the conditions for living of invasive species. The wild boars, which live outside the limits of natural habitats of their incidence, may be the reason for degradation of species versatility of plants (Sondej and Jaroszewicz 2010). The degree of penetration of agricultural cultivation areas is determined by the seasons of the year, access of feed in forest habitats, being natural for the

discussed species and humidity in the forests, therefore, the phenomenon of intensity of hunting damages is foreseeable.

The level of utilization of wild boar population has been given in Table 2. In the compared seasons, it was similar in the voivodeships form group, including the fact that in seasons of 2011/2012 in the West Pomerania Voivodeship, it occurred to be the highest one and exceeded the level of 100%; in the Warmia and Mazury Voivodeship, it was the smallest one. The level of utilization of the population in group II vs. I was clearly lower; the differences amounted to ca. 40–60%. Flis (2011) expresses the opinion that hunting on the mean level of 85% is not sufficient and results in further increase of the population in the country. It intensifies the consequences, connected with the discussed phenomenon i.e. hunting damages and penetration of urbanized areas. Under the present environmental conditions, in which good feeding conditions are accessible, hunting size should be established on the level of ca. 100% of the spring number of animals. In exceptionally good habitats for the discussed animal species, the

TABLE 2. Level of utilization of wild boar population in voivodeships from group I and II in hunting seasons of 2000/2001 and 2011/2012. Explanations the same as in Table 1

Group	Voivodeship	Hunting season	
		2000/2001	2011/2012
I	West Pomerania	82.07	101.7
	Warmia and Mazury	84.90	73.95
	Wielkopolskie	84.68	88.02
	Lubuskie	82.62	85.26
II	Świętokrzyskie	39.88	39.31
	Małopolskie	44.52	62.70

increase of the population may reach to 150%, therefore, hunting size should be established on the level of the implemented gain (Flis 2011).

As it was given by Bieber and Ruf (2005), one of the solutions of the problem of the increase of wild boar number in Poland includes management of the population depending on the habitat conditions of animals; it should be diversified. When the population lives in worse environmental conditions, it should be managed via increase of the pressure to hunt sows. In case of correct management, the increase of the population is equal to 130–160%. It is supplemented by structural shooting, as recommended by the Supreme Hunting Council (Uchwała 1999). The goal of the structural shooting of wild boars is to make the population older via directing of hunting pressure to the youngest animals who, at early sexual maturation, cause a double increase of the population; another aim includes improvement of gender structure (females : males) as 1 : 1.1 in favour of male individuals. Shooting of squeakers should reach to 60% of all hunted heads during the hunting season, and it should constitute 2–3 times more than shooting of young animals (piglets). Shooting of the young individuals should amount to 30% and that of the oldest animals – 10% of the plan of hunting size (Uchwała 1999). Structural shooting will result in limitation of negative effects of the increase of the wild boar population in Poland, *inter alia*, decrease of the level of hunting damages in agricultural cultivations, and betterment of biotope utilization by the wild boars. It will have also an influence on the increase on the number of obtained animals.

CONCLUSION

There was recorded an increase in wild boar population in the hunting season of 2011/2012 vs. 2000/2001, including the highest one in the Świętokrzyskie Voivodeship (256%) and in the Małopolskie Voivodeship (264%), with the simultaneous high increase of hunting result (250 and 413%, respectively). The density of wild boar population increased in six examined voivodeships, irrespectively of the basic level of the population (size of the population in 2000/2001 in the voivodeships from group I >10 thousand heads and group II <1.5 thousand heads). The moderate level of utilization of the population occurred to be insufficient what caused a constant progression of the number of the population in the examined voivodeships in the years 2000–2011.

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- Uchwała Nr 106/99 Naczelnej Rady Łowieckiej z dnia 27 kwietnia 1999 roku w sprawie zasad gospodarowania populacjami dzika.

Streszczenie: *Zmiany liczebności populacji dzika europejskiego (Sus scrofa L.) w wybranych województwach w Polsce w latach 2000–2011.* Celem pracy była analiza zmian populacji dzika europejskiego w wybranych województwach w Polsce w latach 2000–2011 oraz określenie ich kierunku z uwzględnieniem pozyskania łowieckiego i poziomu eksploatacji. Dane do analizy udostępniła Stacja Badawcza PZŁ w Czempiniu. Stwierdzono wzrost populacji dzika w sezonie łowieckim 2011/2012 vs. 2000/2001, w tym największy w województwach świętokrzyskim (256%) i małopolskim (264%), przy równocześnie znacznym wzroście pozyskania łowieckiego (250 i 413%). Zagęszczenie populacji dzika wzrosło w sześciu badanych województwach, niezależnie od bazy wielkości populacji (liczebność populacji w sezonie 2000/2001 w województwach z grupy I (>10 tys. szt.) i grupy II (<1,5 tys. szt.). Umiarkowany poziom eksploatacji populacji okazał się być niewystarczający, co spowodowało stałą progresję liczebności populacji w badanych województwach w latach 2000–2011.

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The welfare of horses assessed by the investigations of chosen parameters of the stable microclimate

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Abstract: *The welfare of horses assessed by the investigations of chosen parameters of the stable microclimate.* The performed investigations aimed at assessing the welfare of horses on the basis of the examination of chosen parameters of the stable microclimate. The investigations were carried out at the Wolica horse riding complex of Warsaw University of Life Sciences. The objects of the investigations were two buildings with breeding environments. The investigations were carried out during three seasons: summer, autumn and winter. The investigated basic parameters of the microclimate were: air temperature, relative humidity, air cooling force and movement as well as the type and intensity of lighting. The results were compared with the binding norms. The obtained results show that the parameters agree with the recommendations of animal hygiene exceeding the norms only sporadically. The welfare was maintained.

Key words: welfare of horses, stable, microclimate

INTRODUCTION

Since 1997 The Law on the Protection of Animals (Ustawa 2003) has been in effect in Poland. It regulates the legal situation of animals, point 1 of the Law says that animal as a living creature, capable of suffering is not a thing. The human being should respect, protect and provide care to it as well as humane treatment. Detailed laws, regulating horse breeding can be found in the Regulation of the Minister of Agriculture and Rural Devel-

opment on the minimum conditions for the maintenance of livestock for which protection norms are not defined in the UE regulations which has been in effect since 30 June 2010 (Rozporządzenie 2010).

The main principle of animal welfare is to ensure that animals are hungry or thirsty, free from pain, traumas and diseases as well as from fear, stress and discomfort and are able to behave in their normal way (Kończak and Bodak 1999). While assessing the welfare of animals we should remember the inventive act of March 1928, signed by the President of Poland I. Mościcki (Obwieszczenie 1932) concerning the protection of animals and forbidding their ill-treatment.

At present one can observe the division of the welfare indexes into four groups: physiological, behavioural, health and productive (Moberg 1985, Broom and Johnson 1993). There are also some supplementary indicators such as barn parameters including its microclimate (Kończak and Bodak 1999).

The barn should maintain the optimum microclimate for the animals which ensure their welfare, productivity and effective use. Badly designed or constructed appliances of the building do not allow obtaining satisfactory productive effects, the animals show health

problems and their welfare cannot be fully ensured (Lewandowski 1997, Kołacz 2000, Fiedorowicz et al. 2004).

The performed investigations aimed at assessing the welfare of horses on the basis of chosen parameters of the stable microclimate.

MATERIAL AND METHODS

The investigations were carried out at Wolica stables of Warsaw University of Life Sciences from 21 June 2006 to 16 February 2007. The investigated objects were two stables (new and old) built in the same area where the measurements were also taken. The objects comprised physically separate breeding environments. The evaluation concerned the microclimate of the buildings. An attempt at assessing the conformity of zoohygienic parameters with the binding standards (Rozporządzenie 2010). The current order concerning horse management is very general, so other zoohygienic standards accepted for stables were also used (Rozporządzenie 2003, Fiedorowicz et al. 2004, Kołacz and Dobrzański 2006, Kośla 2011).

The examined period was divided into three measuring seasons: summer, autumn and winter. In each season, at a week's interval, measurements were taken for 5 days with three series of measurements every day (at 7 a.m., 1 and 7 p.m.). The measurements were taken in both neighbouring stables and outside between the buildings.

The measurements included the air temperature, relative humidity, temperature of the dew-point, atmospheric pressure, air cooling force and movement. In each season the light intensity was

measured in the buildings with natural and artificial lighting and outside for the comparison.

Temperature (°C), relative humidity (%), dew-point (°C) and atmospheric pressure (hPa) were measured with a thermo-hygrometer LB-707B with a built-in barometric modulus (produced by LAB-EL). In the stables the panel was installed in the middle of the corridor and the probe was tested at the height of 80 cm from the floor. The measurements of the air cooling force were done with the Hill Katathermometer and the results were also used for calculating the air movement velocity (Kośla 2011). At the same time the air movement velocity was measured with the help of the vane anemometer with digital readout. Illumination (lx) was measured with a light meter. The measurements were taken inside the new and old stable with attention paid to the reading place, i.e. the middle of the corridor, in a sunny box (considered as light) and in a dark box. Also two alternatives were considered: measurements taken with a natural light source (function "S") and artificial light source (function "F"). The photocell was installed in six positions: parallel to the ceiling and floor and to four walls/barriers and the mean result was calculated (Janowski 1978, Kośla 2011). On the basis of the results of light intensity measurements in the buildings and outside, the room lighting coefficient was calculated (Kołacz and Dobrzański 2006, Kośla 2011).

Statistical analysis was done using the Statistica 5.0™ programme, ANOVA module. The significance of differences between the experimental groups was calculated using the LSD test (least significant differences) or Tukey's test.

RESULTS AND DISCUSSION

The new stable was built in 2003 together with the adjacent riding school. A shorter wall of the school adjoins the long stable wall from the north-east side. Due to that there are no windows on that side of the stable so one row of boxes is lighted only with artificial light. Hopper windows (18) are only on the south-west side at the height of 2.15 m from the floor. It is a safe height, so the window panels do not need any extra protection. However, their location near the roof limits the natural light inflow (Janowski 1978, Kołacz and Dobrzański 2006, Kośła 2011).

The location of windows only on one wall caused the uneven lighting with natural light of the entire stable. Boxes under the windows are clearly lighter than those situated on the other side. Results of the intensity of lightening confirm that phenomenon (Table 1). Artificial lightening decreases light deficit, however, it results from the observations that artificial light was turned on only when the light was needed by the users of horses (for grooming or saddling a horse before the

training). Each window is of a rectangle shape with the glass surface of 0.68 m². There are two windows in each box.

The building interior lightening was calculated from the ratio of the window surface to the floor and it amounted to 1 : 21.9. Lightening standards for breeding horses and for competitive horses is 1 : 10 (Jodkowska 2007) or 1 : 12 (Kośła 2011) and for other horses it is 1 : 15 (Jodkowska 2007). Only for working horses which most of the day spend outside the stable the accepted ratio can be 1 : 20 (Kośła 2011).

The old stable is an adapted farm building. Windows are on both long walls and on a short south-west wall. Their shape is a standing rectangular of 122 cm over the floor on the short wall (7 windows) and 172 cm on the long walls (12 windows). Due to a low location of windows they are protected with metal bars. The surface of glass panels is 0.42 m² (7 windows) and 0.62 m² (12 windows). The degree of stable lightening calculated as a ratio of window to the floor surface amounted to 1 : 46.7. It shows that the window surface is too

TABLE 1. Seasonal average light intensity (lx)

Light	Corridor		Dark box		Sunny box		Outside
	the new stable	the old stable	the new stable	the old stable	the new stable	the old stable	
The summer season							
Natural	37.0	114.0	4.2	75.0	68.8	98.3	8 850.0
Artificial	138.8	106.7	13.8	49.8	61.3	95.8	8 850.0
The autumn season							
Natural	20.5	10.7	2.5	34.5	103.7	57.3	1 360.0
Artificial	138.3	41.3	13.0	40.7	118.2	71.0	1 360.0
The winter season							
Natural	1.4	11.0	0.5	30.3	5.6	36.8	780.0
Artificial	13.8	46.8	1.7	67.2	7.3	80.0	780.0

small in relation to the recommended standards (Kołacַz and Dobrzański 2006, Jodkowska 2007, Pirkelmann et al. 2010, Kośla 2011). Table 1 shows a comparison of results describing light conditions inside and outside the stables depending on the site of the measurements taken distinguishing between natural and artificial light. The obtained results confirm the division into the, so-called, light and dark side of the stable (Fig. 1).

ference in temperature between the new and old stable at 7 a.m. in autumn is significant at $p \leq 0.05$. The remaining values of temperature in the new and old stable did not show any significant differences. In winter the temperature drop below the required minimum value was observed a few times but it was more frequent in the old stable. On the other hand, the tendency to exceed the maximum value in summer was revealed by the results of

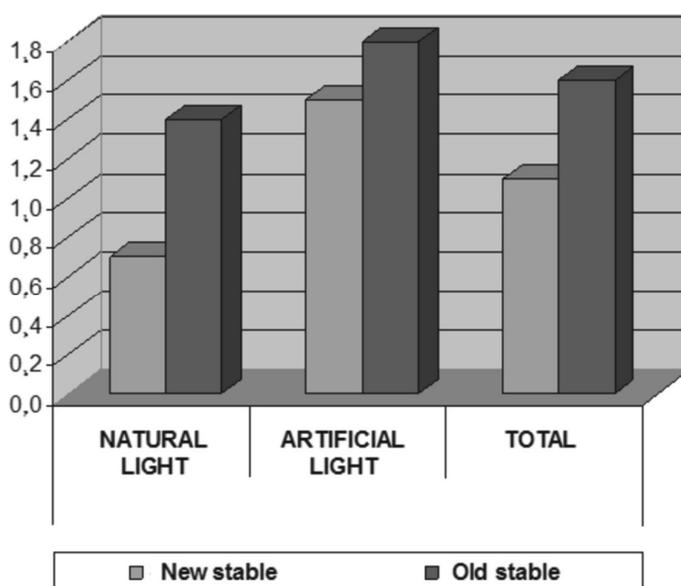


FIGURE 1. Room lighting index (%)

Good stable conditions ensuring animal welfare include a complex of physico-chemical parameters of the building microclimate. The most important of them from the animal hygiene point of view are: air temperature and humidity, air movement and its cooling force, atmospheric pressure and lightening.

The averaging results of the temperature measurements in the new and old stable and outside are presented in Table 2. Statistical calculations show that the dif-

ference in temperature between the new and old stable at 7 a.m. in autumn is significant at $p \leq 0.05$. The remaining values of temperature in the new and old stable did not show any significant differences. In winter the temperature drop below the required minimum value was observed a few times but it was more frequent in the old stable. On the other hand, the tendency to exceed the maximum value in summer was revealed by the results of

the measurements taken in the new stable. The optimum scope of temperatures recommended for horses which do not cause any welfare disturbances is from 5 to 28°C (Rozporządzenie 2003, Kołacַz and Dobrzański 2006). Horses tolerate lower values of temperature much better than higher both while resting, moving or during activities. In the moderate values of temperature the mechanisms and processes responsible for the thermal balance are

TABLE 2. Seasonal values of air temperature (°C) at 7 a.m., 1 and 7 p.m.

Air temperature (°C)	07:00			13:00			19:00		
	the new stable	the old stable	outside	the new stable	the old stable	outside	the new stable	the old stable	outside
The summer season									
Minimum	21.8	20.7	23.7	23.8	25.2	33.1	24.7	23.9	21.2
Average	23.0	22.2	26.0	26.0	22.5	37.0	27.0	26.1	25.9
Maximum	25.2	23.3	29.8	28.5	29.25	40.1	28.6	28.1	28.6
The autumn season									
Minimum	12.5	4.5	1.0	3.9	4.0	1.6	7.6	5.5	0.9
Average	13.9	8.7	4.6	12.6	11.6	11.0	12.4	10.8	7.7
Maximum	15.9	11.8	9.2	16.8	16.5	16.2	16.0	16.0	13.3
The winter season									
Minimum	4.6	2.9	-0.2	5.3	2.6	0.2	2.5	3.5	-1.3
Average	8.3	5.6	1.5	6.3	4.9	2.5	5.8	5.1	1.4
Maximum	11.3	7.4	2.9	7.5	7.0	4.1	9.6	7.5	3.7

able to maintain the organism temperature within a safe range (Wolski 1987, Golachowski 2005).

Air humidity and temperature in the farm buildings are particularly important for the welfare and health state of horses (Kończak and Dobrzański 2006, Kośla 2011). In case of the air temperature drop its relative humidity increases. A very thorough measure of air humidity is the comparison of air temperature and dew-point temperature (Kończak and Dobrzański 2006, Kośla 2011). The determined upper limit of the relative humidity for horses in Poland is 80% (Lewandowski 1997, Kończak and Dobrzański 2006, Kośla 2011).

A too high humidity level affects unfavourably the microclimatic conditions in the building and affecting negatively

the elements of its construction may cause biodegradation of the building materials (Wiśniewska 2005).

Mean relative humidity values for stables and seasons are compared in Table 3. No significant differences were noted in the air relative humidity values in the new and old stable. Air humidity showed an increasing tendency in the afternoons and evenings reaching in one of the measurements the acceptable value of 80% – Figure 2 (Fedorski 2003, Fiederowicz 2006, Kończak and Dobrzański 2006).

The dew-point phenomenon affects in a significant way the humidity conditions in the farm building and thus the animal welfare. In the case when cold atmospheric air gets into the stable through the ventilators then the dew-point

TABLE 3. The mean values for relative humidity (%) in different seasons at 7 a.m., 1 and 7 p.m.

Season	07:00			13:00			19:00		
	the new stable	the old stable	outside	the new stable	outside	the old stable	the new stable	the old stable	outside
Summer	68.79	64.94	52.60	46.35	48.38	26.32	48.48	50.16	45.13
Autumn	73.58	68.80	86.01	65.82	67.92	66.81	64.82	69.25	75.54
Winter	70.60	72.00	86.76	68.46	73.44	80.34	70.40	71.36	82.20

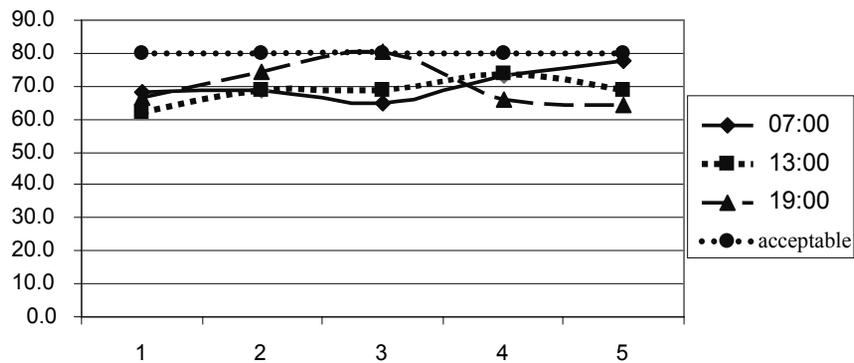


FIGURE 2. The values of relative humidity (%) in the new stable in the winter season

temperature may be exceeded, especially when the horse density is high (Kořla 2011). It leads to the cooling of their organisms and their susceptibility to cold increases. A significant difference of the dew-point temperature (Table 4) in the new and old stable was observed in the autumn season at 7 a.m. ($p \leq 0.05$).

The results of the LSD test confirm the statistically significant differences in the air cooling force (Table 5). A highly significant difference ($p \leq 0.01$) between the cooling force in the new and old stable was noted in the summer season at 7 a.m. and in the autumn season also at

7 a.m. In the winter season no statistically significant differences were observed. While comparing the air movement velocity in the new and old stable (Table 6), the obtained results proved to be statistically significant at 7 a.m. in the summer season at $p \leq 0.01$ and in the autumn season at the significance level $p \leq 0.05$.

Mean values of atmospheric pressure for a given season formed a characteristic pattern. The highest readings were obtained in the summer season, a bit lower in the autumn season and the lowest in winter.

TABLE 4. Seasonal values the temperature of dew point (°C) at 7 a.m., 1 and 7 p.m.

Temperature (°C)	07:00			13:00			19:00		
	the new stable	the old stable	outside	the new stable	the old stable	outside	the new stable	the old stable	outside
The summer season									
Minimum	15.3	12.7	12.7	9.2	12.0	7.7	11.5	12.0	7.6
Average	17.2	15.3	15.3	14.0	14.7	13.1	14.8	14.5	12.3
Maximum	19.5	18.2	17.5	19.8	20.3	19.6	18.2	18.3	17.2
The autumn season									
Minimum	6.8	-1.4	-1.9	-0.4	0.0	-0.5	0.4	-1.1	-3.5
Average	9.3	3.3	2.6	6.3	4.8	4.7	5.9	5.3	3.9
Maximum	12.1	7.2	6.8	11.6	10.9	10.3	11.0	11.5	10.5
The winter season									
Minimum	-0.6	-1.6	-2.2	0.0	-1.1	-1.9	-3.3	-0.9	-4.2
Average	2.7	0.5	-0.7	0.7	0.3	-0.7	0.1	0.1	-1.7
Maximum	6.7	4.1	2.0	3.2	3.5	2.1	3.6	2.3	2.2

TABLE 5. The average values of cooling from katathermometer ($W \cdot dm^{-2}$) at 7 a.m., 1 and 7 p.m. in different seasons

Season	Cooling ($W \cdot dm^{-2}$)								
	07:00			13:00			19:00		
	the new stable	the old stable	outside	the new stable	the old stable	outside	the new stable	the old stable	outside
Summer	1.82	2.52	2.14	1.99	1.53	1.57	2.12	1.95	2.68
Autumn	3.22	4.80	6.87	4.75	3.88	6.02	4.07	4.03	6.61
Winter	3.76	4.67	8.96	4.45	4.85	8.43	4.71	4.94	7.43

TABELA 6. The average and maximum values air speed ($m \cdot s^{-1}$) at 7 a.m., 1 and 7 p.m.

Air speed ($m \cdot s^{-1}$)	07:00			13:00			19:00		
	the new stable	the old stable	outside	the new stable	the old stable	outside	the new stable	the old stable	outside
The autumn season									
Average	0.13	0.29	0.71	0.54	0.22	0.79	0.27	0.20	0.80
Maximum	0.28	0.46	1.58	1.42	0.62	1.28	0.46	0.41	1.32
The winter season									
Average	0.10	0.17	1.13	0.16	0.18	1.01	0.19	0.22	0.65
Maximum	0.25	0.32	2.09	0.33	0.28	2.03	0.46	0.56	1.68

CONCLUSIONS

1. Thermal conditions in both stables do not always agree with the standards. In the winter season a temperature drop below the required minimum value was observed a few times, more frequently in the old stable. On the other hand, in summer a tendency to exceed the maximum value was observed in the new stable.
2. Low intensity of natural light caused by architectural causes should be compensated with artificial light. Unfortunately most of the day horses were kept in the stable with the lights turned off. Especially in the new stable boxes without windows should be additionally lighted.

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Streszczenie: *Dobrostan koni oceniony z wykorzystaniem badań wybranych parametrów mikroklimatu stajni.* Celem przeprowadzonych badań była ocena dobrostanu koni na podstawie badania wybranych parametrów mikroklimatu stajni. Badania i pomiary przeprowadzono na terenie kompleksu hippicznego Wolica SGGW. Obiektami badań były dwa budynki stajni wykazujące różnice pod względem pierwotnego przeznaczenia, położenia, wymiarów, oświetlenia, warunków mikroklimatycznych. Badania zostały przeprowadzone w okresie trzech sezonów roku: letnim, jesiennym i zimowym. Oceniono czynniki kształtujące mikroklimat stajni, tj. temperaturę powietrza,

wilgotność względną, prędkość i siłę oziębającą powietrza, rodzaj i intensywność oświetlenia, a otrzymane wyniki zestawiono z obowiązującymi normami. Uzyskane wyniki wskazują, iż parametry są zgodne z zaleceniami zoohigieny i tylko sporadycznie przekraczają normy. Dobrostan był zachowany.

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The welfare of horses assessed by the zoohygienic inventory method

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Abstract: *The welfare of horses assessed by the zoohygienic inventory method.* The performed investigations aimed at assessing the welfare of horses in accordance with the rules and methods of zoohygienic inventory. The investigations were performed and the measurements taken at the Wolica riding complex of Warsaw University of Life Sciences. The basic parameters creating the microclimate were evaluated in the research described in the paper “The welfare of horses assessed by the investigations of chosen parameters of the stable microclimate” published in the present issue of Animal Science. Using the rules and methods of zoohygienic inventory the current paper presents the farm buildings, their equipment, ventilation system and closest surroundings in which the horses are kept. The investigations included the concentration of ammonia in the stables, to demonstrate the effectiveness of drains and ventilation. The obtained results were compared with the binding standards. The obtained results show that the parameters agree with zoohygienic recommendations and exceed the standards only sporadically. The welfare of animals was maintained.

Key words: welfare of horses, stable, zoohygienic inventory, ventilation, ammonia concentration

INTRODUCTION

Since 28 June 2010, horse management has been regulated in Poland by the Regulation of the Minister of Agriculture and Rural Development on the minimum conditions for the maintenance of livestock for which the protection norms are not defined in the UE regulations (Rozporządzenie 2010). Horses in the

stable should be kept on the bedding in boxes, tying stalls or loose housing without tying. The surface of the box for adult horse with the height at withers of over 1.47 m should amount to at least 9 m² and for the mare with foal – 12 m². The air cycle, degree of dust loading, and concentration of gases is maintained at the level harmless for animals.

At present the assessment of the animal welfare includes the, so-called, supplementary indexes, namely parameters of the farm building, efficiency of the ventilation system and the way of the movement restriction of animals (Kończak and Bodak 1999). The possibility of ensuring the proper management conditions for the animals including the minimized content of ammonia additives is a criterium of a proper structure of the farm building (Lewandowski 1997). In order to prevent mechanical injury, the stables as well as paddocks should meet the safety rules preventing mutilations, injuries and other factors dangerous for the health and life of horses (Fiedorowicz et al. 2004).

The aim of the investigations was the assessment of the welfare of horses kept in the Wolica stable in accordance to the rules and methods of zoohygienic inventory. The zoohygienic inventory was performed and the ventilation functioning and ammonia concentrations were evaluated.

MATERIAL AND METHODS

The investigations were performed in the Wolica stables of Warsaw University of Life Sciences from 21 June 2006 to 16 February 2007. The investigation objects comprised two stables in the same area. An attempt to assess the compatibility of zoohygienic and zootechnical parameters in relation to the binding standards (Kończak and Dobrzański 2006, Rozporządzenie 2010, Kośła 2011). The basic dimensions, location of the stables, paddocks, stud accommodations (riding school, lungeing space), their position relative to each other and cardinal points were defined. In order to assess the buildings the measurements of the doors, windows, floors, boxes, partitions, ventilation openings, the width of passages and corridors were taken. It was checked whether the mangers and water-bowls are placed at the proper height. It was assessed whether the applied solutions are functional and meet the safety requirements and whether they do not upset the horse state of welfare. The method and frequency of the change of bedding and general state of cleanness in the stables were evaluated. The paddocks were also assessed. It was checked whether the horses had access to water both in boxes and paddocks and the hygiene of mangers and water-bowls was also examined. The functioning of the gravity ventilation was estimated. To demonstrate the effectiveness of drains and ventilation was conducted measurement of the ammonia concentration. The measurement of the ammonia concentration was taken in both stables in the middle of the corridor at 7 a.m. using the gas detector WG-2 and gauge tubes (Kośła 2011).

Statistical analysis of ammonia concentration was done using the programme Statistica 5.0™, ANOVA modulus. The significance of differences between experimental groups was calculated with the help of the LSD or Tukey's tests.

RESULTS AND DISCUSSION

Stables, riding schools, paddocks and additional buildings form the didactic-breeding complex of Warsaw University of Life Sciences situated at Wolica, near Warsaw.

The complex of stables and paddocks is situated on the escarpment, in a relatively level area. The driveway is hard-surfaced. The entire area is fenced and secured. Horses which are kept in the new and old stable are mainly taken care of by students who are interested in horse riding. The staff has the basic knowledge necessary for proper performing the stable work, feeding the horses, cleaning boxes, turning out horses to the paddocks and everyday grooming. In the stable one can notice that everything is kept in order, feeding times are observed and any improprieties or happenings are immediately taken care of. During the performed investigations no cases of aggression or harassment were noted. Horses were well treated and the work load connected with riding is rationally planned. Animals showing any alarming health signs are immediately examined by a veterinary surgeon. Physical and psychological condition of horses does not arouse suspicion as to the correctness of looking after them (Pirkelmann et al. 2010).

The new stable was built in 2003 together with the adjoining riding school. The stable is made of brick, with a tiled roof and the attic is used for farm purposes. The building is located with its longitudinal axis in the south-east direction, it is of 10.5 m in width and 38.0 m in length. The height of the building measured inside is 3.0 m. The building can be entered through a double door with the total measurement of 2.2 m in width and 2.9 m in height. Additionally the door is barred which allows opening the door which protects the horses against the unfavourable temperature increase. Behind the door there are the special rooms: saddle-room, scrubbing room, locker room and utility room. Inside the stable there two rows of loose-boxes for horses. Between them there is a corridor of 2.5 m in width. The floor in the corridor and driveway is paved with the concrete blocks and wooden blocks are used in the boxes. Between the corridor and the line of boxes, there is a dung channel of 14 cm in width protected with a grid from above.

Boxes are situated in two rows: 9 boxes on the north-west side and 8 boxes on the other side. The boxes are of the same size – 2.9 m in width and 3.9 m in length which makes the total surface of 11.31 m² per horse. It agrees with the standards (Kołac and Dobrzański 2006, Rozporządzenie 2010). Box structure is based on the steel-wooden construction which can be easily disassembled. Partitions and doors up to 120 cm in height are made of wooden boards and above them there is an openwork part of 105 cm in height which agrees with standards (Kołac and Dobrzański 2006).

Every other partition is built of bricks up to 120 cm and has a heater installed.

Through the verticale bars between boxes the horses can see each other and air circulation in the stable is easier which agrees with the principle of the horse welfare (Kołac and Dobrzański 2006). The boxes have sliding doors of 1.1 m in width. Kołac and Dobrzański (2006) report that they cannot be narrower than 1.3 m. Their upper barred part has a hinge allowing opening it in such a way that the horse may lean its head out to the corridor. Inside each box there are a rotating manger, an automatic water-bowl and a rock-salt installed about 100 cm over the floor which allows their welfare (Kołac and Dobrzański 2006). Rotating manger allows quick and efficient feeding the horses with bulky food without the necessity of entering each box. Automatic water-bowl assures permanent access to fresh water. Horses are kept on the bedding which is cleaned every day, i.e. excrements and wet bedding are removed systematically. Hay is delivered directly on the bedding in the corner of a box. Feeding the concentrate is done three times a day. Halter is screwed to each box as well as the folding (for safety reason) saddle rack.

A system of gravity ventilation was installed in the stables – there are square ventilatory openings in the ceilings whose side is 14 cm. They are located perpendicular to the long walls 4 openings in a row situated over every second partition in such a way that they collect the air from two neighbouring boxes. There are 4 rows of the exhausting ventilation openings.

The old stable is a farm building which was rebuilt and adapted for

a stable in the 1970s. The long axis of the stable runs from north-east to south-west.

The object is made of bricks with the attic used as store-room for bulky feed (hay) and bedding (straw). The roof is made of wood covered with roofing paper. The stable is 55.0 m in length and 9.5 m in width. The height from the floor to the ceiling is 2.95 m. The stable can be entered from three sides: the main doors on the edges of the building are 2.1 m in width and 2.4 in height and a double door in the middle of the building on the south-east side. Similarly as in the new stable the doors have an additional grating installed. On the north-east end of the stable the utility and social rooms are located as well as the saddle room and sanitation facilities which take up about 10.8 m of the entire building. There are two doors to the utility room, one from the outside and one from the inside. There is a comfortable driveway for a tractor with a trailer. Hay and straw are systematically thrown down manually onto the middle of the corridor through the chute opening in the roof. Inside the stable there was a feeding and dunging passage of 2.26 m in width and two rows of boxes located on its both sides. On the junction of corridor and boxes there is a shallow (3 cm in depth) sewage groove. Floor in the corridor is paved with the concrete blocks and boxes have concrete floors. Horses are kept on the bedding with the same method of cleaning them as in the new stable. Boxes are also located in two rows along the long walls. Their measurements are not the same varying from 8.5 to 14.1 m² which means that in the case of the smallest boxes the standards are not met (Kořacz and Dobrzański

2006, Rozporządzenie 2010). Partitions between boxes are made of a solid wood up to the height of 117 cm and the barred part is of 135 cm in height over the solid part which disagrees with the standards because the solid partitions should be of 140 cm in height (Kořacz and Dobrzański 2006, Pirkelmann et al. 2010). Front walls of the boxes from the side of the corridor are of the same measurements, i.e. the solid part up to 142 cm and the barred part over it up to 113 cm. In each box there are a rotating manger, an automatic water-bowl and a rock-salt installed about 90 cm over the floor. The ventilation in the building is of a gravity type. There are four exhaust shafts with the surface of 65 × 55 cm. These shafts end with a roof ventilator.

The results of the measurements of the air movement in the ventilation shafts in the new and old stable are compared in Table 1. In the summer season the gravity ventilation did not work in both stables, it worked better in the autumn and winter seasons when the difference in temperature values inside and outside the buildings was bigger than in summer. More often the air movement was noted in the exhaust shaft in the old stable than in the new one.

In the old stable a treatment surface of the 3.6 × 4.1 m is separated. It is parallel to the corridor and it is used for shoeing of horses, grooming, saddling and veterinary treatments.

Between the old and new stables there is a paddock of 1,440 m² in size (60 × 24 m). The fencing is made of a metal tube whose upper border is at the height of 100 cm. Directly behind that paddock there is another, bigger paddock of the 1,875 m² (75 × 25 m). Fencing is made

TABLE 1. The air movement in the ventilation shafts in autumn and winter season at 7 a.m., 1 and 7 p.m.

Date of measurement	Air movement					
	07:00		13:00		19:00	
	new stable	old stable	new stable	old stable	new stable	old stable
Autumn season						
12/10/2006	0.00	0.00	0.04	0.00	0.00	0.00
19/10/2006	0.00	0.00	0.00	0.00	0.00	0.00
26/10/2006	0.00	0.00	0.08	0.00	0.00	0.00
02/11/2006	0.00	0.00	0.00	0.00	0.00	0.00
09/11/2006	0.00	0.00	0.00	0.44	0.04	0.08
Winter season						
01/02/2007	0.04	0.41	0.62	0.22	0.04	0.27
07/02/2007	0.00	0.22	0.00	0.08	0.00	0.27
09/02/2007	0.00	0.22	0.22	0.27	0.04	0.04
14/02/2007	0.04	0.04	0.00	0.00	0.00	0.00
16/02/2007	0.00	0.22	0.00	0.41	0.00	0.27

of the same material but the height was 130 cm with an additional tube at the 70 cm in height (Sasimowski 1984). The two level fencing does not allow the horses to get out (Zwoliński 1983). Corners of the paddocks are cut. It prevents the horses to bunch together in the corners which could be dangerous during establishing the social hierarchy or during the horse panic (Jodkowska 2007). While building the new stable and the riding school, the paddock surface was improved and covered with coarse sand mixed with gravel. Unfortunately in the ground there are also some stones of several centimeters in diameter which increase the danger of crippling the horse's foot. Also the smaller stones of the gravel are dangerous because they may become stuck between the shoe and the hoof, inducing a pressure. During warm days plastic portable water-bowls kept full with water are placed on the paddock. All horses use the accessible paddocks (Hansen

2004) and their rotation is frequent and time consuming. There are no green pastures for horses.

Behind the new stable there is a dung pit. It has a concrete bottom and a meter high walls in accordance with the rules of environmental protection (Runowski et al. 2006). Next to both stables, on the paved surface the washing stand is situated. A corridor connects the riding school with the stable. It has a separate gallery, a mirror on a part of the long wall, oblique bands and elastic surface made of sand fraction and sawdust. The riding complex also include a horse exerciser and a roofed lunging ring.

The results of the investigations of ammonia concentration in the stable are presented in Table 2. The obtained values do not show the overstepping of the accepted standards for that compound in the stable air. The norm amounts to 20 ppm (Kołac and Dobrzański 2006, Kośla 2011).

TABLE 2. Seasonal maximum values of ammonia concentration in the stable air at 7 a.m.

Season	Ammonia concentration		
	new stable		old stable
	mg·m ⁻³	ppm	mg·m ⁻³
Summer	5.0	7.1	0.0
Autumn	3.1	4.4	0.0
Winter	5.1	7.2	0.0

CONCLUSIONS

1. The conditions of horse keeping in the stables of Warsaw University of Life Sciences – Wolica are correct and do not disturb in a significant way their welfare either in the physical or psychological state. On the basis of the performed investigations it can be stated that the horses are kept at a good level of their welfare.
2. The building a new stable, despite its high aesthetic qualities, is not functional in respect to its feed storage. There is no utility attic.
3. The system of gravity ventilation in the new stable did not function properly or effectively.
4. The presence of ammonia in the of the air new stable was detected, however, its level did not exceed the permissible standards.
5. The total surface of the paddocks is too small in relation to the number of horses kept in both stables. Horses use them for a short time because the rotation is high. The gravel on the paddock ground is too coarse and contains unwanted stones.
6. Horses have no access to pastures which is improper in view of their ethological needs

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Streszczenie: *Dobrostan koni oceniony metodą inwentaryzacji zoohigienicznej.* Celem przeprowadzonych badań była ocena dobrostanu koni zgodnie z zasadami i metodyką inwentaryzacji zoohigienicznej. Badania i pomiary przeprowadzono na terenie kompleksu hippicznego Wolica SGGW. Podstawowe parametry tworzące mikroklimat oceniono w „Dobrostan koni oceniony z wykorzystaniem badań wybranych parametrów mikroklimatu stajni” opublikowanym w aktualnym numerze *Animal Science*. W niniejszej pra-

cy, postępując zgodnie z zasadami i metodyką inwentaryzacji zoohigienicznej, opisano budynki inwentarskie, ich wyposażenie, system wentylacji i najbliższe otoczenie, w którym utrzymywane były konie. Zbadano stężenie amoniaku w pomieszczeniach, a otrzymane wyniki zestawiono z obowiązującymi normami. Uzyskane wyniki wskazują, iż badane elementy środowiska koni były zgodne z zaleceniami zoohigieny i tylko sporadycznie przekraczają normy. Dobrostan pod tym względem był zachowany.

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Effect of cow's breed and feeding season on the content of bioactive whey protein of milk produced according to principles of the biodynamic farming

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Abstract: *Effect of cow's breed and feeding season on the content of bioactive whey protein of milk produced according to principles of the biodynamic farming.* The aim of this study was to determine genetic effect of cow breed and feeding season on content of bioactive whey protein of cow's milk. In the selected biodynamic dairy farm, cows of two different breeds, i.e. Brown Swiss (BS) and Holstein-Friesian black and white (HF) were kept under the same environment conditions. The samples of milk were collected twice (in summer and winter) from 30 cows (in the same for number from BS and HF). Content of following whey protein: β -lactoglobuline (β -LG), α -lactoalbumine (α -LA), lactoferrin (Lf), lactoperoxidase (Lp), lysozyme (Lz) and bovine serum albumine (BSA) were determined used RP-HPLC technique. The studies showed a significant effect of breed and feeding season on the content of whey proteins in milk. The average content of whey proteins in milk of cows ranged from 0.73% for the HF breed in winter to 0.84% for the BS breed in summer. Important differences weren't stated in the content of lactoferrin and lactoperoxidase in the milk, and for her the content fluctuated from 0.201 to 0.259 g/l and from 0.111 to 0.154 g/l appropriately at examined breeds. There were no statistically significant differences in the level of α -lactoalbumine in the milk of cows. Its content was ranged from 1.663 to 1.994 g/l. Statistically important differences were shown in conducted experience for the breeds and the feeding season ($P \leq 0.01$) for the content of β -lactoglobuline from BS cows in the period of summer

feeding was 5.38 g/l, however reduced to the level herself by the winter 4.62 g/l. The similar trend was shown in the milk of HF cows in the summer season the concentration of this white was 4.60 g/l, and in the winter season underwent lowering up to 4.41 g/l. The experiment demonstrated statistically significant differences for the breed and feeding season for the content of β -lactoglobuline in cow's milk.

Key words: whey protein, β -lactoglobuline, RP-HPLC, breed, season

INTRODUCTION

The main purpose of the biodynamic farming is production functional food, rich in bioactive components important to humans health. Whey proteins have unique properties, beyond their importance in nutrition; they exhibit chemical, physical, physiological, functional and technological usefulness (McIntosh et al. 1998). The term of whey proteins has been used to describe the group of milk proteins that remain soluble in milk serum or whey after precipitation of casein at pH 4.6 and temperature 40°C. Traditionally, β -LG, α -LA, bovine serum albumine (BSA), immunoglobuline (Ig),

and proteose-peptone fractions have been considered the major characterized components of this fraction. Probably many factors affect the whey protein composition of bovine milk, including genetic and environmental (Metera et al. 2010).

In the literature available no information was found about whey protein content of milk from Brown Swiss (BR) and Holstein-Friesian (HF) cows kept in a certified organic farm in Poland. This is a new solution and makes it possible to analyze changes occurring in content whey protein in cow's milk caused by the biodynamic farming.

MATERIALS AND METHODS

The experiment was conducted at the biodynamic farm of Juchowo, which is located in a small village of Juchowo (north-western Poland) – about 90 km from the Baltic Sea. The owner of the farm is a public benefit foundation – the Stanisław Karłowski Foundation, founded in 2001. From a herd of about 300 animals, maintained in a free-stall dairy shed, two groups of 15 were selected by the analogue method: BS and HF cows, taking into consideration the stage of lactation (45 ± 9 days), daily milk yield and the age of cows (primiparous). Two dietary treatments were applied: in the summer feeding season: hay meadow; yellow lupine, pea; oats and field pea; forage grass with red clover and spelled. In the winter season: corn meal; oats with field pea meal, rye meal, grass silage with red clover and grass hay with red clover (Table 1). The diets were formulated using French National Institute for Agricultural Research (INRA) system.

TABLE 1. Components of the treatments in experiment

Composition	Treatment	
	Summer feeding season	Winter feeding season
Ingredient (kg/d)		
Hay meadow	8.68	–
Yellow lupine and pea	0.50	–
Oats and field pea	2.0	–
Forage grass with red clover	50.00	–
Spelt	2.0	–
Corn meal	–	1.0
Oats with field pea meal	–	4.0
Rye meal	–	2.0
Grass silage with red clover	–	24.0
Grass hay with red clover	–	6.0
JPM	18.37	17.21
BTJE	1 381.9	1 370.7
BTJN	1 950.7	1 734.6

Sampling

The cows were milked daily at 05:30 and 17:30 and milk yield was recorded at each milking. Representative milk samples were collected from each cow during milking by means of a milk autosampler in the milking parlour. Milk samples were collected once: in the 45 ± 9 days of lactation. Combined milk from morning and evening milking was placed in sterile bottles, preserved with Mlekostat CC and immediately submitted to the Cattle Breeding Division (Milk Testing Laboratory of Warsaw University of Life Sciences) for composition analysis.

Milk analyses

Total protein concentration of the milk was determined by automated infrared analysis with a MilkoScan FT – 120 instrument (Foss Electric). The samples were heated up to 40°C in a water bath, the number of pump strokes was set to 22, and the pipette shake was set to 5.

Evaluation of hygienic status of the milk was based on somatic cell count on Somacount – 150 (Bentley).

Whey proteins: β -lactoglobuline (β -LG), α -lactoalbumine (α -LA), bovine serum albumine (BSA), lactoferrin (Lf), lysozyme (Lz) and lactoperoxidase (Lp) were examined using: high-performance liquid chromatography (Agilent 1100 series; Agilent Technologies) with a set-up consisting of a quaternary pump, an UV detector, an auto-sampler and Chemstation software. The analytical standards were purified proteins, i.e. β -lactoglobuline (β -LG; 90%), α -lactoalbumine (α -LA; $\geq 85\%$), lactoferrin (Lf; 90%), lysozyme (Lz; 95% from hen egg whites) and lactoperoxidase (Lp), which were purchased from Sigma Aldrich (Germany). Acetonitrile super gradient and trifluoroacetic (TFA) were obtained from Merck and Sigma Aldrich; membrane filters 0.45 μm were manufactured by Pall Laboratory. The chromatographic conditions were as follows:

- chromatographic columns with SupelcosilTM LC-318 Supelco and Supelguard, with particle size 5 μm , pore size 300 Å;
- conditions for linear gradient elution: 0% B – 32% for 10 min, 32–52% B for 25 min, 52–80% for 3 min;
- flow rate of mobile phase 1.2 ml per min;
- ambient temperature of separation.

Chromatographic separation was performed using the following eluents: A – 0.1% TFA in the solution of acetonitrile : water (5 : 95), B – 0.1% TFA in the acetonitrile.

Statistical analyses

The data obtained were analyzed statistically using a multi-factor analysis of variance (least squares) by means of the SPSS 12.0 packet software. Only interactions between factors whose influence was statistically significant ($P \leq 0.001$, $P \leq 0.01$ or $P \leq 0.05$) were considered in the study. The level of significance was determined after performing preliminary statistical analyses.

The model used was: $Y_{ijk} = \mu + A_i + B_j + (A_i \times B_j) + e_{ijk}$
where:

Y_{ijk} – dependent variable,

A_i – breed effect ($i = 1 - 2$),

B_j – season of the feeding ($j = 1 - 2$),

$(A_i \times B_j)$ – fixed interaction effect between breed and season of the feeding.

RESULTS AND DISCUSSION

Content of total protein and composition of protein fraction of cow's milk is very important for the dairy industry. It is also fundamental for its nutritive value as well as processability (Barłowska et al. 2011). For the first time in Poland Reklewska et al. (2003) showed, that content of biological active components in milk of cows, especially from protein fraction, depend on system and season of feeding, that is environmental conditioning. Considering environmental ones, nutrition is the most important determinant

TABLE 2. The composition of whey protein in milk of cows independent of breed and of feeding season

Parameters	Significant			Season											
	season	breed	season × breed	Summer feeding				Winter feeding							
				BS	HF	BS	HF	BS	HF	BS	HF				
Milk yield (k/d)	*	NS	NS	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
SCC (thou./cm ³)	NS	NS	NS	162	297.04	126	235.74	189	235.54	135	159.92				
Protein (%)	NS	NS	NS	3.93	0.89	3.65	1.07	3.75	0.59	3.59	0.53				
Whey protein (%)	**	*	NS	0.84	0.09	0.77	0.11	0.75	0.87	0.73	0.05				
Lz (µg/l)	NS	*	NS	34.35	20.16	44.39	20.10	29.65	17.69	44.30	23.28				
Lf (g/l)	NS	NS	NS	0.200	0.11	0.250	0.21	0.260	0.15	0.204	0.10				
Lp (mg/l)	NS	NS	NS	0.154	0.12	0.111	0.25	0.149	0.10	0.154	0.23				
BSA (g/l)	NS	NS	NS	0.412	0.87	0.351	0.81	0.348	0.10	0.363	0.12				
α-LA (g/l)	NS	NS	NS	1.994	0.45	1.897	0.63	1.906	0.48	1.663	0.25				
β-LG (g/l)	**	**	NS	5.385	0.65	4.603	0.69	4.621	0.58	4.411	0.58				

SCC – somatic cell count, SE – standard error of the mean; NS – no significant, *P ≤ 0.05, **P ≤ 0.01.

of milk chemical composition, followed by production system, health status, as well as the effect of herd and labour. Within genetic factors, breed of the cow maintained in our country seems to have substantial influence on milk compounds (Brodziak et al. 2012). Comparison study quality of milk from BS and HF breeds found a higher content of protein and of fat in the milk (Kuczyńska et al. 2011). The studies showed a significant effect of selected breed and feeding season on the content of whey proteins in milk (Table 2). Similar results were obtained in Italy (De Marchi et al. 2008).

The average content of whey proteins in milk of cows ranged from 0.73% for the HF breed in winter to 0.84% for the BS breed in summer. The level of functional whey proteins in milk is strongly influenced by the breed of cows (Brodziak et al. 2012). Breed of cows significantly affect the content of lysozyme in cow's milk. No significant differences in the content of lactoferrin and lactoperoxidase in the milk was recorded. There were no statistically significant differences in the level of α -lactoalbumine in the milk of cows. Its content was ranged from 1.663 to 1.994 g/l. The experiment demonstrated statistically significant differences for the breed and feeding season for the content of β -lactoglobuline in cow's milk.

CONCLUSIONS

To sum up season and breed had significant influence in created of content of bioactive components of whey protein of cow's milk produced according to principles of the biodynamic farming.

The average content of whey proteins in milk of cows ranged from 0.73% for the HF breed in winter to 0.84% for the BS breed in summer.

The content of β -lactoglobuline from BS cows in the period of summer feeding was 5.38 g/l, however reduced to the level herself by the winter 4.62 g/l.

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Streszczenie: *Wpływ rasy krów i sezonu żywienia na zawartość bioaktywnych białek serwatkowych w mleku produkowanym według zasad biodynamicznego rolnictwa.* Celem pracy było określenie zawartości białek serwatkowych w mleku w zależności od rasy i sezonu żywienia. W wybranym gospodarstwie specjalizującym się w produkcji mleka według zasad rolnictwa biodynamicznego utrzymywane są w tych samych warunkach środowiskowych dwie rasy krów, tj. Brown Swiss (BS) i Polska Holsztyńsko-Fryzyska (PHF). Próbkę mleka od 30 krów (w tej samej liczbie od BS i PHF), były pobierane dwukrotnie w sezonie żywienia letniego i zimowego. Przy wykorzystaniu wysokosprawnej chromatografii cieczowej w odwróconym układzie faz RP-HPLC oznaczono następujące białka serwatkowe: β -laktoglobulinę (β -LG), α -laktoalbuminę (α -LA), laktoferynę (Lf), laktoperoksydazę (Lp), lizozym (Lz) i bydlęcą albuminę serum (BSA). W badaniach wykazano istotny wpływ rasy ($P \leq 0,05$) i sezonu żywienia ($P \leq 0,01$) na zawartość białek

serwatkowych w mleku. Wykazano większą ogólną zawartość białek serwatkowych w mleku obu ras bydła w okresie żywienia letniego w porównaniu z żywieniem alkierzowym. Średnia zawartość białek serwatkowych w mleku krów wahała się od 0,73% dla rasy PHF zimą do 0,84% dla rasy BS latem. Nie stwierdzono istotnych różnic w kształtowaniu się zawartości laktoferyny i laktoperoksydazy w mleku, a ich zawartość wahała się od 0,201 do 0,259 g/l i od 0,111 do 0,154 g/l odpowiednio w mleku badanych ras. Nie stwierdzono również statystycznie istotnych różnic w poziomie α -laktoalbuminy w mleku badanych krów. Jej zawartość kształtowała się na poziomie wartości referencyjnych w zakresie od 1,663 do 1,994 g/l. W przeprowadzonym doświadczeniu wykazano statystycznie istotne różnice dla rasy i sezonu żywienia ($P \leq 0,01$) dla zawartości β -laktoglobuliny. Koncentracja β -laktoglobuliny w mleku krów rasy BS w okresie żywienia letniego wynosiła 5,38 g/l, jednakże zimą obniżyła się do poziomu 4,62 g/l. Podobną tendencję wykazano w mleku krów rasy PHF. W sezonie letnim stężenie tego białka wynosiło 4,60 g/l, a w sezonie zimowym uległo obniżeniu do 4,41 g/l.

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The quality of camel wool held in the Tunisian Sahara Desert

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Abstract: *The quality of camel wool held in the Tunisian Sahara Desert.* The goal of present work was to investigate wool quality of camels breeding in the Tunisian part of Sahara Desert. The study was carried out with 10 camel males at the age of three years. The samples of wool were taken from the left mid-side of a shoulder. Due to the low regrowth rate of wool, the samples were not split into the external and internal fractions before a measurement. The individual fibers were classified into three groups: 1) fibers with continuous medulla, 2) fibers with intermittent medulla, 3) fibers without medulla. Then the percentage of each type of fibers was evaluated. Thickness measurement was made using the microprojection method according to Polish Standard PN-72/P-04900. At least 600 fibers were measured in each sample. The high fraction of hair from the core constant rate of 78% wool with a thickness of up to 48 μm , and the hair thickness variation of 47% be attributed to the roughness of the wool. The wool of the camels under study should be used for the production of carpets and handicrafts. The occurrence of continuous core both in the thin and thick fibers confirmed the adaptation of camels to a large diurnal temperature variation, as in llamas and alpacas.

Key words: camel, wool, quality

INTRODUCTION

The camel population is around 26 million (FAO statistics from 2011). Most of them are dromedaries, whose habitat are dry, semi-arid, or desert areas of Africa. Camel's wool is a valuable and sought-after product. Its value depends mainly on the thickness and length. The most

valuable thin fibers are soft and provide an attractive material for scarves and sweaters of the highest quality. The fibers of medium thickness may be used for the production of outerwear. Thick wool finds its way in the manufacture of tents, carpets, blankets, ropes and halters as well as insulating material (Chand et al. 2011). The quality of camel's wool also depends on the part of body it is obtained from. Thinner wool, taken from a side of the body or from the abdominal, is best suited for spinning. This is the first shearing wool fiber of the diameter from 20 to 23 microns and is obtained from camels under the age of 3 years. Longer (and also rougher) wool comes from the front of the neck and the top of the humps. The color of camel wool is from cream to almost black and is easy to dye (Huebscher 2008, Mathias 2010).

Wool is harvested by combing as well as picking molting fiber or clipping, which takes place once a year in spring. The average yield for an individual in stocks is 5 kg. The fibers of wool obtained from adult male dromedary have a diameter of 31–35 microns. Thinner wool is produced by yearlings and its yield is from 1 to 4 kg (Khan et al. 2003, Huebscher 2008, Mathias 2010).

A typical farm consisting of 46 camels generates about 12% of its annual income from the sale of greasy wool (Mathias 2010).

The goal of present work was to investigate wool quality of camels breeding in the Tunisian part of Sahara Desert.

MATERIALS AND METHODS

The study was carried out with 10 camel males at the age of three years.

The samples of wool were taken from the left mid-side of a shoulder. Due to the low regrowth of wool, the samples were not split into the external and internal fractions before a measurement. The individual fibers were classified into three groups: 1) fibers with continuous medulla, 2) fibers with intermittent medulla 3) fibers without medulla. Then the percentage of each type of fibers was evaluated. Thickness measurement was made using the microprojection method according to Polish Standard PN-72/P-04900. At least 600 fibers were measured in each sample.

RESULTS AND DISCUSSION

The average thickness of the wool was 40.26 μm (Table 1) and ranged from 28.80 to 48.13 μm . The average deviation was 18.33 μm , with 47.61% variance which demonstrates a high diversity of wool thickness in Tunisian camels. The wool in our samples appeared thinner compared to the study of Rozbicka (2006). Other authors reported that 80% of wool derived from adult camel had a thickness of 17–20 μm . This difference could be due to the quality of feed. The best fibers of inner fractions have been derived in central Mongolia, and their thickness ranges from 19 to 24 μm , while the thickness of fibers in the outer fraction can vary between 20 and 120 μm (Petrice 1995, Huebscher 2008).

Most of the tested samples consisted of continuous and discontinuous hair core (Fig. 1), and their fractions were, respectively, 78 and 17%. Coreless hair was only 5% but their fraction was

TABLE 1. Mean thickness of fiber per wool sample

Number of sample	Fiber diameter		
	mean thickness (μm)	standard deviation (μm)	variation (%)
1	37.01	22.65	61.19
2	40.00	17.21	43.03
3	28.80	24.57	85.31
4	46.98	25.95	55.24
5	47.18	15.96	33.82
6	48.13	10.70	22.23
7	46.20	17.71	38.33
8	41.78	15.22	36.43
9	34.87	16.51	47.35
10	31.66	16.84	53.19
Mean	40.26	18.33	47.61

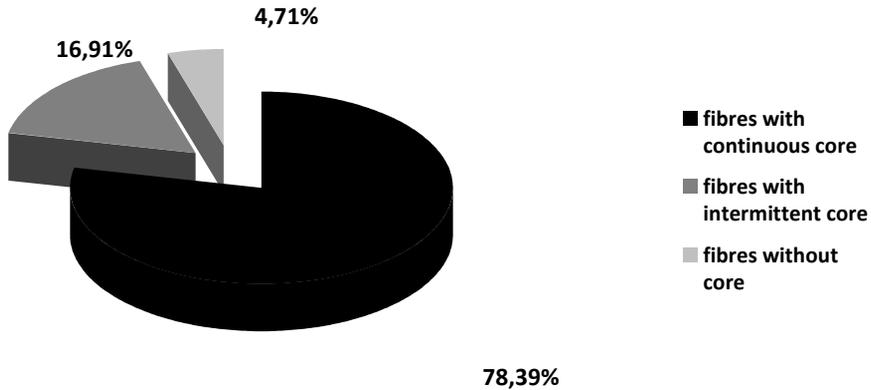


FIGURE 1. The percentage of fibres with continuous core, intermittent core, and without core in all samples under test

greater than that reported by Rozbicka (2006). The fraction of continuous-core hair in a sample varied between 50 and 100%; the share of intermittent-core hair was from 0.2 to 33%, and the coreless hair comprised 1 to 18% of the sample. In three samples, there was no hair without a core, and their share in the remaining samples was very small (Fig. 2).

The occurrence of continuous core both in the thin and thick fibers results from the adaptation to a large diurnal

temperature variation, as in llamas and alpacas (Kujaszewska and Kuźnicka 2012).

CONCLUSIONS

The high fraction of hair with continuous core amounting to 78% wool with a thickness of up to 48 microns, and hair thickness variation of 47% is a proof of high roughness of the wool. The wool of

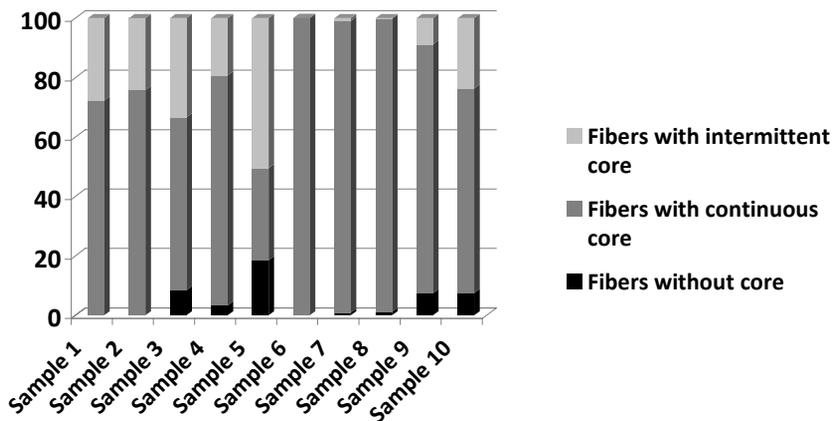


FIGURE 2. The participation of fibers without core, with continuous core, and intermittent core in each trial

the camels under study should be used for the production of carpets and handicrafts. The occurrence of continuous core both in the thin and thick fibers confirms the adaptation of camels to a large diurnal temperature variation, as in llamas and alpacas.

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Streszczenie: Jakość wełny wielbłądów utrzymywanych na obszarze tunezyjskiej Sahary. Analizie poddano wełnę pochodzącą od 10 wielbłądów utrzymywanych na obszarze tunezyjskiej Sahary. Próby pobrano z boku za łopatką od samców w wieku 3 lat. Ze względu na mały odrost wełny pomiary zostały przeprowadzone na całym zespole włosowym, nie rozdzielano włókien na frakcję zewnętrzną i wewnętrzną. W trakcie pomiarów grubości poszczególne włókna klasyfikowano do 3 grup: 1) włókna o rdzeniu ciągłym; 2) włókna o rdzeniu przerywanym; 3) włókna bez rdzenia. Pomiar grubości dokonywany był metodą projekcyjną za pomocą lanametru na wyodrębnionej frakcji (pęczku). Obliczono procentowy udział włosów rdzeniowych, bezrdzeniowych i z rdzeniem przerywanym występujących w badanych próbkach. Bardzo duży udział włosów z rdzeniem ciągłym, wynoszący 78% przy grubości wełny dochodzącej do 48 μm i zmienności grubości włosów wynoszącej 47%, może świadczyć o szorstkości wełny. Wełna badanych wielbłądów powinna być wykorzystywana do produkcji dywanów i rękodzieła ludowego. Występowanie rdzenia ciągłego zarówno we włosach cienkich, jak i grubych wskazuje na przystosowanie do dużej dobowej zmienności temperatury, podobnie jak u lamy i alpaki.

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Canine von Willebrand's disease – knowledge and awareness among dog breeders and owners in Poland

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Abstract: *Canine von Willebrand's disease – knowledge and awareness among dog breeders and owners in Poland.* Von Willebrand's disease (vWD), one of the most common congenital bleeding diatheses caused by a deficient or defective von Willebrand's factor (vWF), is found in different breeds of dogs. The objective of the study was to perform a preliminary analysis of the knowledge and awareness of von Willebrand's disease among dog breeders and owners in Poland. The online survey was addressed to dog breeders and owners, comprised 25 questions and was voluntary and anonymous. It was placed on 12 thematic Internet forums dedicated to different breeds of dogs. The respondents provided 231 answers, 76 of which were complete. The results of the survey indicate that the dog breeders and owners are highly interested in genetic tests and want more general access to scientific information. By their own admission, the dog owners and breeders have partly insufficient knowledge about vWD. Considerable gaps in their knowledge about other issues (including breeding work) were also evidenced. Being a contribution to the discussion about canine vWD, this article highlighted the need to improve the education of dog breeders and owners, and to make them aware of their role in canine health care.

Key words: von Willebrand's disease, coagulation disorders, *Canis lupus familiaris*

INTRODUCTION

Von Willebrand's disease (vWD) is one of the most common haemorrhagic diatheses in dogs (Denis and Wagner 1999). It has been identified in over 50 canine

breeds (Latimer 2011), with a high prevalence (from 15 to 60%) in 10 breeds, e.g. Dobermann and Airedale Terrier, (Ginsburg and Bowie 1992), whereas mixed breed dogs are rarely affected (Brooks 1999).

This blood coagulation disorder is associated with hereditary platelet dysfunction caused by presence of defected von Willebrand's factor (vWF) in plasma, which is necessary for platelet adhesion. This leads to defects of primary haemostasis (Mischke 2012). Three types of the disease with different space of mutations and different modes of inheritance are distinguished depending on whether vWF deficiency is qualitative or quantitative (Mischke 2012). vWD variants differ in inheritance pattern, heterogeneity of gene defects, and variation in expression of the mutated gene, thus resulting in different levels of vWF : Ag concentration (Brooks et al. 2001) and heterogeneity in the clinical signs of the disease (Brooks et al. 1992). Furthermore, the heterogeneity of the disease forms can also be influenced by a number of factors that regulate vWF levels, e.g. the level of thyroid hormones and adrenaline (Meyers et al. 1990). Antithrombotic drugs or the age of puppies have little effect on vWF concentrations (Moser et al. 1996).

Reduced vWF levels increase the tendency towards spontaneous and prolonged bleeding from mucous membranes during the exchange of teeth, injury or surgery (Stokol 2012). In animals affected with vWD there is bleeding on the surface of the skin and mucous membranes. The duration of bleeding due to tissue trauma is prolonged and out of proportion to the type of injury sustained (Brooks 1992). Owners should be aware that some diseases concomitant with vWD, as well as certain drugs, infections and endocrinological disorders may aggravate the bleeding (Brassard and Meyers 1991, Latimer 2011).

Especially dog owners and breeders should have a knowledge about vWD, because it is essential not only to eliminate affected animals and vWD carriers, but also to mate animals properly based on genetic tests, after determining possible risk of the disease in puppies. However, access to relevant information is insufficient and there are no reports about the knowledge and awareness of dog breeders and owners about vWD. Moreover, in Poland the literature regarding vWD disease is scarce (Wessely-Szponder 1999, 2001 and 2003, Wessely-Szponder and Szponder 2001). Therefore, the aim of this study was to determine the level of knowledge and awareness about canine vWD among dog breeders and owners in Poland.

MATERIAL AND METHODS

Respondents

The survey was addressed to dog owners and breeders, interested in von Willebrand's disease as well as to the owners

of affected dogs. It was open to active members and occasional users of Internet forums. The survey was anonymous and completely voluntary.

Survey design

The respondents were asked to complete a survey containing 25 questions. Eight of them were general questions that focused on the respondents' dogs (e.g. breed and origin) and tested their knowledge of breeding. Thirteen questions pertained to various aspects of von Willebrand's disease (vWD) in dogs: predispositions to disease, knowledge about genetic background, diagnostic methods and treatments. Four questions concerned the respondents' subjective assessment of their knowledge and awareness regarding vWD and dog breeding methods in their broadest sense. The study was performed online and was generated and conducted through website www.moje-ankiety.pl. The survey was made available, between May and October 2012, at 12 internet forums for breeders of dog breeds in which the disease was detected and at forums dedicated to the breeding of purebred dogs, in thematic sections on canine health. Once the web survey was accessible (<http://moje-ankiety.pl/respond-24155.html>), the respondents were able to complete it and their answers were automatically sent to the platform, while the authors could monitor, correct and gather the data for later processing. Most questions were closed-ended (18) and the participants were allowed to choose one of the suggested answers, most of which were Yes or No. Open questions were in the minority (7 out of 25). Some semi-open questions provided space to allow participants to choose their own answers

or to justify their choice. Some questions were of the multiple-choice type and allowed the respondents to select several answers they felt were appropriate. The respondents were also permitted to give no answer to a question. In case the survey question was unclear, the respondents could leave a comment or note in the designated area. All participants were asked the same questions.

In the survey the names of the breeds followed the FCI Nomenclature of Dog Breeds, taking into account Polish nomenclature of breeds (http://www.zkwp.pl/zg/regulaminy/Systematyka_ras_FCI.pdf, 2012).

Data analysis

Data were analysed based on the respondents' answers. Surveys sent to the designated area on the platform were taken into account, even if they were incomplete. Because all the answers from all the surveys were analysed, a percentage result for the total number of answers obtained was given for some of the questions. The results of the survey were used not only to estimate the knowledge and awareness levels, but also to gather maximum information based on the individual experiences of the respondents. Basic statistical parameters, the proportion of particular answers and figures were prepared using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

A total of 231 respondents (breeders and dog owners) filled in the survey. Unfortunately, only 76 people (33%) returned completely filled surveys. Dog owners

and breeders were less willing to answer open and semi-open questions, which required more of their time and effort, especially with regard to the questions concerning vWD. However, many more respondents answered multiple-choice and closed-ended questions.

Below we present a summary of the survey results, paying special attention to the answers concerning vWD. The answers obtained from the questionnaire survey show that it is not possible to determine the incidence of canine vWD in Poland or to make definitive conclusions about the attitudes of dog owners and breeders.

Out of 94 respondents, 11% had Shetland Sheepdogs while the others owned other breeds (82%) or mixed breed dogs (7%). Seventy-three percent out of 107 respondents had dogs from breeders.

Ninety-three percent of the respondents (out of 71 people) agreed that information obtained from molecular diagnostic tests and provided in the pedigree can be of benefit to dog breeders or owners. According to the International Breeding Rules of the FCI, a stud book can be published by the Polish Kennel Club or another national kennel club recognized by the FCI, and although it does not guarantee the quality of the dog, it states its origin (provides information about the dog's ancestors), which prevents buying a dog from a puppy mill. By buying a puppy with a pedigree certificate, we know that it meets the breed standard. What is more, litter information sheet contains all notes about possible defects and imperfections.

All the respondents knew that dogs are afflicted with genetic diseases, but almost half of them considered

their knowledge on that subject to be insufficient, especially with regard to vWD. The available literature provides information about the dog breeds in which a given disease was noted (Latimer 2011) and which breeds of dogs are predisposed to certain types of vWD and which breeds are sporadically affected (Ackerman 2011). The majority of respondents knew that some breeds of dogs are predisposed to particular genetic conditions. Sixty-one percent of the respondents (out of 76 people) heard that genetic molecular tests (DNA analyses) for canine genetic diseases can be performed in Poland, 13% of which concerned vWD analyses. The respondents were aware of the benefits that breeders have using genetic test in breeding practice as well as their increasing popularity and usefulness in diagnosing many serious conditions, and in identifying the carriers and affected dogs early in life. However, they pointed out that the tests are expensive, there are no accredited laboratories that perform these tests, and some veterinary doctors have incomplete knowledge about the disease. Furthermore, unlike in Great Britain, for example, dog owners and breeders in Poland are not obliged to perform genetic profiling of particular animals. Unfortunately, the respondents mistakenly believe that genetic tests are universal regardless of species or breed. The respondents' answers demonstrate the need to prepare training programmes for breeders and veterinary doctors, and to make available regularly updated information to the public for educational purposes.

It follows from the questions concerning vWD that most respondents (70% out of 76 people) would not de-

cide to buy or intentionally mate dogs affected with vWD or dogs carrying the vWD gene. The fear of genetic defects (including von Willebrand's disease), which eliminate potential sires from breeding, discourages buying and mating a dog carrying the vWD gene.

The results obtained showed that knowledge in this area is insufficient and suggested that more attention should be devoted to these issues, because half the respondents had no knowledge of the frequency of vWD in certain breeds of dogs. Yet, the respondents were correct in indicating the most vulnerable breeds (Fig. 1). The largest percentage of answers pointed to Dobermann as the breed genetically predisposed to vWD. Johnstone and Crane (1981), Ginsburg and Bowie (1992), Riehl et al. (2000) and Mattoso et al. (2010) estimated the incidence of vWD in this breed to exceed 60%. Bell (2011) showed that inbreeding affects more than 60% of the Dobermann population due to deliberate increase in homozygosity. Most of the 76 respondents (86.84%) knew that inbred mating, which increases homozygosity (inbreeding) in offspring, can be a deliberate breeding practice, while 92.11% of the 76 respondents realized that this carries the risk of genetic defects. In fact, the genetic tests and the screening programme developed for Dobermann dogs make it possible to identify affected dogs and carriers so as to replace these dogs with healthy individuals through proper selection of offspring for further breeding. The incidence of vWD in Dobermann dogs in Poland was found to be similar to that in other countries (Wessely-Szponder and Szponder 2001). The diagnostic tests used in Dobermann dogs

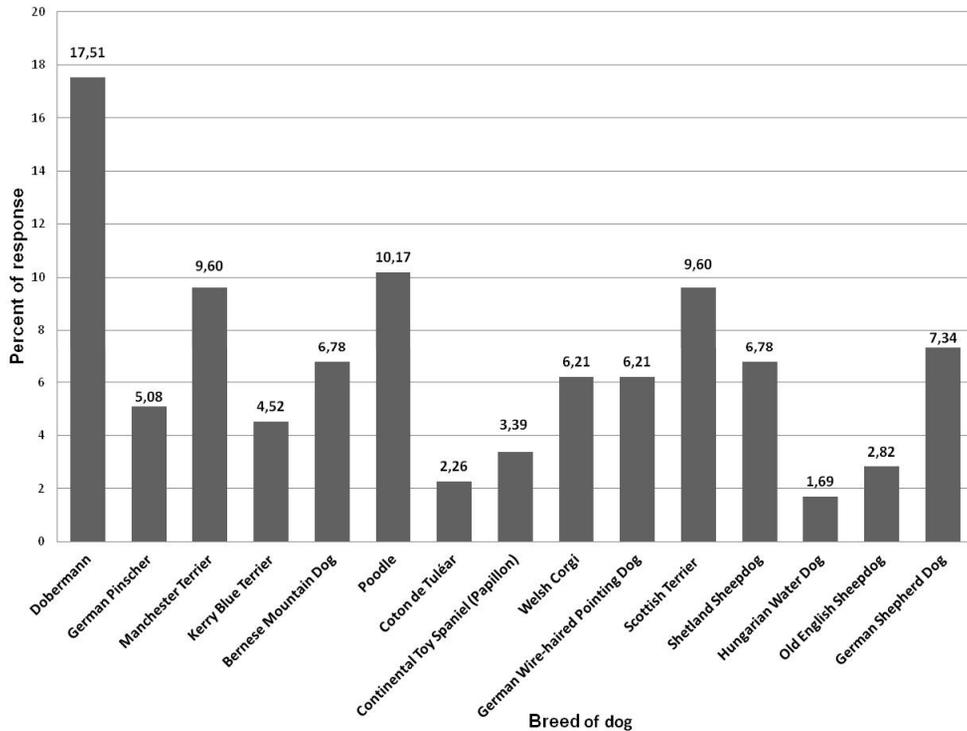


FIGURE 1. Percentage of answers to the question “Do you know that some breeds are genetically more/less predisposed to vWD?” (a total of 177 respondents)

enabled the identical mutations to be detected in Poodle and Manchester Terrier dogs (Venta et al. 2004, Boudreaux 2012). Along with Scottish Terrier, German Shepherd Dog, Shetland Sheepdog and Bernese Mountain Dog, these breeds were indicated by the respondents to be particularly predisposed to vWD (Fig. 1). This is in agreement with the results of Johnstone et al. (1993) for Scottish Terrier, Raymond et al. (1990) and Pathak (2004) for Shetland Sheepdog, Arnold et al. (1997) for Bernese Mountain Dog, and <http://fallingbranch.com/library/vonwill.htm> for German Shepherd Dog.

The answers sent by the 76 respondents show that the disease was diagnosed in 6 dogs, which included three cases of

type 1 (the mildest and most common form of the disease; depending on mutation type, it may have either recessive or dominant inheritance), one case of type 2 (bleeding is much more serious; associated with an autosomal recessive gene) and two cases of type 3 vWD (the most acute and life-threatening form; associated with an autosomal recessive gene). Most respondents admitted that no vWD was diagnosed in their dogs, but the possible reason was that the dogs were not tested for these conditions. This was probably due to the fact that the respondents were unaware of vWD, and did not need and had no possibility of conducting such tests in their breeding practice. Only one person performed tests at

a commercial laboratory, and the more active group of breeders declared that they only choose properly tested animals for breeding and refuse to mate dogs if they have no test results.

Most out of the 76 respondents (72%) admitted that their current knowledge of vWD is inadequate (Fig. 2). In addition, 39% of them have difficult access to information of interest and 71% of respondents would be interested in expanding their knowledge of vWD. Therefore, the respondents' answers show that dog owners and breeders realize that access to information about vWD is difficult.

The results of the present survey show that the dog owners and breeders have, by their own admission, little knowledge about vWD. Considerable gaps in their knowledge about other issues (includ-

ing breeding work) were also revealed. The Internet definitely serves as one of the main sources of information about the possible symptoms of vWD, the diagnostic methods and curative therapies, but it is not always the appropriate route to reach potential dog owners and breeders. Because the level of respondents' knowledge is low, it might be necessary to use other methods of disseminating the knowledge of vWD. Perhaps the introduction of mandatory routine tests identifying different types of vWD and distinguishing this syndrome from other bleeding diseases, especially in the representatives of genetically predisposed breeds, would make the breeders aware of the scale of the problem (Wessely-Szponder 2001).

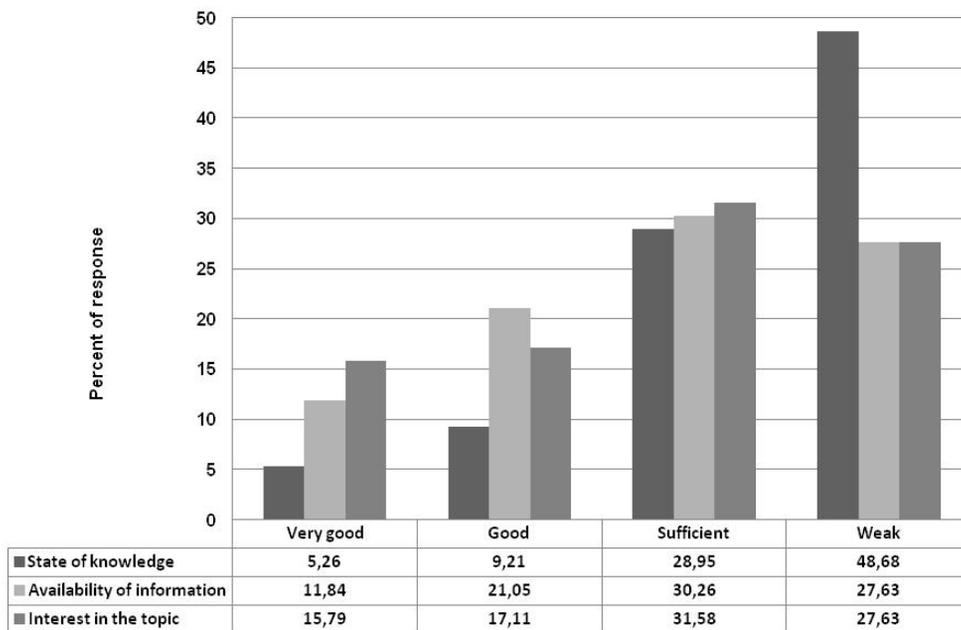


FIGURE 2. Percentage of answers to the question “Please summarize your observations and determine the degree that reflects your opinion on this subject. Use space under the table for possible comments” (a total of 76 respondents = 100%; percentages do not add up to 100 because respondents were allowed to select 4 answers)

To date, studies on canine vWD in Poland have been very rare (Wessely-Szponder and Szponder 2001). If conducted, they usually covered a small group of patients and failed to approach the problem comprehensively, often disregarding the key aspects of the disease. In contrast, international studies are conducted on a much greater scale and represent higher standards. Tightness of funds is only one of the factors limiting the development of scientific research and new technologies in Poland. Many laboratories are very well equipped and can perform such genetic tests provided that there is demand and interest from breeders. The results of studies conducted in foreign centres are used to create databases, which are valuable sources of information for breeders. Being a contribution to the discussion about canine vWD, the present article highlighted the need to improve the education of dog breeders and owners, and to make them aware of their role in canine health care.

CONCLUSIONS

Analysis of the results enabled us to make the following conclusions:

1. The respondents stated that pedigree certificate is considered an important document which guarantees the dog's origin, while the results of molecular tests included in the certificate provide valuable information for dog breeders and owners.
2. The respondents mistakenly believe that genetic tests are universal regardless of species or breed.
3. In the analysed group, the respondents with low knowledge and awareness regarding vWD showed their willingness to expand their knowledge in this area.
4. It was considered necessary to create a platform for exchanging data and observations between dog owners, dog breeders and veterinary doctors, and for researchers to provide updated information about the availability of diagnostic tests.

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Streszczenie: *Choroba von Willebranda u psów – stan wiedzy i poziom świadomości hodowców i właścicieli psów w Polsce.* Choroba von Willebranda (vWD) jest jedną z najczęściej występujących wrodzonych skaz krwotocznych, spowodowaną niedoborem bądź zaburzeniem czynnościowym czynnika von Willebranda, odnotowaną wśród psów różnych ras. Celem pracy była wstępna analiza stanu wiedzy i poziomu świadomości na temat choroby von Willebranda wśród hodowców i właścicieli psów w Polsce. Dlatego też w jej realizacji przeprowadzono ankietę skierowaną do hodowców i właścicieli psów. Ankieta on-line zawierała 25 pytań, wypełnienie jej było dobrowolne i anonimowe. Została ona umiesz-

czona na 12 wybranych tematycznych forach internetowych, dotyczących różnych ras psów. Od respondentów otrzymano 231 odpowiedzi, z czego 76 pełnych. Wyniki ankiety wskazują, iż hodowcy oraz właściciele psów są niezwykle zainteresowani problematyką testów genetycznych, pragną bardziej powszechnego dostępu do informacji naukowej. W pewnym stopniu właściciele i hodowcy psów posiadają niewystarczający zasób wiedzy dotyczący vWD, co sami przyznali w ankiecie. Także w zakresie innych zagadnień (m.in. pracy hodowlanej) stwierdzić można znaczne jej braki. Niniejszy artykuł jest głosem w dyskusji na temat vWD u psów, który jednocześnie ujawnił potrzebę szerszej edukacji hodowców i właścicieli psów, a także konieczność uświadomienia im roli, jaką pełnią w ochronie zdrowia psów.

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Effect of classic massage therapy on the heart rate of horses working in hippotherapy. Case study

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Abstract: *Effect of classic massage therapy on the heart rate of horses working in hippotherapy. Case study.* The objective of this study was determine the effect of classical massage therapy on heart rate in horses working in hippotherapy. We hypothesised that massage can be used to improve the horse's welfare, because it mentally relaxes the horse. The relationship between variables were examined to determine whether the selected factors (use or non-use of massage, the method of securing the patient on the horse, way of getting on a horse, place of treatment) influenced the heart rate of two horses working in hippotherapy. The results showed significant but very different effects on heart rate of particular horses subjected to a massage. It was also shown that heart rate of horses working in hippotherapy is highly influenced by environmental factors.

Key words: massage, horses, hippotherapy

INTRODUCTION

Massage is defined as the systematic therapeutic stroking or kneading of the body or one of its parts (Blood and Studert 1999). It has a wide spectrum of effects. It allows to relieve the body from pain and stress, reduces pressure, helps to achieve relaxation. Massage is currently widely used in veterinary physiotherapy for both its physiological and psychological effects (Valberg 1996, Roetting 1999). Horses have a very large muscle mass compared to their body structure. Massage is a manual therapy that can be

used therapeutically to reduce muscle tone, restore muscle flexibility, improve joint range of motion and, as a result, has the potential to improve the horse's locomotor function. Thus it allows the horse to move and perform in its full movement potential. Hill and Crook (2010) explore the effects of massage to the caudal muscles (retractors) of the equine hindlimb on active and passive limb protraction and stride length and found that massage significantly increased passive ($P = 0.01$) and active limb ($P = 0.01$) protraction. In human patients massage therapy reduces feelings of anxiety and improves self-esteem (Hernandez-Reif et al. 1998). It is likely emotions also play an important role in the horse, particularly with respect to his work. The horse may not function optimally unless it is psychologically comfortable and confident (Denoix and Pailloux 1996) and massage may be able to promote this feeling of well-being in work. This study is one of the first attempt to utilise objective outcome measures to determine the effectiveness of classical massage on welfare of hippotherapy horses.

The objective of this study was to determine the effect of classical massage therapy on heart rate of horses working in hippotherapy. We hypothesised that massage have positive psychological effect on hippotherapy horses reducing their

heart rate and mentally relaxing horse, and may therefore play a valuable role in the development of strategies used to improve a horse's welfare.

MATERIAL AND METHODS

The research was carried out in the facility for blind and low-vision people in Laski, Poland, where hippotherapeutic activities for pre-school and school children are organized. The facility in Laski has been under the patronage of the Polish Hippotherapeutic Association since 2006.

The subjects comprised two geldings with different records in hippotherapy work:

- Horse A: hucul, 15 years old, withers height 138 cm, calm and composed; working for 9 years as hippotherapy horse.
- Horse B: fjord, 13 years old, withers height 130 cm, lazy but courageous; working for 2 years as hippotherapy horse.

All horses were in regular work in hippotherapy and identically managed at the same hippotherapy center. Both geldings received the massage treatment. We attempted to ensure that the experimental conditions were the same for both horses. The intention was to minimize any physical or emotional stress in the subjects, which may have affected the results of heart rate measurements.

The trait under examination in the horses was the heart rate measured by the constant heart rate monitor (beats per minute – bpm). The analysis of this feature is usually applied in research on changes in the balance of the sympath-

etic nervous system and the vagus nerve connected to disorders, in research on psychological and environmental stressors (Rietmann et al. 2004, Łojek and Poszepczyńska 2006), behavioural disfunctions (Bachmann et al. 2003), training methods or for individual evaluation of the animals for temperament (Visser et al. 2002), emotional status and the strategies of coping with environmental conditions.

The examination of the heart beat was carried out with the Heart Rate Monitor model S 810 manufactured by POLAR ELECTRO OY. The analysis and correction at the level of 6.0 (average level of correction) was performed with Polar Precision Performance for Windows which allows to record and analyze the data in graphical form.

The research lasted from the end of April till the end of June 2012, during hippotherapeutic activities with children at pre-school age with various degrees of vision disability. Heart rate of both horses was measured during their participation in the hippotherapeutic activities during the following periods:

- in the last week of April, when the horses were not massaged,
- in May, when the horses were massaged,
- in the first two weeks of June, when the horses were massaged.

The organization of the research comprised the following procedures:

- from 8:30 a.m. to about 9:40 a.m. the horses were groomed and consequently taken for a ride in the countryside;
- after returning the horses had some rest and subsequently they started activities with children at the pre-

- school, which lasted from 10:15 a.m. to 12 a.m. During these activities the heart rate was measured;
- After returning from the therapy around 12 a.m. the first horse was let out on a paddock and the second one was massaged for about 1 hour. Then the horses were changed.

The heart rate measurements (bpm) of both horses working in hippotherapy were taken with reference to the conditions of the conducted therapy. In the analyses the SPSS Statistical Package ver. 19.0 (2012) was used. The four chosen factors of the analysis were as follows:

1. Month of therapy – so the use or non-use of massage:
 - April – no massage applied,
 - May – massage applied,
 - the first two weeks of June – massage applied.
2. The method of securing the patient on the horse:
 - from the horseback,
 - from the ground.
3. The method of mounting the horse:
 - from a ramp,
 - from the ground.
4. The place where the therapy were conducted:
 - in the forest,
 - in the lime-tree alley.

The data were checked for normality of distribution with Kolmogorov–Smirnov Test (K-S test) for one sample. Due to the fact, that the value of the test probability turned out to be lower than the accepted level of significance ($P \leq 0.05$) the hypothesis that the analysed distribution is identical with normal distribution was rejected. Logarithmic transformation of

the data did not result in normalization of the distribution.

The Pearson Chi-square test and the precise Fischer tests were used to examine the interdependence between the features in order to decide whether the chosen factors resulted in the change in heartrate of the two horses working in hippotherapy.

The small sample size limited our statistical analysis to nonparametric tests: Kruskal–Wallis test and Mann–Whitney U test.

RESULTS AND DISCUSSION

One of the most important and most popular types of massage is the classical massage. Depending on its intensity it can be healing, stimulating or relaxing. Massage is a common method for “wellness” purposes, its efficacy for several indications is still under debate. The psychological effects of massage in humans are well documented (Hernandez-Reif et al. 1998, Hemmings et al. 2000). However, effects on behavior or stress parameters described for man (Hemmings 2001, Moyer et al. 2004) have been found also in horses, where grooming or massage reduced heart rate and caused positive behavioral responses, when performed on preferred sites of allogrooming (Feh and de Mazieres 1993, McBride et al. 2004).

This study demonstrates that massage of the horses had significant influence on their heart rate during hippotherapy. The results of the Kruskal–Wallis test allowed to reject the null hypothesis on lack of influence of massage on the heart rate in both horses used for hippo-

therapy. The results of the Mann–Whitney U test showed significant differences ($P \leq 0.01$) between the heart rates depending on the month when the therapy was performed (and thus on applying or not applying massage), the method of securing the patient on the horse, the method of getting on the horse and the place of therapy (Table 1).

The results indicate significant differences between the horses. This is shown by the total average heart rate of Horse A (56.2 bpm) and Horse B (43.5 bpm), as well as the horses individual reaction to the conditions of conducting of the therapy, including applying massage (Table 1). In the case of Horse A, no relaxing effect of massage on the heart beat during hippotherapeutic activities with children was observed. In April the heart rate of this horse was low-

est, in May, when massage was applied throughout the month, it clearly increased (Table 1 – The month of conducting therapy). The heart rate decreased slightly in June – the month when massage was applied only for the first two weeks. Different reaction to applying massage as a relaxing factor was observed in Horse B. The horse's heart rate significantly decreased in May compared to April. This tendency continued in June, when the horse was massaged only for half of the month. This could result from the individual preferences of the horses, as far as the sense of touch is concerned, although Horse A has never showed negative reaction to touch (e.g. during grooming), but the research shows that the horse's heart rate increased as a result of massaging. The horses' reaction to massage indicates, that different horses may react

TABLE 1. The results of the analysis of the heart rate (bpm) of two horses working in hippotherapy depending on factors connected with the conditions of the conducted therapy

Factors	Horse A				Horse B			
	<i>n</i>	<i>x</i>	SEM	P	<i>n</i>	<i>x</i>	SEM	P
Month of therapy (use or non-use of massage)								
April – no massage applied	953	41.2	0.289	<0.001	1273	53.2	0.535	<0.001
May – massage applied	1963	62.8	0.197		1057	35.9	0.151	
June – massage applied	649	58.5	0.198		585	35.8	0.179	
Method of securing the patient on the horse								
From the horseback	726	65.1	0.335	<0.001	585	35.8	0.179	<0.001
From the ground	2839	54.0	0.227		2330	45.4	0.349	
Method of mounting the horse								
From a ramp	2842	54.0	0.226	<0.001	1198	37.8	0.257	<0.001
From the ground	723	65.1	0.336		1717	47.4	0.435	
The place where the therapy were conducted								
Forest	1015	53.3	0.469	<0.001	1056	35.4	0.129	<0.001
Lime-tree alley	2550	57.4	0.217		1859	48.1	0.413	
Total	3565	56.2	0.207		2915	43.5	0.290	

The level of significance assumed as highly significant: $P \leq 0.01$; SEM – standard error of the mean.

differently to this procedure. It should be pointed out that the massage does not always cause the positive result. Massage increases endorphin release, which may explain the reported sensation of well-being post massage (Kaada and Torsteimbo 1987). Massage may also have had a beneficial psychological effect on the horses used in this study, aiding relaxation and improving their sense of well-being.

Both horses reacted differently to the method of securing the patient (Table 1). Horse A reacted with increasing heart rate in the case of securing from the horse's back and Horse B showed the same increase in heart rate while securing the patient from the ground. This may be due to the fact, that Horse B was working as hippotherapy horse for only 2 years and may not be accustomed to the securing person, walking at his side. Moreover, the presence of two people – the person leading the horse and the person securing the patient – may have caused his anxiety. On the other hand, Horse A has been used in hippotherapy for a long time and has already been accustomed to greater number of persons around him during the therapy. It must be also taken into consideration, that the activities with securing the patient from the horseback have been conducted very rarely and this method of securing the patient might have been something unexpected for the horse, thus causing his heart rate to rise.

The reactions of both horses to the method of mounting and the location of performing the therapeutic activities were similar. The method of putting the patient on the horse depends on the type of illness and its advancement. Introduc-

tion of this factor into the research aimed at defining, whether the moment of putting the patient on the horse is perceptible for the animal. Horses were more tolerant to mounting from a ramp rather than from the ground (Table 1) which can be explained by the fact, that mounting from a ramp is definitely less strenuous for the horse's back and is more comfortable for the animal. The average heart rates measured at this method of mounting were lower than the total average of horses A and B. This could also be due to the fact that the horse waited a while for the patient while standing near the ramp and only then was he starting the work. When mounting from the ground, the work started immediately.

Lower average heart rates were recorded in both horses during work in the forest compared to the lime-tree alley (Table 1). This result may be contributed to fact, that the forest is a much calmer environment for work. It is a natural environment, isolated from external influences and from wind. The conditions of work in the lime-tree alley were totally different. Very often strong wind occurred, and on adjacent areas field works were carried out, which was connected with noise from the tractors and other machines. Both the results of this study, and results of studies performed by other Authors indicate, that the environment plays a crucial role in conducting hippotherapy, which directly influences the main condition of successful therapy – the patient's safety.

The results of our work should be viewed as a preliminary insight into the effectiveness of classical massage on heart rate of horses working in hippotherapy. It is recommended that further

experiments be undertaken using a large number of animals to confirm the findings of this work.

CONCLUSIONS

1. Significant influence of massage on the heart rate during the hippotherapeutic activities was observed.
2. The reaction of the horses to massage indicates, that different horses may have a totally different reaction to this procedure and one has to bear in mind that this procedure will not bring the expected result in any case.
3. A crucial role of the environmental conditions of therapy was demonstrated. The conditions of conducting the therapy, i.e. the method of securing the patient, the method of mounting the horse and the location of the therapeutic activities had a significant influence on the heart rates of the horses at work.
4. The highly significant individual differences between horses in terms of their reaction to the same conditions of conducting the therapy emphasize the importance of proper selection of horses for hippotherapy.

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- na konia, miejsce prowadzenia terapii, wpływają na częstotliwość tętna koni pracujących w hipoterapii. Masaż wpłynął odmiennie na każdego z badanych koni. U jednego z nich nie stwierdzono rozluźniającego wpływu masażu na pracę serca w czasie zajęć hipoterapeutycznych z dziećmi, zaś u drugiego zastosowanie masażu spowodowało znaczący spadek tętna. Wskazuje to, że różne konie mogą zupełnie odmiennie reagować na zabieg masażu i należy się liczyć z tym, że nie w każdym przypadku przyniesie on spodziewany efekt. Także warunki realizowania terapii (sposób asekuracji pacjenta, sposób wsiadania na konia i miejsca prowadzenia terapii) w sposób istotny wpływały na częstotliwość tętna pracujących koni.

Streszczenie: *Wpływ masażu klasycznego na parametry pracy serca koni pracujących w hipoterapii. Studium przypadku.* Celem badań była ocena wpływu masażu na częstotliwość pracy serca koni pracujących w hipoterapii. Zakładano, że masaż, mający pozytywny wpływ na stan psychiczny koni, może służyć jako zabieg relaksujący konie w ich trudnej i monotonnej pracy z pacjentami. Określano też jak wybrane czynniki: sposób asekuracji pacjenta, sposób wsiadania

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Histological profile of breast and leg muscles of Silkies chickens and of slow-growing Hubbard JA 957 broilers

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Abstract: *Histological profile of breast and leg muscles of Silkies chickens and of slow-growing Hubbard JA 957 broilers.* The experiment was conducted with 60 slow-growing Hubbard JA 957 broilers and with 60 Silkies chickens reared until the 63rd day of life, in three replications, 20 birds each, in a closed building on litter. A three-stage feeding program was applied in the rearing period. Feed mixtures used in the experiment did not differ in contents of nutrients. In the first stage of rearing (till 21 days of life) the chickens were fed a standard starter feed mixture containing: 20.98% of total protein, 2,845 kcal/kg of metabolizable energy, 1.17% of lysine and 0.98% of met. + cys. In the period from 22 to 56 days of life they were receiving a grower type mixture containing: 20.0% of total protein, 2,900 kcal/kg of energy, 1.04% of lysine and 0.88% of met. + cys., whereas contents of these components in the finisher type mixture administered till the end of the rearing period were as follows: 18% of protein, 2,965 kcal/kg of EM, 0.90% of lysine and 0.78% of met. + cys. On day 63 of rearing, 12 cockerels and 12 hens were selected random from each group. The birds were slaughtered in a poultry slaughter house, and specimens of their major breast muscle (*m. pectoralis superficialis*) and thigh muscle (*biceps femoris*) were sampled for analyses. The breast and leg muscles of the birds were characterized by diversified diameters of muscle fibers depending on bird genotype. The greatest diameters were found in breast and leg muscles of the slow-growing Hubbard JA 957 broilers, which was confirmed statistically

($P \leq 0.01$). It was also demonstrated that the mean diameter of muscle fibers of Silkies chickens was more than two times smaller compared to the Hubbard JA 957 chickens.

Key words: Silkies and Hubbard chicken, meat, histology, morphometry of muscle fibers

INTRODUCTION

Contemporarily only highly-selected hybrids of slaughter chickens and turkeys are used in the production of poultry meat in Poland. White meat of chickens, compared to red meat, is deemed as basic in a healthy diet of man owing to, among other things, relatively low contents of fat and cholesterol. In addition, consumers are convinced by a relatively low price and usually convenient portions of this meat as well as by a lack of religious restrictions in its consumption (Jaturasitha 2004).

Quality traits of poultry meat are determined by many factors, including species, breed or genetic line of birds, rearing conditions, pre-slaughter handling and meat processing procedures. The key one include the origin of birds and

their age at slaughter. These two factors influence mainly the organoleptic traits of meat, but also affect its technological properties. A significant factor determining meat quality is the rearing system. In the intensive production of slaughter chickens, constituting the main source of poultry meat in Poland, use is mainly made of hybrids with a high genetically-determined production potential. Consumers of poultry meat are increasingly more interested in the welfare of animals as well as in the quality and safety of final products. Many of them pay attention to housing conditions, rearing system and color of carcass. Today also Polish consumers are considering the purchase of meat originating from chickens reared in less intensive systems and additionally having access to free ranges, as they believe that this meat is characterized by higher quality. Furthermore, they search for meat with good flavor and health-promoting values. Meat of slow-growing chickens is characterized by a higher content of protein and a low fat content desired by consumers (Khantaprab et al. 1997, Połtowicz et al. 2003). The elongation of rearing period affects the concentration of chemical compounds in breast and leg muscles, their more attractive aroma, taste and, thus, sensory properties (Fujimura et al. 1994, Qinghua 1994). A faster rate of these birds body weight gain, at unequal development of their entire bodies, often leads to metabolic disorders, exerts an adverse effect on their health status and – most of all – on meat quality, which is especially significant to consumers.

Meat and animal products are essential components of a man diet. According to PN-65/A-82000, meat constitutes

“skeletal muscles and adherent adipose, connective and bone tissues, derived from carcasses, half carcasses or quarter carcasses of particular types of animals for slaughter”. One of the most valuable, not only from the dietetic point of view, is poultry meat being a source of balanced protein, amino acids (e.g. leucine, isoleucine, valine or arginine), fatty acids and minerals (Wangang et al. 2010). High digestibility of poultry meat, its assimilability, availability and low price make it very popular amongst consumers (Nowak and Trziszka 2010). The histological structure of muscles is subject to some changes depending on their functions (Klont et al. 1998). Factors that affect changes in the character of muscle fibers include: gender (Ozawa et al. 2000), age (Candek-Potokar et al. 1998), breed (Ryu et al. 2008) and physical activity (Karlsson et al. 1999). The quality of meat is determined by the content of three tissues: crosswise striated muscle tissue, connective tissue and fatty tissue. Mutual proportions and type of particular muscle fibers may lead to changes in the sensory assessment of meat (Damez and Clerjon 2008).

Meat of Silkies chickens has been for years appreciated in the Asian cuisine owing to its health-promoting values. Meat of these chickens is characterized by dark (blue) color of skin and tissue (Siriwan et al. 2004). Nowadays, they are also becoming popular in America where their meat is deemed to be “super food” and “exclusive meat”. They owe this status to, among other things, high contents of antioxidants, proteins, vitamins (mainly B-group vitamins and fat-soluble vitamins) as well as macro- and microelements (calcium, iron, phosphorus).

The Silkies chickens came to Europe from China or from eastern India, whereas old literature says also about Singapore. Amateur breeders keep these hens mainly due to their strong hatching instinct and great care over chickens. The Silkies hens are trustful and are reared for their atypical fluffy plumage (silky). They are characterized by a dark mulberry color of comb and wattles turquoise color of earlobes, dark eyes and exceptional crop of hair. Worthy of special attention is dark (blue) color of their skin as well as five toes in each foot. Today, this ornamental hen in several colors is also reared in Europe.

The goal of this study was to determine differences in the histological and morphological structure of fibers of breast and leg muscles of Silkies chickens and slow-growing Hubbard JA 957 chickens.

METHODS AND MATERIALS

The experiment was conducted with 60 slow-growing Hubbard JA 957 broilers and with 60 Silkies chickens reared until the 63rd day of life, in three replications 20 birds each, in a closed building on litter. A three-stage feeding program was applied in the rearing period. Feed mixtures used in the experiment did not differ in contents of nutrients. In the first stage of rearing (till 21 days of life) the chickens were fed a standard starter feed mixture containing: 20.98% of total protein, 2,845 kcal/kg of metabolizable energy, 1.17% of lysine and 0.98% of met. + cys. In the period since 22 to 56 days of life they were receiving a grower type mixture containing: 20.0% of total

protein, 2,900 kcal/kg of metabolizable energy (EM), 1.04% of lysine and 0.88% of met. + cys., whereas contents of these components in the finisher type mixture administered till the end of the rearing period were as follows: 18% of protein, 2,965 kcal/kg of EM, 0.90% of lysine and 0.78% of met. + cys.

One-day chicks, after weighing and tagging, were allocated to two groups (each of 60 birds), in three replications (20 birds each). On day 63 of rearing, from each group 12 cockerels and 12 hens were selected random from each group. The birds were slaughtered in a poultry slaughter house, and specimens of their major breast muscle (*m. pectoralis superficialis*) and thigh muscle (*biceps femoris*) were samples for analyses. The samples (0.5×0.5×1.0 cm) were collected within 15 minutes since slaughter after appropriate chicks bleeding, and subjected to 24-hour fixing. Next, the samples were rinsed in ethyl alcohol to remove the fixing agent and dehydrated using a series of ethyl alcohols with increasing concentration. The dehydrated preparation were saturated with paraffin. The saturation process was conducted in an incubator, at a melting point of paraffin. Its duration was adjusted to the sampled specimens of muscles and lasted a few hours. In result, paraffin blocks were formed. Paraffin preparations were sectioned using a Leica RM 2265 rotary microtome (Leica Microsystems, Nussloch, Germany) by cutting the muscles crosswise into 6 µm thick sections that were next stained with a standard H+E method (Ostaszewska et al. 2008). In each preparation, diameters of 300 muscle fibers were measured with a Nikon Ellipse E200 light microscope equipped

in a Nikon DS-Fi2 camera and COOL view 2.7 software.

Results were developed statistically using the variance analysis, computed with the method of the least squares using statistical software SPSS 19.0 PL for Windows (SPSS Inc., Chicago, IL, USA). Differences were found significant at $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

Results of microstructural analysis of the major breast muscle (*m. pectoralis superficialis*) and thigh muscle (*biceps femoris*) of chickens were presented in Table 1. Both the breast (Figures 1 and 2) and leg muscles of the experimental birds were characterized by diversified diameters of muscle fibers depending on genotype. Cross sections showed a distinct shelf structure in most of the fibers. The largest diameter was determined in the breast muscle of slow-growing Hubbard JA 957 birds, which was confirmed statistically ($P \leq 0.01$) – Table 1. The diameter of breast muscle fibers in the group of Hubbard JA 957 birds reached 75.61 μm , whereas in the group of Silkies chickens it was more than two times smaller (33.23 μm). More advantageous to consumers is fine-fiber meat as fine

fibers improve its juiciness. In the group of Hubbard JA 957 chickens, diameters of breast muscle fibers were relatively equal, 75–90% of the fibers in particular bundles had diameters in the range of 70–80 μm , and 10–30% of the fibers had diameters in the range of 25–40 μm . In the group of Silkies chickens, diameters of breast muscle fibers were more equalized and reached 25–35 μm in ca. 80–90% of the fibers and less than 25 μm in 10–15% of the fibers.

The same tendency was observed in case of leg muscles – larger diameters were demonstrated in Hubbard JA 957 birds. Diameters of leg muscle fibers in the group of Hubbard JA 957 chickens were relatively equalized, i.e. 70–90% of fibers in particular bundles had diameters between 60 and 70 μm , and 10–30% of fibers had diameters in the range of 25–50 μm . In the group of Silkies chickens, the diameters of leg muscle fibers, likewise these of breast muscles, were more equalized compared to the Hubbard JA 957 chickens because 80–90% of fibers in particular bundles had diameters in the range of 20–35 μm , and in 10% of the fibers diameters were lesser than 20 μm .

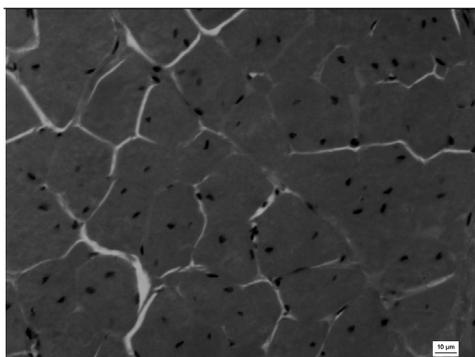
According to Dransfield and Sośnicki (1999), the fast-growing chickens are characterized by a larger diameter of muscle fibers compared to the chickens with a slow growth rate. As reported by other authors, increased diameter and length of muscle fibers may be due to intensive selection (Brocka et al. 1998, Guernec et al. 2003) and changes appearing in the size and shape of muscle fibers (Bogucka and Kapelański 2004).

Skeletal muscles are a heterogeneous tissue that is characterized by multiple types of muscle fibers that affect

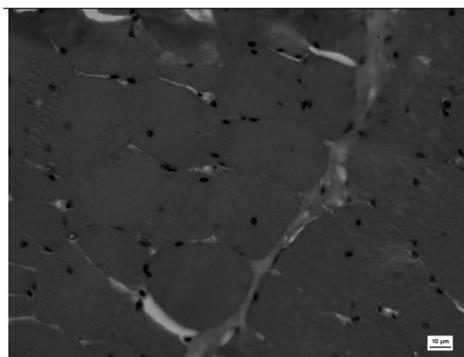
TABLE 1. Fiber diameter of breast and leg muscles of chickens (♀ + ♂)

Group	Fiber diameter (μm)	
	breast muscles	leg muscles
Silkies	33.23 ^B	28.73 ^B
Hubbard JA 957	75.61 ^A	62.51 ^A
SEM	1.311	1.262

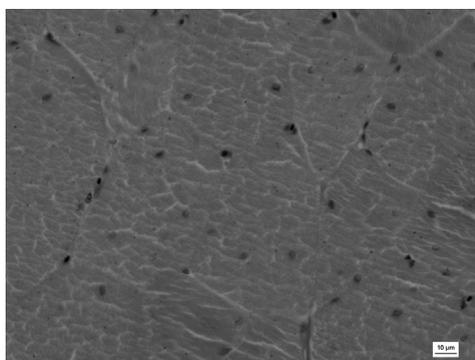
A, B – statistically significant differences at $P \leq 0.01$.



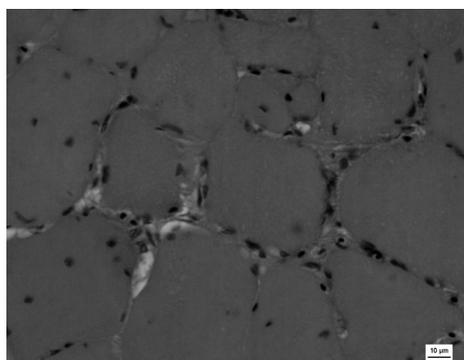
breast muscle – Silkies chickens



leg muscle – Silkies chickens



breast muscle – Hubbard JA957 chickens



leg muscle – Hubbard JA957 chickens

FIGURE 1. Cross-section of breast and leg muscles of Silkies and Hubbard JA 957 chickens

diversified characteristics of particular muscles (Bottinelli and Reggiani 2000). There are many factors that contribute to changes in the character of muscle fibers, these including: gender (Ozawa et al. 2000), age (Candek-Potokar et al. 1998), breed (Ryu et al. 2008), and physical activity (Jurie et al. 1999). Investigations conducted by Castellini et al. (2002a, 2002b) and Branciarri et al. (2009) demonstrate that the access to free runs may also influence the character of muscle fibers.

The number and type of muscle fibers are determined already in the fetal life (Petersen et al. 1998, Karlsson et al.

1999). Dankowiakowska et al. (2012) showed that an increased temperature of hatching to 38.5 or 39.0°C resulted in an increased number of fibers with a larger diameter in the breast muscle of broiler chickens. The experiment conducted by Sobolewska et al. (2011) indicates that in the period of 35-day rearing of broiler chickens, a more intensive increase of muscle fiber diameters was observed between days 8 and 21. The breast muscle fibers of chickens are fully developed already around the 8th week of birds life.

The impact of muscle fibers character on meat quality has been investigated for years. The character of muscle fibers is

determined genetically and is typical of particular hybrids and breeds. Histological and biochemical properties of muscles, including the type, number, proportions and diameter of muscle fibers as well as their metabolic character affect the pH value and water absorbability of meat, whereas these traits influence meat quality (Bereta and Eckert 2010). From a consumer's point of view, proportions and diameters of particular types of fibers may lead to changes in the sensory assessment. A higher number of fibers with small and medium diameters improves meat quality (Choi and Kim 2009). Additional increase in the thickness of white muscle fibers has a positive impact on tenderness but a negative impact on juiciness of meat. In case of red fibers, this dependency is opposite (Cameron et al. 1998, Migdał et al. 2005). In hens, almost entire breast muscle is constituted by white fibers.

CONCLUSION

Results of histomorphometric analyses demonstrate that breast and leg muscles of slow-growing Hubbard JA 957 chickens were characterized by a significantly greater diameter of muscle fibers compared to muscles of Silkies chickens. These differences were most of all due to genetic factors. Though slowly growing, the Hubbard JA 957 chickens are selected for body weight gains. The Silkies chickens are a native breed of Chinese hens that have for years been valued for the health-promoting properties of their meat (higher content of carnosine). Chickens of this breed could be successfully used for small-scale production.

The end product may be especially appreciated by a taste-loving consumer, a connoisseur or a dietitian owing to its originality and to the fact of being a valuable source of animal protein.

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- Streszczenie:** Profil histologiczny mięśni piersiowych i nóg kurcząt Jedwabistych oraz wolno rosnących Hubbard JA 957. Badanie przeprowadzono na 60 brojlerach wolno rosnących Hubbard JA 957 oraz 60 kurcząt Jedwabistych odchowywanych do 63. dnia życia, w trzech powtórzeniach po 20 szt., w zamkniętym budynku na ściółce. W okresie odchowu zastosowano trzyfazowy program żywienia. Mieszanki użyte w doświadczeniu nie różniły się pod względem zawartości składników pokarmowych. Kurczęta w pierwszym okresie odchowu do 21. dnia życia żywiono standardową mieszanką typu starter: o zawartości 20,98% białka ogólnego, 2845 kcal na kg energii metabolicznej, 1,17% lizyny i 0,98% met. + cys. W okresie od 22. do 56. dnia stosowano mieszankę typu grower, zawierającą 20,0% białka ogólnego, 2900 kcal na kg energii metabolicznej (EM), 1,04% lizyny i 0,88% met. + cys. Zawartość wcześniej wymienionych składników w mieszance finisz, stosowanej do końca odchowu, wynosiła odpowiednio: 18% białka, 2965 kcal na kg EM, 0,90% lizyny i 0,78% met. + cys. W 63. dniu odchowu wybrano z każdej grupy po 12 kogutów i 12 kur o masie ciała zbliżonej do średniej w grupie. Kurczęta ubito w ubojni drobiu i pobrano wycinki mięśnia piersiowego powierzchniowego (*m. pectoralis superficialis*) oraz dwugłowego uda (*biceps femoris*). Mięśnie piersiowe i nóg ptaków doświadczalnych charakteryzowała zróżnicowana średnica włókien mięśniowych w zależności od genotypu. Największą średnicą charakteryzowały się mięśnie piersiowe i nóg ptaków wolno rosnących Hubbard JA 957, co zostało potwierdzone statystycznie ($P \leq 0,01$). Stwierdzono, że średnica włókien mięśniowych kurcząt Jedwabistych była o ponad połowę mniejsza w porównaniu do kurcząt Hubbard JA 957.
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Comparison of components and number of *Nosema* sp. spores of wintering Carniolan and Italian bees debris

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Abstract: *Comparison of components and number of *Nosema* sp. spores of wintering Carniolan and Italian bees debris.* The aim of the experiment is to investigate and compare the components of the winter debris of two breeds of bees: Carniolan and Italian and microscopic tests of the bees samples from the experimental debris for the presence of *Nosema* sp. The study was carried on 20 wintering bee colonies in the apiary of the Apiculture Division of Warsaw University of Life Sciences – SGGW.

The research material was debris falling during the winter at the bottom of the hive that was bees, wax cappings, eggs, wax, pollen pellets, dark hardened fungal clods and fecal stains on the pads. The examination was conducted during the overwintering 2008/2009. In the spring of 2009 microscopic examination was conducted (samples of bees from the experimental debris for the presence of *Nosema* sp.). Pollen pellets are originated from bee bread, which bees use to feed the brood and was collected from two different breeds of bees. The presence of eggs in debris testifies to the fact that in winter queen of two studied breeds of bees are also laying eggs. The presence of fecal spots in debris is sporadic but it is the sign of nosemosis. Microscopic examination confirmed the assumption that the appearance of the fecal spots was a symptom of *Nosema* sp. presence. *Nosema* sp. infects bees of both breeds. Carniolan breed bees tend to self-medicate from nosemosis. Lack of feces does not indicate the lack of nosemosis in the case of both mentioned breeds of bees.

Key words: honeybee, winter debris, nosemosis, breeds of bees

INTRODUCTION

During winter bees do not leave the nest and accumulate feces in the rectum waiting for warmer days and for an opportunity to leave the hive and to empty bowels (Muszyńska and Bornus 1981). When the colonies in the autumn and winter time create a cluster, the activity of bees is reduced. They move at the small space within the comb and collect only a small amount of food needed to maintain the proper conduct of the necessary processes of life. Flights of bees are rare, but only during the transient (usually fleeting) warming. Sometimes at the end of February in our climatic conditions, also happen that temperature rises above 10°C, which bees use for the first flights for purification and water supply of hive (Muszyńska and Bornus 1981).

Bees do not clean their nests and do not throw away trash during winter. Falling to the bottom of the hives dead insects, food debris (pollen, honey, sugar), wax cappings, dried crumbs, pieces of propolis and other various organic molecules and inorganic contaminants (sand) accumulate in the form of debris (Chmielewski 1992). The practice

of wintering bees of different races in areas with harsh winters shows that the first debris is made up of bees that have fallen to the bottom and froze. The highest daily debris is observed during the first two months of overwintering hives, when there is the peak concentration of bees in the cluster and there is continuous replenishment of outer shell by the bees from a center of the comb. Debris in the second half of wintering consist of the bees died due to excessive physiological overload, with overfilled gut. Next appears the stability of thermoregulation violation in comb, increased food consumption, death of older bees in the colony, which is an important evaluation index of wintering colonies and their possible start to the new beekeeping season (Skubida 1994). According to Chmielewski (1991, 1992), on the basis of qualitative and quantitative debris composition can be conducted observations on the state of health of the colony. Small debris reflects good overwintering of bees, and large and moist one, the improper overwintering. Annual examination of debris allows the early detection of certain diseases and the possibility of potential therapeutic interventions such as (Chmielewski 1992):

- Debris – dead bees and the mentioned earlier ingredients are organic matter, which is the breeding ground and the substrate for the development of different species of mites as house dust mite, dried fruit mite, brown mite, storage mite, narrow mite, flour mite;
- The presence of fungal clods indicates the occurrence of tinea;
- Traces of feces are a sign of diarrhea in the colony, which is caused by nosemosis infection.

The inner and outer walls of the hive contaminated by feces indicate the presence of nosemosis in the colony. It should be done a study to determine the debris infection of bees with *Nosema* sp. spores.

Nosemosis of bees is well-known for beekeepers and veterinarians as a chronic of the honey bee (*Apis mellifera*) caused by intraspecific parasite *N. apis* specie – Eukaryota, Fungi, Microsporea (Gajda 2010). The parasite locates and develops in the cells of the midgut epithelium of workers, queens, and drones (Gliński and Rzedzicki 1983). *Nosema* spores are ingested along with food and water by adult honey bees. Symptoms of the disease include dysentery, increased winter loss of bees, reduced honey production, and shortened life span of the worker bees. These symptoms are most noticeable in the spring. The disease usually fades away in the summer and briefly returns again in the fall of the year (Reed 2010). Worker bees and queen bees have been found to become infected at similar rates when inoculated with *Nosema* spores (Thomas et al. 2004). The clinical symptoms appear only in severe course of disease (Table 1).

From a clinical point of view, nose-mosis is a disease of older bees working outside the hive. Young bees, up to 15 days of age, were infected less often. The greatest severity of disease occurs in early spring – April, May (Gliński and Rzedzicki 1983). The disease spreads easily in a bee colony as bees licking the feces of sick bees infect themselves. Nosemosis causes death of bees (Marcinkowski 1994).

In the case of notice the symptoms of the disease it is proved it can be treated

TABLE 1. Summary of symptoms and changes occurring in nosemosis

Disease	Etiology and occurrence	Bees age	Clinical symptoms	Post-mortem lesions
Nosemosis	<i>Nosema apis</i> winter, early spring, spring	older bees	– loss of fly ability, abdominal distension, diarrhea – feces yellow loam color, grit-like consistency	midgut strongly inflated, pearly white

with many different preparations (Topolska et al. 2008, Nanetti 2009, Thrasyvoulou et al. 2009, Gajda 2010). At the low level of colonies contamination disease resolves spontaneously. In a time of generous food base and of radical replacement of source of infection – alive infected bees, it disappears (Tomaszewska 1995).

Results of apiary wintering is determined by the number of dead colonies during this period of time, as well as the state of health of those colonies which survived the wintertime. Taking care of health control and condition status of colonies during the overwintering it is recommended a debris composition and number of *Nosema* sp. spores inspection. Each year a number of colonies in the apiary cannot stand the wintering what is resulting in their weakening or death. On the strength of the colony during the wintering significant impact has breed of bees.

The aim of the experiment was to investigate and compare the components of the winter debris of two breeds of bees: Carniolan and Italian and microscopic tests of bees samples from the experimental debris for the presence of *Nosema* sp.

MATERIAL AND METHODS

The experiment was conducted on honey bee (*Apis mellifera*) Carniolan and Italian during the overwintering 2008/2009.

The study was carried on 20 wintering bee colonies (10 of Carniolan bees and 10 of Italian bees) in the apiary of the Apiculture Division of Warsaw University of Life Sciences – SGGW. There were selected colonies of similar force, in the same types of hives (Wielkopolska bee hive), with the same number of frames and with a similar number of bees in colonies measured accordingly to the method described by Aimdorf and Gerig (1999).

The bottom boards of examined 20 hives were checked eight times during the wintering season. The measurements were taken at two-week intervals starting from 19 November 2008. Therefore, plastic pads were used, which have previously been properly fitted to the shape and dimensions of the bottom board of the hive so that they could be easily removed and replaced to and from hives (600 × 375 mm). Pads were placed in hives on bottom boards under the frames (5.11.2008) and all debris could fall freely directly to them. After each debris collection plastic pads were cleaned out and again placed on the bottom of the hive.

Winter debris was collected and the description of the components (such as bees, wax cappings, eggs, wax, pollen pellets, dark hardened fungal clods and fecal stains on the pads) measurement was made. Each test accurately determined the quantitative and qualitative composition of debris. To test the

significance of differences of the breeds of bees in each group of debris components (such as number of bees, number of eggs and number of wax cappings mean values) Student's t-test was used. This test applies to the statistics made on studied samples and is sufficient for used in the research groups size. Differences between the means in all groups were calculated at the 0.05 significance level (Olech and Wiczorek 2012).

There were also observed all abnormal changes that could be symptoms of diseases such as fecal spots or dark hardened fungal clods. Then, the tested material was packed into separate envelopes with pre-marked number of hives, the date and the information about the debris. Samples were stored in a freezer (at -20°C).

In the spring of 2009 microscopic examination was conducted (samples of bees from the experimental debris for the presence of *Nosema* sp.). *Nosema* detection method used in the study relied mainly on the procedures developed by Wilson and Ellis (1966), Cantwell (1970), Fingler et al. (1982), Kauko et al. (2002), Rogers et al. (2002), OIE (2004), Topolska and Hartwig (2005).

Examination was carried out as follows: to each sample were collected 30 bees to test it for the presence of *Nosema* sp. Abdomens were detached with tweezers and were homogenized with distilled water in a ratio of 1 ml per bee. A drop of the homogenate was transferred to a chamber used for counting blood cells (Fusch Rosenthal) and calculated the number of possible *Nosema* sp. spores in four small squares. In the case of infection number of *Nosema* sp. was calculated in a single bee according to the following formula:

$$\begin{array}{ll} 10 \text{ squares} & 2 \text{ mm}^3 \\ 1 \text{ square} & 0.2 \text{ mm}^3 \\ 1/4 \text{ squares (4 - small squares)} & 0.05 \text{ mm}^3 \end{array}$$

$$X = \frac{\text{calculated number of spores} \times 10,000}{0.05} \quad (1)$$

It should be noted that the tested colonies were not treated for disease.

RESULTS AND DISCUSSION

In Carniolan bees hives on the bottom board was found on average less bees and wax cappings but more eggs than in Italian bee hives. Because of the wide variation in results (some hives had really small amounts of debris, but some hives were heavily littered) the standard deviations were high. There were not found significant differences between the means in any group (at the 0.05 significance level) of tested components between Carniolan and Italian race (Table 2).

In debris there was generally very small amount of wax itself, but pollen pellets was very common in almost every hive and in almost whole season (from November 2008 till March 2009). Especially in February when pollen pellets were found in all examined hives. Feces were observed only in three hives with various intensity and in one case the trace of faces did not indicated nosemosis infection (which was verified later by the test for *Nosema* sp. presence).

Fungal clods were found in January, in February and in March in four hives, but not too many of them. Generally it was more often observed in Italian bees hives (Table 3).

TABLE 2. The average number of some debris components in the whole wintering season in Carniolan and Italian bees hives

<i>Apis mellifera</i> breed	Value	Number of bees	Number of eggs	Number of wax cappings
Carniolan	mean	236.40	48.00	59.60
	SD	23.02	94.65	39.30
Italian	mean	326.50	33.00	61.75
	SD	110.41	34.30	19.00

TABLE 3. The results of the investigation of some debris components in Carniolan and Italian bees hives

Bee breed	Wax	Pollen pellets	Feces	Fungal clods
Carniolan	+	++++	+++	+
Italian	+	++++	-	++

Designations: (-) lack of component, (+) single presence, (++) double or few times presence, (+++) numerous, (++++) high intensity.

TABLE 4. Results of the dead bees samples tested for *Nosema* sp. in 2008/2009 season

Bee breed	Number of observations	The level of infection			
		- (N)	+ (S)	++ (M)	+++ (H)
Carniolan	80	69	3	2	6
Italian	80	76	0	1	3

Designations: (-) no spores, (+) single spores not in each field of vision, (++) single spores in each field of vision, (+++) numerous spores in each field of vision, (N) – not infected, (S) – slightly infected, (M) – moderately infected, (H) – heavily infected.

From that number only few hives showed *Nosema* sp. appearance. More often and more heavily infected were Carniolan bees (Table 4).

It was very interesting that at the beginning of the wintering the number of spores was high and from November till December even rose to the number of 16.6 millions of spores, it seemed to at the same level for some time but later on it seemed to fall and in March it reached about half a million of spores (Fig. 1).

CONCLUSIONS

1. Wax debris consisted of bees, eggs, wax cappings, crumbs of wax, pollen pellets, fecal spots and fungal clods of both bee breeds.
2. The presence of eggs in debris of both breeds testifies to the fact that in winter some queen bees are also laying eggs.
3. The presence of fecal spots in debris is sporadic in studied breeds but it is the sign of nosemosis.

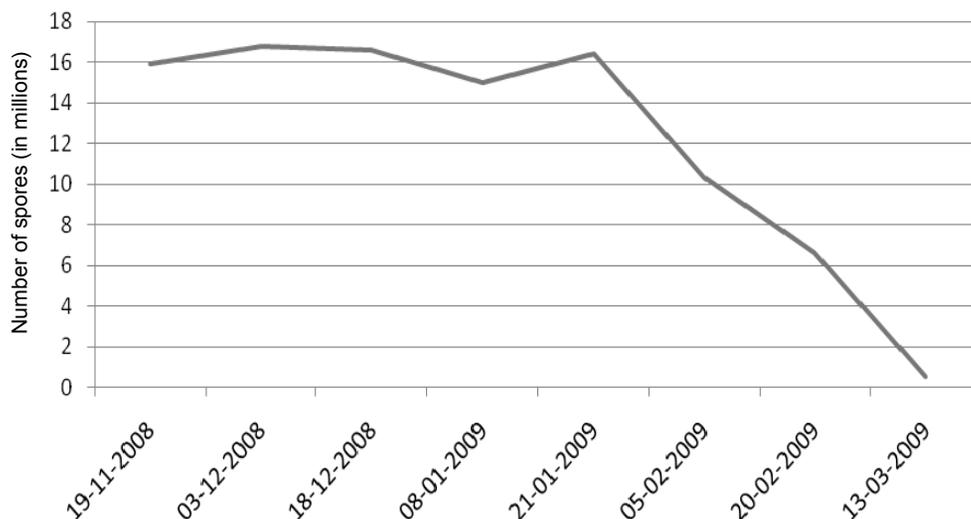


FIGURE 1. Number of spores of *Nosema* sp. in one of the Carniolan bees colony examined in 2008/2009 season

4. Microscopic examination confirmed the assumption that the appearance of the fecal spots was a symptom of *Nosema* sp. presence.
5. *Nosema* sp. infects bees of both breeds.
6. Lack of feces does not indicate the lack of nosemosis.

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Streszczenie: Porównanie części składowych osypu zimowego oraz ilości spor *Nosema* sp. pszczoł rasy kraińskiej i włoskiej. Celem doświadczenia jest zbadanie i porównanie części składowych osypu zimowego pszczoł dwóch ras kraińskiej i włoskiej oraz przeprowadzenie badań mikroskopowych prób pszczoł z osypów doświadczalnych pod kontem występowania *Nosema* sp. Doświadczenie przeprowadzono na pszczole miodnej (*Apis mellifera*). Do badań użyto pszczoł z 20 zimujących rodzin pszczelich w pasiece Pracowni Hodowli Owadów Użytkowych SGGW w Warszawie. Materiałem badawczym był osyp spadający w czasie zimy na dno ula, tzn. pszczoły, łuseczki woskowe, jaja, wosk, obnóża pyłkowe, ciemne stwardniałe grudki grzybiące oraz plamy kału na podkładkach. Doświadczenie przeprowadzono podczas zimowli 2008/2009. W okresie wiosennym 2009 roku przeprowadzono badania mikroskopowe (prób pszczoł z osypów doświadczalnych na występowanie *Nosema* sp.). Obecność obnóż pyłkowych świadczy o obecności czerwiu w rodzinach obydwu ras. Obnóża pyłkowe pochodzą z pierzgi, którą pszczoły wykorzystują do karmienia czerwiu. Obecność jaj w osypie obydwu ras pszczoł świadczy o tym, że w zimie niektóre matki też czerwią. Kał występuje sporadycznie i jest symptomem nosemy. Badania mikroskopowe potwierdziły przypuszczenie, że pojawienie się kału świadczy o obecności *Nosema* sp. *Nosema* sp. poraża pszczoły obydwu ras. Pszczoły rasy kraińskiej wykazują tendencje do samoleczenia. Brak kału u obu ras pszczoł nie wskazuje na brak nosemozy.

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New sites of the rare Microlepidoptera species on Warsaw basin

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Abstract: *New sites of the rare Microlepidoptera species on Warsaw basin.* An inventory studies on Microlepidoptera were performed in 2008 on meadows neighbouring the then planned Warsaw-Modlin Airport. The studies revealed the occurrence of 165 species representing 26 families in the area including 37 species first noted in Masovian Province. Species that deserve special attention due to a low number of sites in Poland include: *Depressaria pimpinellae* (Zeller, 1839), *Scythris seliniella* (Zeller, 1839), *Schiffermuelleria schaefferella* (Linnaeus, 1758), *Pseudatemelia flavifrontella* (Denis et Schiffermüller, 1775), *Nothris verbascella* (Denis et Schiffermüller, 1775), *Ptocheuusa inopella* (Zeller, 1839), *Epinothia rubiginosana* (Herrich-Schäffer, 1851), *Lobesia artemisiana* (Zeller, 1847), *Phtheochroa pulvillana* (Herrich-Schäffer, 1851), *Anania verbascalis* (Denis et Schiffermüller, 1775), *Epascestria pustulalis* (Hübner, 1823).

Key words: Microlepidoptera, new records, Warsaw basin

INTRODUCTION

Mazowiecka Lowland is a region poorly recognised in terms of the occurrence and distribution of species from many families of tiny moths (Microlepidoptera); moreover, many data have only an historical importance. The only complex study that has recently appeared from this region pertains to Gracillariidae in the Skarpa Ursynowska Reserve (Jaworski 2009), several rare species of Microlepidoptera were reported by Mazurkiewicz and Wrzesień (2008) from

typically anthropogenic Warsaw habitat (Pola Mokotowskie).

Study area involved grounds neighbouring the then planned Warsaw-Modlin Airport covered mainly by xeric meadow communities with single dwarf specimens of trees and shrubs (mainly hawthorn and oaks). The communities were extensively used (mown twice a year without undersowing and fertilisation).

MATERIAL AND METHODS

Studies were carried out since March till the end of August 2008. Basic methods applied included: sweeping with moths net over herb vegetation, catching specimens by a single stroke, flushing out the moths by shaking them off from tree and shrub branches. Moreover, 6 times (once a month) a light trap was used in places most interesting with respect to plant cover. 250 W mercury lamp used for this purpose was powered by 750 W Honda EX7 generator. Pre-imaginal stages were also searched for to breed them until obtaining imagines.

RESULTS AND DISCUSSION

Performed field studies in the Warsaw-Modlin Airport revealed the occurrence of 165 species of Microlepidoptera,

which is ca. 8.5% of this group in Poland. Caught species belonged to 26 families. From among caught species 37 were new for Masovian Province (Table 1). Eleven species described below deserve special attention – they are most rare from among species found in the area and known from a few sites in Poland. The description of some species is supplemented by a short diagnose of the state of their population in the study area.

Systematic order and nomenclature is presented based on a study “The Lepidoptera of Poland. A distributional checklist” (Buszko and Nowacki 2000).

Depressariidae

1. *Depressaria pimpinellae* (Zeller, 1839). Caterpillars of this species develop on umbels of the burnet saxifrage and the greater burnet saxifrage (*Pimpinella saxifraga* and *P. major* Toll, 1964). In the last 50 years, the species has been

TABLE 1. A list of recorded species of Microlepidoptera new for Masovian Province

No.	Species
1	2
Eriocraniidae	
1.	<i>Eriocrania semipurpurella</i> (Stephens, 1835)
2.	<i>Eriocrania sparrmannella</i> (Bosc, 1791)
3.	<i>Eriocrania subpurpurella</i> (Haworth, 1828)
Tischeriidae	
4.	<i>Emmetia gaunacella</i> (Duponchel, 1843)
5.	<i>Emmetia marginea</i> (Haworth, 1828)
Tineidae	
6.	<i>Monopis monachella</i> (Hübner, 1796)
7.	<i>Tinea semifulvella</i> (Haworth, 1828)
Yponomeutidae	
8.	<i>Argyresthia trifasciata</i> (Staudinger, 1871)
Plutellidae	
9.	<i>Eidophasia messingiella</i> (Fischer v. Röslerstamm, 1840)
10.	<i>Ethmia bipunctella</i> (Fabricius, 1775)
Depressariidae	
11.	<i>Agonopterix alstromeriana</i> (Clerck, 1759)
12.	<i>Depressaria pimpinellae</i> (Zeller, 1839)
13.	<i>Luquetia lobella</i> (Denis et Schiffermüller, 1775)
14.	<i>Semioscopis oculella</i> (Thunberg, 1794)
15.	<i>Semioscopis steinkellneriana</i> (Denis et Schiffermüller, 1775)
Elachistidae	
16.	<i>Elachista albidella</i> (Nylander, 1848)
17.	<i>Elachista argentella</i> (Clerck, 1759)

Table 1 cont.

1	2
18.	<i>Elachista poae</i> (Stainton, 1855)
19.	<i>Elachista pollinariella</i> (Zeller, 1839)
Scythrididae	
20.	<i>Scythris seliniella</i> (Zeller, 1839)
Oecophoridae	
21.	<i>Schiffermuelleria schaefferella</i> (Linnaeus, 1758)
Coleophoridae	
22.	<i>Coleophora colutella</i> (Fabricius, 1794)
Amphisbatidae	
23.	<i>Pseudatemelia flavifrontella</i> (Denis et Schiffermüller, 1775)
Gelechiidae	
24.	<i>Aristotelia brizella</i> (Treitschke, 1833)
25.	<i>Dichomeris derasella</i> (Denis et Schiffermüller, 1775)
26.	<i>Neofaculta infernella</i> (Herrich-Schäffer, 1851)
27.	<i>Nothris verbascella</i> (Denis et Schiffermüller, 1775)
28.	<i>Pseudotelphusa scalella</i> (Scopoli, 1763)
29.	<i>Ptocheuusa inopella</i> (Zeller, 1839)
Tortricidae	
30.	<i>Epinotia rubiginosana</i> (Herrich-Schäffer, 1851)
31.	<i>Eucosma metzneriana</i> (Treitschke, 1830)
32.	<i>Lobesia artemisiana</i> (Zeller, 1847)
33.	<i>Phtheochroa pulvillana</i> (Herrich-Schäffer, 1851)
Choreutidae	
34.	<i>Anthophila fabriciana</i> (Linnaeus, 1767)
Pyralidae	
35.	<i>Anania verbascalis</i> (Denis et Schiffermüller, 1775)
36.	<i>Epascestria pustulalis</i> (Hübner, 1823)
37.	<i>Sciota fumella</i> (Eversmann, 1844)

reported from several sites in various parts of Poland (Buszko and Nowacki 2000). Several specimens were caught with the butterfly net. Population of this species is probably numerous in the study area.

Scythrididae

2. *Scythris seliniella* (Zeller, 1839). Larvae develop on the field wormwood *Artemisia campestris* and on the fastigi-

ated gypsophila *Gypsophila fastigiata*. This is a one-generation species (moths appear since the middle of May till July). It occurs locally but in sites of occurrence is relatively abundant. Sites of this species known so far in Poland are situated mainly in the south of the country (Baran 2005). Several specimens were caught with butterfly net in few places, so population is probably numerous.

Oecophoridae

3. *Schiffermuelleria schaefferella* (Linnaeus, 1758). Caterpillars of this species live in rotten wood and under the bark of various dead trees (Toll 1964). It was reported from single stands in various parts of Poland (Buszko and Nowacki 2000). One specimen was caught with the light trap.

Amphisbatidae

4. *Pseudatemelia flavifrontella* (Denis et Schiffermüller, 1775). Caterpillars of *P. flavifrontella* feed on dead leaves (Toll 1964). In the last 50 years, the species has been found in only two stands in Poland (Buszko and Nowacki 2000). One specimen was caught with the light trap.

Gelechiidae

5. *Nothris verbascella* (Denis et Schiffermüller, 1775). The species is trophically associated with various species of mullein *Verbascum* spp. Known in Poland from single dispersed sites (Buszko and Nowacki 2000). Several specimens were caught with the light trap and a net at dusk. It may be assumed that the population of this species is quite numerous in the study area.

6. *Ptocheuusa inopella* (Zeller, 1839). Larvae develop on the dwarf everlast plants *Helichrysum arenarium* (Elsner et al. 1999). The species is known in Poland from several sites in various parts of the country (Buszko and Nowacki 2000). One specimen was caught with the light trap.

Tortricidae

7. *Epinotia rubiginosana* (Herrich-Schäffer, 1851). Host plants for this species are pine *Pinus silvestris* and spruce *Picea excelsa*. In the last 50 years the species has been found in only two sites

in Poland (Buszko and Nowacki 2000). One specimen was caught with the light trap.

8. *Lobesia artemisiana* (Zeller, 1847). Host plants for the larvae are mainly the common bugloss *Anchusa officinalis*, the viper's bugloss *Echium vulgare* and field wormwoods *Artemisia* spp. The species is known in Poland from single stands. Several specimens were caught with the light trap.

9. *Phtheochroa pulvillana* (Herrich-Schäffer, 1851). Caterpillars feed on milkvetch plants *Astragalus* spp. The species was noted in single dispersed sites in Poland. Single specimens of this species were caught with the light trap.

Pyralidae

10. *Anania verbascalis* (Denis et Schiffermüller, 1775). Host plants of the larvae are germanders *Teucrium* spp. and mullein *Verbascum* spp. The species has one generation per year – adults appear in June and July. Several specimens were caught with the light trap. One may assume that the species is quite numerous in the study area.

11. *Epascestria pustulalis* (Hübner, 1823). Larvae feed (mine) in parenchyma of leaves of the common bugloss *Anchusa officinalis*. The species is known in Poland from a few dispersed stands. Numerous mines with larvae were observed in many sites of the study area, moreover, several specimens were attracted by light and several specimens were caught with net during the day. The study area is probably inhabited by one of more numerous populations of this species in Poland.

Most of the described species are trophically associated with plants characteristic for dry and open meadow com-

munities which covered a larger part of the study area. These habitats developed there as a result of specific way of management (mowing twice a year) which is recommended in order to maintain them. It would be also recommended to leave trees and shrubs, mainly oaks, trembling poplar, blackthorn and hawthorn, at the edges of meadows (at least in part of the area). These are the host plants for caterpillars of several relatively rare species in Poland (e.g. *Emmetia gaunacella*, *Acrocercops brongniardella* or *Luquetia lobella*).

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- Streszczenie:** Nowe stanowisko rzadkich gatunków *Microlepidoptera* w Kotlinie Warszawskiej. W 2008 roku przeprowadzono badania inwentaryzacyjne dotyczące *Microlepidoptera* na obszarze łąk sąsiadujących z planowanym wówczas portem lotniczym Warszawa-Modlin. W wyniku badań stwierdzono na tym terenie występowanie 165 gatunków reprezentujących 26 rodzin. Wśród nich było 37 gatunków po raz pierwszy wykazanych z województwa mazowieckiego. Do gatunków zasługujących na szczególną uwagę ze względu na niewielką liczbę znanych w Polsce stanowisk zaliczono: *Depressaria pimpinellae* (Zeller, 1839), *Scythris seliniella* (Zeller, 1839), *Schiffermuelleria schaefferella* (Linnaeus, 1758), *Pseudatemelia flavifrontella* (Denis et Schiffermüller, 1775), *Nothris verbascella* (Denis et Schiffermüller, 1775), *Ptocheuusa inopella* (Zeller, 1839), *Epinotia rubiginosana* (Herich-Schäffer, 1851), *Lobesia artemisiana* (Zeller, 1847), *Phtheochroa pulvillana* (Herich-Schäffer, 1851), *Anania verbascalis* (Denis et Schiffermüller, 1775), *Epascestria pustulalis* (Hübner, 1823).

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The effect of density on the breeding optimization of the tropical house cricket *Grylloides sigillatus* (Walker) (Orthoptera: Gryllidae)

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Abstract: *The effect of density on the breeding optimization of the tropical house cricket *Grylloides sigillatus* (Walker) (Orthoptera: Gryllidae).* The study was aimed at testing the density effect in the tropical house cricket breeding on its survival and growth rate when fed *ad libitum*. The tropical house crickets were kept in nine containers of a volume of 81 litres each. Three experimental variants were used: 7.5 ml of crickets were placed in the first container, 15 ml in the second and 30 ml in the third. Temperature in containers was 29°C, the experiment lasted 25 days. Obtained results showed that survival did not depend on the initial density in culture containers while crickets kept at a high density had smaller body length. The results may affect the optimization of house cricket breeding.

Key words: the tropical house cricket, *Grylloides sigillatus*, survival, population density, growth rate, optimization of breeding

INTRODUCTION

Terraristics enjoys increasing popularity. More and more reptiles and amphibians are kept by both experienced and novice breeders. The animals require mainly live food. The nutritive requirements of reptiles, amphibians, but also of popular African pigmy hedgehogs *Atelerix albiventris* (Wagner, 1841) and many bird species, are fulfilled by feeding them with insects. They are the main component or

an additive to animals' diet. Moreover, insects are more and more often considered food for humans. This is because of the need to search for new protein sources for growing human population and of a curiosity and desire to learn new tastes and to break nutritional stereotypes. For the second mentioned reason the first in Poland restaurant "Co To To Je", that serves exclusively insect dishes, has recently been open in Warsaw.

Above mentioned circumstances indicate that the market for food insects has a great potential but needs some improvement. Insects imported are usually weak and soon die of exhaustion and conditions of transportation. Country breeding still needs to be developed since its efficiency may be higher and there is a deficit of insects from local production.

Food insects most often bred include: crickets, cockroaches, locusts, larvae of wood-eaters, mealworms and wax moths. Crickets are bred to the largest extent. They have low requirements compared with other food insects. The tropical house cricket *Grylloides sigillatus* (Walker) has relatively high protein content from among bred insect species (Table 1).

The tropical house cricket is the smallest of bred crickets (18–22 mm of length). It has two transverse black bands on its abdomen and resembles the house cricket. Females are larger than males and wingless. Males have partly reduced wings, hence they contain less chitin and are more easily digested. The tropical house crickets are distinguished by low aggression towards other crickets and cannibalism is rare among them. Biting animals that feed on them is also rare. The insects are very fast, which is an advantage for animals actively hunting for their prey. They also show a high fertility and resistance to viruses (like e.g. *Acheta domesticus* Densovirus) and fungi, which makes them ideal animals for mass production. The development from hatching till imago lasts 8–15 weeks depending on temperature.

productivity and the quality of animal products in food invertebrates.

MATERIAL AND METHODS

Experimental crickets were provided with basic elements of welfare. Having *ad libitum* access to food and water they were free from hunger and thirst. Crickets were free from discomfort – their resting area was made of cardboard egg trays which increased living area and offered a shelter. The animals were free from pain, injuries, diseases and stress. Studies were carried out in the food insect farm “Fabryka owadów” in Warsaw.

Before setting up the experiment, freshly hatched (maximum 24 h after hatching) cricket larvae were mixed to obtain uniform sample. The experiment

TABLE 1. Nutritive value of crickets (<http://www.livefoodsdirect.co.uk/Category/Banded-Crickets>)

Specification	<i>Grylloides sigillatus</i>	<i>Gryllus assimilis</i>	<i>Gryllus bimaculatis</i>
Energy (kcal/kJ per 100 g)	164/687	130/546	153/640
Protein (%)	21.4	15.2	14.7
Fat (%)	6.9	5.7	8.3
Carbohydrates (%)	4.0	4.5	4.9
Water (%)	66.1	73.3	70.8
Ash (%)	1.6	1.3	1.3

Performed studies had to estimate the effect of initial density on survival and body length in crickets. This information is useful to increase the profitability of breeding.

The main aim of performed studies was to intensify breeding. This requires, however, providing animal welfare and hygienic conditions. Like in farm animals, habitat conditions affect health and

lasted 25 days. The initial number of crickets in 1 ml (mean from three samples) was 424.7.

The tropical house crickets were kept in nine containers of a volume of 81 litres each (60×40×34 cm). Temperature in containers was 29°C. All containers had the same ventilation and were placed at the same height on shelves to avoid differences in temperature between cultures.

Ten pieces of cardboard egg trays glued together were placed in each container. They served as the main living area for crickets.

There was a small waterer (a bowl with hydrogel which prevented larvae from drowning) in each container to provide crickets with permanent free access to water. Dry food consisted of a mixture of wheat bran and fishmeal (a protein additive that inhibits cannibalism) in the ratio 2 : 1 and trays with carrot – a base of diet during the experiment. The access to food and water was provided *ad libitum* to exclude food as the growth limiting factor.

calculated by multiplying the final volume of crickets by the number of crickets in 5 ml. Mean body length of crickets was calculated from measurements of 90 randomly selected specimens from each experimental variant.

To calculate the survival of crickets' larvae, their number was counted and calculated per 1 ml before experiment in the same way as in the end of experiment.

ANOVA, non-parametric Kruskal-Wallis rank test and non-parametric Mann-Whitney U test were used to check the statistical significance of differences.

TABLE 2. The relationship between survival and final number of crickets and the initial sample volume after 25 days of experiments

Variant (initial volume)		<i>N</i>	Mean	Standard deviation	Minimum	Maximum	X^2_{emp}	<i>p</i>
Survival	7.5	3	27.6000	2.12838	25.30	29.50	5.600	0.061
	15	3	16.7667	1.70098	15.10	18.50		
	30	3	18.4000	3.96863	15.40	22.90		
Final	7.5	3	879.0000	67.10440	806.00	938.00	7.200	0.027
	15	3	1070.0000	109.05503	963.00	1181.00		
	30	3	2343.6667	505.55349	1966.00	2918.00		

Three experimental variants were used: 7.5 ml of crickets were placed in the first container, 15 ml in the second, and 30 ml in the third. Each variant was triplicated.

On 25th day of experiment crickets were taken off from culture containers and their volume measured with a cylinder with millilitre scale. Than two random 5 ml samples were taken from each culture, transferred to containers and photographed. The photos were used to count individuals with the Adobe Photoshop software. Final number of crickets was

RESULTS AND DISCUSSION

Performed experiments showed that the initial volume of crickets used in each experimental variant did not affect insect survival ($p = 0.061$) – Table 2.

The effect of initial volume of crickets on body length after 25 days of experiment was highly significant ($p < 0.001$). The longest body had the crickets from experimental variant with the initial volume of 7.5 ml, the shortest – those from the variant with initial volume of 30 ml (Table 3).

TABLE 3. The relationship between body length and initial volume of the sample after 25 days of experiments

Initial volume	N	Mean	Standard deviation	Minimum	Maximum	Femp	p
7.5	90	7.2744	0.91216	5.70	10.10	38.247	<0.001
15	90	6.9544	0.95637	5.10	10.00		
30	90	6.1344	0.83305	4.50	8.30		

Initial volume significantly affected the final number of crickets in experimental variants ($p = 0.027$). The highest number of insects was obtained from culture of initial volume of 30 ml (Table 2).

Many studies (pertaining, however, mostly to vertebrates) on density-dependent survival indicate that young individuals may die more often at a high density (Sheperd and Cushing 1980, Van der Veer 1986, Myers and Cadigan 1993, Ohman and Hirche 2001, Holbrook and Schmitt 2002). In view of the breeding efficiency an important finding of our study is that the initial volume does not affect crickets' survival. One may expect that similar mechanisms were involved here as in mealworms which at high densities acquired higher resistance to viruses and fungi (Barnes and Siva-Jothy 2000). Survival of mosquito larvae bred at medium temperature also increased at high densities (Lyimo et al. 1992). Noteworthy, no symptoms of viral or fungal diseases were observed during our experiments.

Performed studies showed that an increase of density resulted in decreased body length. Slower growth rate of larvae and smaller mass of imagines was noted e.g. in mosquitoes (Gimnig et al. 2002). Body mass of female pupae of this species was conversely proportional to density (Hawley 1985). Mosquitoes

kept at 27°C achieved higher survival at high densities but their growth rate declined (Lyimo et al. 1992).

In order to obtain the greatest number of adult crickets in the shortest possible time one may set up a culture of relatively high density but crickets will not achieve as large body size as they would at lower densities.

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Streszczenie: Wpływ zagęszczenia na optymalizację hodowli świerszcza bananowego *Grylloides sigillatus* (Walker) (Orthoptera: Gryllidae). Praca miała na celu zbadanie wpływu zagęszczenia początkowego w hodowli świerszczy bananowych na ich przeżywalność oraz szybkość wzrostu przy

dostępie do pokarmu *ad libitum*. Świerszcze bananowe w czasie eksperymentu były hodowane łącznie w dziewięciu pojemnikach o pojemności 8 l każdy. Zastosowano trzy warianty doświadczeń: w pierwszym pojemniku umieszczono 7,5 ml świeżo wylęgłych świerszczy, w drugim 15 ml, a w trzecim 30 ml. Każdy wariant doświadczenia powtórzono trzykrotnie. Temperatura w pomieszczeniu wynosiła 29°C. Eksperyment trwał 25 dni. W wyniku przeprowadzonych badań stwierdzono, że objętość początkowa świerszczy użytych w każdym wariantcie doświadczenia nie wpływa na przeżywalność owadów, jednak świerszcze hodowane w większym zagęszczeniu miały mniejszą długość ciała. Uzyskane wyniki mogą przyczynić się do zoptymalizowania hodowli tego gatunku świerszcza.

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Effect of nanoparticles of copper and copper sulfate administered *in ovo* on hematological and biochemical blood markers of broiler chickens

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Abstract: *Effect of nanoparticles of copper and copper sulfate administered in ovo on hematological and biochemical blood markers of broiler chickens.* At the first stage of the study the experimental material included 300 clutching eggs of Hubbard Flex chickens. The eggs were divided into three groups: control (without injection *in ovo*), Nano50 (*in ovo* injection of colloidal copper nanoparticles) and NanoCuSO₄ (*in ovo* injection of colloidal nanoparticles of copper sulphate). Experimental solutions were administered by *in ovo* injection using a sterile needle 0.3 mm as follows: Nano50 group – colloid of copper nanoparticles (concentration: 50 ppm), and NanoCuSO₄ group – colloid of copper sulfate (concentration: 50 ppm), to the air cell of the egg. The eggs were incubated under standard conditions. After hatching, 50 chicks were selected from each group for 42-day rearing. Birds were fed standard complete feed mixtures for broilers. On the last day of rearing (day 42), 12 females and 12 males were selected from each group and their blood was sampled for assays of hematological and biochemical markers. Hematological analyses included determinations of: WBC, RBC, Hb, heterophils, lymphocytes, monocytes, eosinophils and basophils, whereas biochemical analyses included assays of the following markers in blood serum: glucose, cholesterol, triglycerides, HDL-cholesterol, urea, calcium, magnesium, phosphorus, iron, AS-PAT, and ALAT. The use of copper nanoparticles evoked an increase in blood levels of RBC, HGB, HTC, heterophils, monocytes and basophils. In addition, in blood serum in contributed to reduced

concentrations of glucose and cholesterol and increased levels of selected microelements: calcium, phosphorus and iron.

Key words: *in ovo*, nanoparticles, colloid, hematological and biochemical markers in chicken blood

INTRODUCTION

Copper is an element commonly occurring in organisms of plants and animals. As a microelement, it is a constituent of active centers of many enzymes being of key significance to metabolic processes. In addition, it is a component and activator of enzymes in many redox reactions, however its major roles are seen in the synthesis of red blood cells and assistance in connective tissue formation. Also, this element plays an important role in iron metabolism – it facilitates its absorption and binding to transferrin, which transfers iron to erythrocytes, where hemoglobin is being synthesized. Copper present in ceruloplasmin (serum protein) is one of the most active forms of this element in organisms and in this form it regulates iron metabolism and

transport (Witkiewicz 2008). By activating the enzyme indispensable of erythrocytes synthesis, it influences the proper functioning of the erythropoietic system. Deficiency of copper and iron leads to anemia and low utilization of vitamin C. Significant is also its impact on the regeneration of connective tissue by, e.g., synthesis of collagen and elastin and on the development of the nervous system by synthesis of dopamine. Furthermore, copper and zinc prevent damages induced by free oxygen radicals. This element plays also a significant role in the regulation of glucose and cholesterol metabolism. It is a constituent and an activator of a numerous group of enzymes, particularly of oxidases: cytochrome, lysyl, ascorbic, ketochole superoxide dismutase and tyrosinase (Makarski and Zadura 2006). Copper deficiency induces osteoporosis, reduced body immunity, increases blood level of cholesterol and anemia. In chickens it mainly contributes to: growth retardation, disorders of the nervous and cardiovascular systems, anemia, disorders in plumage pigmentation, disorders in ossification, and myocardial fibrosis. When copper deficiency is also a reduced production of eggs, but the larger mass. Moreover, the eggs are often without the shell (in the membrane of the egg) or with a thin shell, or distorted. Deficiency revealed disturbances in reproduction and development of sperm and high mortality of embryos during hatching. In addition, results in poor pigmentation colorful feathers feathered breeds, slow growth, reducing body weight, anemia and ultimately death. Symptoms of copper deficiency include poor production results in dairy cattle, loss of hair color and hair loss as well

as reproduction disorders. Copper deficit deteriorates keratin synthesis and, as a result, causes more frequent incidence of lameness and increased incidence of mastitis. Poultry demand for copper is not that big, but necessary for the proper development of the organism. In poultry, is from 5 to 25 mg in 1 kg of the mixture (Smulikowska 1996), depending on the species, age and the production direction. Why or copper in 1924 was included in the essential micronutrients. Furthermore, it is one of two major transition metals present in the body.

Copper is an important microelement, indispensable for vital processes and for the proper development of living organisms as it plays significant metabolic functions. For instance, it facilitates iron absorption from the gut, takes part in hemoglobin synthesis and therefore has a great impact on red blood cells production (erythropoiesis), it is also an indispensable element of many enzymes (Brzozowski 2007). Both its deficiency and excess may evoke adverse and toxic effects. However, information is lacking on the boundary between its necessary and toxic concentrations in a body. It is common knowledge that intracellular copper occurs mainly in mitochondria and in the nucleus. It is capable of establishing links with nucleic acids wherein it may induce permanent structural changes. It forms links especially easily with various sulfur-containing proteins, particularly with low-molecular metallothionein.

Nanotechnology – which is one of the most intensively developing sciences – has entered into many research disciplines including: chemistry, physics, bioengineering and biomedicine.

Nanotechnological processes have enabled structural modifications of many simple and complex substances, owing to which they can be transformed into submicroscopic objects. What is more, it has been quite recently discovered that the submicroscopic fragments of the matter are characterized by exceptional biochemical traits. Examples of these substances include nanoparticles constituted by atoms of silver, chromium or copper. Nanoparticles are microscopic molecules the size of which is measured in nanometers (nm). They are defined as particles sized less than 100 nm. A nanometer equals one millionth of a millimeter or the width of three or four atoms. By comparison, a human hair is ca. 10–50 nm in width, whereas red blood cells are 2–6 nm and DNA is 2–12 nm in diameter.

The objective of this study was to determine the effect of copper and copper sulfate nanoparticles injected *in ovo* on hematological and biochemical blood markers of broiler chickens.

MATERIAL AND METHODS

Characterization of copper nanoparticles

The shape and size of the copper nanoparticles were inspected (Fig. 1) using a JEM-1220 (JEOL, Tokyo, Japan) transmission electron microscope (TEM) at 80 KeV, with a Morada eleven-megapixel camera (Olympus Soft Imaging Solutions, Münster, Germany). Samples for the TEM were prepared by placing droplets of hydrocolloids onto Formvar-coated copper grids (Agar Scientific, Stansted, UK). Immediately after drying

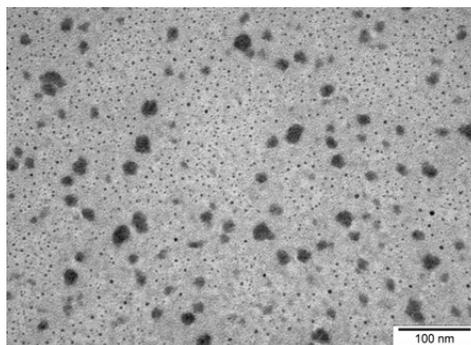


FIGURE 1. Copper nanoparticles visible under electron microscope

of the droplets in dry air, the grids were inserted into the TEM. The test was performed in triplicate. The zeta potential in water was measured using a Zetasizer Nano ZS model ZEN3500 (Malvern Instruments, Malvern, UK).

In ovo injection and incubation conditions of clutching eggs

At the first stage of the study, the experimental material included 300 clutching eggs of Hubbard Flex chickens (average weight 62.25 ± 2.2 g), that were stored for 4 days at a temperature of 12°C and humidity of 73%. The eggs were weighed and divided into three groups: C (control), NanoCuSO₄, and Nano50 (each of 100 eggs). Experimental solutions were administered by *in ovo* injection using a sterile needle 0.3 ml as follows: Nano50 group – colloid of copper nanoparticles (concentration: 50 ppm), and NanoCuSO₄ group – colloid of copper sulfate (concentration: 50 ppm), to the air cell of the egg. Injection orifices were tight-sealed and eggs were fixed in an incubator under standard conditions (temperature 37.8°C, humidity 60%, egg revolution by 90° once a day

for 18 days). The eggs were incubated in a hatching apparatus by Heka company equipped in a temperature, air humidity and egg rotation control module. During incubation, the eggs were light-exposed twice (in day 6 and 18 of incubation) and weighed in order to determine egg weight loss. On day 19 of incubation, the eggs were transferred to a hatcher with a temperature of 37.0–37.5°C and relative air humidity of 75–80%. After hatching, one-day chicks were evaluated and healthy birds with healed navels were selected for further rearing.

Rearing, housing conditions and feeding

A further stage of the study included 150 Hubbard Flex chickens (50 birds from each group) that were kept on litter till 42 days of age under typical zoohygienic conditions, in a room without daylight. One-day chickens after weighing and tagging were divided into three groups (control – C, Nano50, and NanoCuSO₄) in two replications, 25 birds each. Stock density in a henhouse reached 11 birds per 1 m². Immediately after moving to a production hall, the chickens from all groups were vaccinated against Marek's disease, infectious bronchitis and coccidiosis.

The mean air temperature in the room accounted for 24°C, and under radiators – for 34.5°C. The following parameters of the microclimate were measured since the 1st week to the end of rearing: humidity, temperature and contents of toxic gases: ammonia, hydrogen sulfide and carbon dioxide. A three-stage feeding program was applied in the rearing period: starter (crumb), grower and finisher (granulate) – Table 1. The birds were fed

ad libitum. Body weight of the birds (on day: 1, 14, 35, 42 day), their mortality and feed intake were monitored in the rearing period.

Determinations of hematological and biochemical blood markers

In week 6 of rearing, 12 hens and 12 cocks with body weight similar to the average body weight in a group were selected from each group for slaughter. Blood was sampled from their brachial vein to 3-ml-heparinized test tubes. Hematological analyses were conducted in full blood for: WBC (white blood cells), RBC (red blood cells), Hb (hemoglobin), HCT (hematocrit), heterophils, lymphocytes, monocytes, eosinophils, and basophils. The following biochemical markers were assayed in blood serum (30 min after sampling 3 ml of blood were centrifuged at 3,000 rpm for 10 min): glucose, cholesterol, triglycerides, HDL-cholesterol, urea, calcium, magnesium, phosphorus, iron, ASPAT, and ALAT using a CORMAY kit.

Analytical results were subjected to a statistical analysis by computing mean values and standard deviation using the analysis of variance calculated with the least squares method in a statistical software SPSS 19.0 PL (SPSS Inc., Chicago, II, USA).

RESULTS AND DISCUSSION

Right after mercury, cadmium and copper represent the most toxic heavy metals (Dojlido 1995). Even their minute quantities affect the course of biological processes (Swędrzyńska and Sawicka 2010). Results of assays of hematological blood

TABLE 1. Composition and nutritive value of feed mixtures

Specification	Starter (1–14 day)	Grower (15–35 day)	Finisher (36–42 day)
Content (%)			
Corn	10.0	11.4	10.0
Wheat	53.0	55.0	59.6
Soybean meal	30.6	27.4	23.2
Feeding chalk	1.19	1.20	1.11
Acidic sodium carbonate	0.20	0.14	0.14
NaCl	0.24	0.28	0.28
Stimulant	0.01	0.01	0.01
FOSF 2-Ca	1.18	0.78	0.70
Soybean oil	2.10	2.40	3.60
Methionine	0.48	0.42	0.36
Lysine	0.36	0.34	0.36
Threonine	0.14	0.13	0.14
Premix	0.50	0.50	0.50
Nutritive value			
Metabolizable energy (ME) (MJ/kg)	12.52	12.76	13.20
Fat (%)	3.67	4.00	5.14
CP (%)	21.99	20.78	19.26
Methionine (%)	0.70	0.63	0.57
Methionine + Cysteine (%)	1.08	1.01	0.92
Lysine (%)	1.38	1.28	1.19
Ash (%)	5.83	5.35	4.96

markers are presented in Table 2. The *in ovo* injection of a copper sulfate colloid increased counts of WBC, lymphocytes and eosinophils compared to the other groups. In turn, the Nano50 group was characterized by a decreased number of leucocytes compared to the remaining groups. A similar effect was observed by Dmoch and Polonis (2007), who in their study with turkeys were adding copper chelate with lysine to drinking water for birds.

During the experiment, significant ($P \leq 0.05$) changes were determined in

the number of erythrocytes (RBC) in the peripheral blood of the birds exposed to copper. An increased percentage of RBC was noted in the group Nano50. The copper-exposed chickens were characterized by an increased frequency of changed erythrocytes in the peripheral blood that were effectively compensated for by the increasing number of juvenile erythrocytes in blood, owing to which the number of red blood cells remained unchanged (C, NanoCuSO₄) or even increased (Nano50). Analyses showed also increased concentration of hemoglobin

TABLE 2. Hematological blood markers of chickens (♀ + ♂)

Specification	Group			
	C	Nano50	NanoCuSO ₄	SE
WBC – white blood cells (g/l)	13.77	12.00	14.52	1.62
RBC – red blood cells (t/l)	2.84 ^b	3.44 ^a	2.82 ^b	0.12
Hb – hemoglobin (g/l)	134.66 ^b	154.75 ^a	148.0 ^b	7.74
HCT – hematocrit (l/l)	0.36	0.43	0.40	0.02
Heterophils (%)	6.33 ^B	13.75 ^A	7.75 ^B	2.32
Lymphocytes (%)	84.00	75.25	86.50	4.90
Monocytes (%)	7.33 ^A	8.00 ^A	2.00 ^B	2.12
Eosinophils (%)	1.00	1.50	2.50	1.09
Basophils (%)	1.33	1.50	1.25	0.37

a, b – statistically significant differences at $P \leq 0.05$, A,B – statistically significant differences at $P \leq 0.01$. Conversion factor: $\text{g/dl} \times 10 = \text{g/l}$, $\text{g/l} \times 0.1 = \text{g/dl}$, $\text{g/dl} \times 0.6206 = \text{mmol}$, $\text{mmol} \times 1.611 = \text{g/dl}$; $1/l \times 100 = \%$, $\% \times 0.01 = 1/l$; $10^9/l = \text{thou./mm}^3$, $\text{thou./mm}^3 \times 1 = 10^9/l$, $10^9/l = \text{G/l}$, $\text{thou./mm}^3 = 10^3/\text{mm}^3 = 10^3/\mu\text{l}$; $t/l = 10^{-15}$; $Tl = 10^{12}$; $p/g = 10^{-12}g$.

which allowed the birds to keep oxygen transport at an appropriate level. This group was additionally characterized by increased numbers of heterophils, monocytes and basophils.

Changes in erythropoietic parameters in the chickens from experimental groups referred mainly to an increased concentration of hemoglobin in peripheral blood, which was usually accompanied by an increased number of RBC (Table 2). The increased level of hemoglobin could be due to its continued synthesis by erythrocytes already circulating in the peripheral blood. Speckner et al. (1989) emphasized the capability of mature erythrocytes of fish for hemoglobin synthesis that may continue even for some time after cells release from the erythropoietic tissue. It is also likely that the increased level of hemoglobin in the group Nano50 could be linked with the homeopoetic function of copper. This element was shown to directly stimulate erythrocytes synthesis, as it determines

iron absorption into the body and its incorporation to hemoglobin (Fox 2003, Mullally et al. 2004). Results reported by Dmoch and Polonis (2007), demonstrated a reducing tendency in the levels of hematocrit and hemoglobin after the application of copper chelate.

The analysis of other studies on the impact of metals on erythropoietic parameters of fish demonstrated a decreasing tendency in the number of erythrocytes, hematocrit value and hemoglobin concentration in blood. Reduced values of these parameters upon the influence of cadmium were reported by Vincent et al. (1996) and upon the influence of copper – by Ates et al. (2008). Decreased values of these parameters were consequently leading to anemia development in fish (Ruperelia et al. 1990).

Furthermore, analyses demonstrated a reduced percentage of monocytes in the NanoCuSO₄ group, which was accompanied by a suppressed metabolic activity of these cells. Reduction was also observed

in the phagocytic activity in the group of chickens characterized by WBC number increase. This indicates that NanoCuSO₄ could contribute to accelerated damage of phagocytes and to inhibited metabolic activity of these cells.

Results of analyses of biochemical blood markers are provided in Table 3. The *in ovo* injection of copper nanoparticles resulted in decreased blood levels of glucose and cholesterol. The reduced concentration of glucose confirms findings of Makarski and Zadura (2006) who were administering copper chelate to turkeys with drinking water. The applied element had some effect on lipid and cholesterol metabolism as well as on properties of myelin sheaths of nervous fibers. Turnlund et al. (1988) and Bakalii et al. (1995) claim that copper addition contributes to a decreased level of triglycerides and reduced cholesterol synthesis in blood plasma and tissues of animals. In our experiment, the lowest level of triglycerides was determined in the

NanoCuSO₄ group, whereas the highest concentration of HDL was assayed in the control group. The administration of copper nanoparticles caused an increase in the blood level of uric acid. A similar effect on increased levels of glucose and uric acid was observed by Dmoch and Polonis (2007), applying a copper-lysine chelate to chickens. The addition of copper contributed also to increased serum levels of calcium, phosphorus and iron compared to the remaining groups. The increased levels of these elements may be reflected in production effectiveness as they contribute to improved bone mineralization. Upon the use of Cu-Lys chelate Dmoch and Polonis (2007) noted an increase in the level of calcium and to, a lesser extent, in the level of phosphorus. In the case of hepatic enzymes, a reduced level of ALAT was observed in the groups subjected to *in ovo* injection with copper and copper sulfate colloids. An opposite effect was reported for ASPAT concentration which in the NanoCuSO₄

TABLE 3. Biochemical blood markers of chickens (♀ + ♂)

Specification	Group			
	C	Nano50	NanoCuSO ₄	SE
Glucose (mmol/l)	13.29	12.85	12.47	1.03
Cholesterol (mmol/l)	3.82	3.43	3.50	0.39
Triglycerides (mmol/l)	0.59	0.62	0.51	0.55
HDL (mmol/l)	2.39	1.95	2.11	0.44
Urea (mmol/l)	2.70	3.10	2.80	0.12
Calcium (mmol/l)	2.27	2.72	2.29	0.33
Magnesium (mmol/l)	0.80	0.85	0.98	0.14
Phosphorus (mmol/l)	1.93	2.27	1.87	0.23
Iron (mmol/l)	24.40	30.50	20.50	3.17
ALAT (mmol/l)	16.00	12.00	12.00	4.96
ASPAT (mmol/l)	368.50 ^B	498.50 ^B	911.00 ^A	100.78

A,B – statistically significant differences at $P \leq 0.01$.

group was almost threefold higher than in the control group. Such a result may be indicative of liver lesions, however histopathological examination of liver excluded the pathological effect of copper on this organ.

CONCLUSION

Results obtained in this study demonstrate that the erythropoietic system of chickens is susceptible to effects of metals, including copper (nanoparticles), which shows that changes in blood may be used as an indicator of the impact of toxic substances on chickens. Simultaneously, the analysis of changes in the peripheral blood enables more precise evaluation and interpretation of the effect a given factor has on bird body.

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Streszczenie: *Wpływ nanocząstek miedzi i siarczynu miedzi podawanych in ovo na wskaźniki hematologiczne i biochemiczne krwi kurcząt brojlerów.* Materiał doświadczalny w pierwszym etapie stanowiło 300 jaj lęgowych kurcząt Hubbard Flex. Jaja podzielono na trzy grupy: kontrola, Nano50 i NanoCuSO₄, z czego jaja z grupy Nano50 i NanoCuSO₄ poddane zostały zabiegowi iniekcji *in ovo*. Eksperymentalne roztwory podano poprzez wstrzyknięcie *in ovo*, przy użyciu sterylnej igły 0,3 mm kolejno do grup: Nano50 (koloid nanocząstek miedzi, stężenie 50 ppm), NanoCuSO₄ (koloid siarczynu miedzi, stężenie 50 ppm) do komory powietrznej jaja. Jaja inkubowano w standardowych warunkach. Po wykluciu z każdej grupy wybrano po 50 piskląt do odchowu trwającego 42 dni. Ptaki żywiono standardowymi mieszankami pełnoporcjowymi dla brojlerów. W ostatnim 42. dniu odchowu z każdej grupy wybrano po 12 samic i 12 samców, od których pobrano krew celem określenia wskaźników hematologicznych i biochemicznych. Wykonano oznaczenia hematologiczne, tj.: WBC, RBC, Hb,

heterofile, limfocyty, monocyty, eozynofile, bazofile. Do oznaczenia wskaźników biochemicznych pobrano krew na surowicę, w której oznaczono wskaźniki biochemiczne: glukoza, cholesterol, triglicerydy, HDL-cholesterol, mocznik, wapń, magnez, fosfor, żelazo, ASPAT, ALAT. Zastosowanie nanocząstek miedzi wpłynęło na wzrost poziomu RBC, HGB, HTC, heterofili, monocytów i bazofili. Dodatkowo w surowicy stwierdzono obniżenie stężenia glukozy i cholesterolu przy jednoczesnym wzroście poziomu wybranych mikroelementów: wapnia, fosforu i żelaza.

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Carbon synthesized by RF PACVD method enhances the activity of antioxidants

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Abstract: *Carbon synthesized by RF PACVD method enhances the activity of antioxidants.* The aim of the presented study was to evaluate the reductive activity of antioxidants in the presence of carbon manufactured by Radio Frequency Plasma Activated Chemical Vapor Deposition method (RF PACVD). 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was employed to estimate antioxidants reductive potential. Based on the experimental results, the antioxidant activity of carbon colloid was not confirmed. Nevertheless the reductive activity of antioxidants measured in time manner, in the presence of carbon, was significantly elevated comparing to the antioxidants alone. The presented results suggest that the surface of carbon synthesized by RF PACVD method suspended in water, creates more friendly reductive environment for antioxidants with hydroxyl groups (ascorbic acid – AA and butylated hydroxyanisole – BHA) then with sulfhydryl groups (glutathione – GSH).

Key words: carbon powder particles, RF PACVD method, antioxidant, ascorbic acid, BHA, glutathione, DPPH

INTRODUCTION

The external environmental factors like UV, optical radiation, temperature, organic and inorganic oxidants as well as internal cell oxidant factors are the potential reasons of the oxidation stress, which interfere the cell redox homeostasis. Such interferences may cause

the serious antioxidant deficiencies and they may initiate tumor processes or cell apoptosis. The limited amount of reducers in the organic matter leads to the unstable redox homeostasis. Growing oxidative strength of the environmental factors causes the rapid decrease of the antioxidant potential. The strength of the reducers in living organisms is limited by the outside factors and endogenous biosynthesis. That is why, the organism diet rich with the natural antioxidants (AA and GSH) is necessary for the regular organism functioning. The organic matter should be protected by synthesized antioxidants (BHA) to extend their time of stability. The environment may also be protected by slowing the oxidation reactions. In the living organism this may be achieved by the establishment of proper ratio between the lipid antioxidants and unsaturated acids. In the organic matter it may be achieved by proper storage conditions like the controlled temperature or the light protection. There is a large demand for the research on the factors which possess both the characteristics of the antioxidant and the ability of sustaining the proper environment for reduction reactions.

The recent study presents the additional biological benefits of carbon particles which are commonly considered as the potential carrier of the molecules. There are also a few studies evaluating the specific characteristics of RF PACVD carbon particles which could be used in biomedicine or pharmaceutical industry. Czerniak-Reczulska et al. (2010) noted the influence of RF PACVD carbon on the process of the proliferation of endothelial cells. Bakowicz-Mitura et al. (2007) observed that RF PACVD carbon may act as an antioxidant or anti-inflammatory factor. Catalytic properties of this type of carbon in organic environment, have not been precisely studied yet. Perhaps, as in the case of diamond obtained by detonation method used for proteolytic enzyme immobilization, the immobilization reducers may modulate redox reaction in simple *in vitro* systems. We suspect that carbon synthesized by RF PACVD method may actively transfer electrons in oxidoreductive reactions in the organic matter. Therefore we presume that RF PACVD carbon can protect antioxidant activity from environmental stress. The hypothesis was tested by determining the effect of RF PACVD carbon colloid on ascorbic acid, glutathione and tert-butyl-4-hydroxyanisole activity by measurements of reducing power of antioxidants on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the iron ions.

MATERIAL AND METHODS

Manufacturing parameters of RF PACVD carbon powder were described by Czerniak-Reczulska et al. (2010). The samples were divided into three groups.

Carbon particles was suspended in water. Investigated antioxidants (AOX): AA, GSH were dissolved in water. BHA was dissolved in ethanol. In the solution of $0.5 \text{ m}\cdot\text{mol}^{-1}$ AA, $0.7 \text{ m}\cdot\text{mol}^{-1}$ BHA and $100 \text{ m}\cdot\text{mol}^{-1}$ GSH, carbon particles (RF PACVD) were suspended in the concentration of $50 \text{ mg}\cdot\text{l}^{-1}$. The experiment was also performed with the incubation of all the solutions in 100°C for 10 min.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging activity of the mix sample was measured by the decrease in absorbance of methanolic DPPH solution at 517 nm (Krings and Berger 2001) in time manner (after 0.25; 0.5; 1; 2; 3; 4; 5 min). The antioxidant activity was expressed as: % disappearance = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\%$ (A – absorbance).

The results were subjected to statistical analysis by two-way ANOVA and Duncan's range test.

RESULTS AND DISCUSSION

According to Mitura (1987) and Bakowicz (2003), RF PACVD particles exhibits the reducing activity. In our study, the analytical methods did not identified reducing strength of the RF PACVD carbon. AA possesses two active hydroxyl groups that participate in the redox reactions (Niemiec et al. 2005). RF PACVD carbon catalyzes reaction with AA in the DPPH test. Carbon elevates AA activity after 15 s incubation and significantly increases the vitamin activity after 3 min incubation. High temperature decreased the activity of AA from 50 to 10%. How-

ever in the presence of carbon powder, the activity of ascorbic acid decreased in about 30% (Table 1).

Carbon particles did not affect the change in activity of glutathione in any of the experiments. BHA synthetic antioxidant strength reduction depends on the active -OH groups. Carbon significantly elevates antioxidant activity of BHA from 30 s to 2 min incubation. High temperature decreased activity of BHA as it was expected. However within first minute, reduction reaction was elevated comparing to the group without carbon particles (Table 2).

GSH is a multifunctional thiol group containing tripeptide that is a powerful antioxidant (Table 3), found in most aerobic organisms (Banhegyi et al. 1997).

The living organisms are exposed to the external environmental factors which is the source of oxidative stress. This is the one of the harmful principal issues in the healthcare, where excess of ROS (chemical species with unpaired electrons on the molecular orbitals) generated in various pathogenic processes are recognized as an indicator in cytotoxicity and the cellular disorder. At the intracellular level, ROS are balancing between the biochemical antioxidants such as ascorbic acid or glutathione. In the organic matter (food) redox homeostasis is remains unstable due to the limited of amount of reducers. Growing oxidative strength of the environmental factors leads to rapid decrease in the antioxidant potential. The strength of the reducers in living organisms is limited by delivered factors from the organism diet and possibility of endogenous biosynthesis. Simultaneously, the known strategies for the preservation of the organic matter (food products) are

TABLE 1. Reducing activity of AA on DPPH radical in time manner

TIME (min)	Reducing activity (%) of						ANOVA influence					
	RF PACVD CARBON		ascorbic acid (AA)		RF PACVD CARBON + AA		RF PACVD CARBON		temperature		inter-action	
	100°C	100°C	100°C	100°C	100°C	100°C	SEM	p	SEM	p	SEM	p
0.25	0.13 ^a	0.40 ^a	52.04 ^b	10.47 ^b	59.11 ^c	29.56 ^c	2.033	0.0191	0.798	0.0000	0.0000	0.0000
0.5	0.14 ^a	0.27 ^a	1.90 ^b	0.18 ^b	4.72 ^c	2.83 ^c	0.426	0.0004	0.195	0.0000	0.0000	0.0000
1	0.16 ^a	0.09 ^a	0.38 ^b	0.15 ^b	6.02 ^c	1.86 ^c	0.203	0.0000	0.329	0.0000	0.0000	0.0000
2	0.16 ^a	0.05 ^a	0.38 ^b	0.16 ^b	5.08 ^c	0.76 ^c	0.359	0.0033	0.316	0.0017	0.0079	0.0079
3	0.14 ^a	0.13 ^a	0.12 ^a	0.08 ^b	0.32 ^b	0.28 ^b	0.0232	0.0063	0.500	0.0011	0.0235	0.0235
4	0.13 ^a	0.12 ^a	0.11 ^a	0.10 ^b	0.17 ^b	0.34 ^b	0.0241	0.0021	0.060	0.1380	0.3045	0.3045
5	0.17	0.11 ^a	0.05	0.15 ^b	0.10	0.29 ^b	0.0229	0.6231	0.047	0.1217	0.3246	0.3246

a,b,c – RF PACVD CARBON + AA > RF PACVD CARBON and AA (p < 0.05).

TABLE 2. Reducing activity of BHA on DPPH radical in time manner

TIME (min)	Reducing activity (%) of						ANOVA influence				
	RF PACVD CARBON		BHA		RF PACVD CARBON + BHA		RF PACVD CARBON		temperature		inter- -action p
	–	100°C	–	100°C	–	100°C	SEM	p	SEM	p	
0.25	0.10 ^a	0.197 ^a	4.94 ^b	3.751 ^b	5.60 ^b	8.941 ^c	0.306	0.0000	0.306	0.0000	0.0000
0.5	0.00 ^a	0.097 ^a	5.26 ^b	5.092 ^b	6.74 ^c	6.843 ^c	0.178	0.0000	0.178	0.0000	0.0000
1	0.01 ^a	0.060 ^a	7.02 ^b	7.134 ^b	8.66 ^c	8.246 ^c	0.154	0.0000	0.154	0.0000	0.0000
2	0.16 ^a	0.222 ^a	12.12 ^b	9.509 ^b	13.82 ^c	9.523 ^b	0.189	0.0000	0.189	0.0000	0.0000
3	0.03 ^a	0.272 ^a	12.32 ^b	7.461 ^b	12.81 ^b	6.970 ^b	0.148	0.3110	0.148	0.3110	0.2235

a,b,c – RF PACVD CARBON + BHA > RF PACVD CARBON and BHA (p < 0.05).

TABLE 3. Reducing activity of GSH on DPPH radical in time manner

TIME (min)	Reducing activity (%) of			ANOVA	
	RF PACVD CARBON	glutathione (GSH)	RF PACVD CARBON + GSH	SEM	P
0.25	0.013 ^a	45.33 ^b	43.07 ^b	1.832	0.0211
0.5	0 ^a	12.87 ^b	13.65 ^b	1.945	0.0000
1	0 ^a	10.46 ^b	12.57 ^b	1.734	0.0000
2	0 ^a	10.80 ^b	13.21 ^c	0.956	0.0014
3	0 ^a	4.05 ^b	3.44 ^b	0.531	0.0088

a, b, c – means with different superscripts are significantly different (p < 0.05).

the supplemented synthetic antioxidants such BHA. The effectiveness of antioxidants depends on the accompanied carriers employed to reach the target. Number of studies presents the improvement of the antioxidant efficiency, in the way of binding them to the nano-carriers consisting of different carbon allotropes which exhibit reducing properties. According to Kato et al. (2009), water-soluble derivative of fullerene (C60) exhibit antioxidant activity. Flavonoids bind to hydroxyl nanotubes revealed significant increase in the antioxidant properties *in vitro* (Nichit and Stamatina 2013).

We observed the significant increase in the reducing force of the ascorbic acid and BHA in the RF PACVD colloidal environment. Probably the high reactivity of the obtained complexes is the result of molecular self-assembly into supramolecular structures. Self-assembly begins at the level of atoms, where it relies on the chemical complementarity. On the molecular level, it allows the materials to precipitate and form highly organized structures. We presume that non-covalent interactions (such as hydrogen bonding and ionic interactions, hydrophobic interactions) between carbon and antioxidants, enhance the thermal stability of the new formed complexes. This happens in the process of stabilizing the engaged electrons on the carboxylic acid groups by electronic properties of RF PACVD carbon structure, especially as the active interaction occurs between the experimental factors. Many allotropes of carbon (graphite, diamond, fullerenes, nanotubes or graphen) are presented as the interesting materials for electrochemical applications, like the energy storage. Moreover, the amphoteric

character of carbon allows for applying its electrochemical properties from the donor to the acceptor state (Frackowiak and Beguin 2001).

CONCLUSIONS

According to the present results, carbon colloid increases the activity of antioxidants, especially in the environment that affect their activity (high temperature). Moreover, the effect of RF PACVD carbon powder depends on the biochemical structure of the antioxidant. The presented results suggest that the surface of carbon particles suspended in water, creates friendly reductive environment for antioxidants with hydroxyl group (AA and BHA), but not GSH.

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Streszczenie: *Węgiel wytworzony metodą RF PACVD zwiększa aktywność antyoksydantów.* Celem doświadczenia było zbadanie aktywności redukującej wybranych antyoksydantów (kwasu askorbinowego, butylowanego hydroksyanizolu i glutationu) w obecności cząstek węgla wytworzonego metodą RF PACVD testem DPPH. Test ten polega na pomiarze kinetyki reakcji między

przeciwutleniaczem a wolnymi rodnikami. Im skuteczniejszy przeciwutleniacz, tym stała szybkości powstawania zredukowanej formy difenylolopikrylohydrazyny (DPPH-H) jest większa. Jakkolwiek nie stwierdzono aktywności redukującej hydrokoloиду węgla RF PACVD to jednak rozpuszczone w nim antyoksydanty istotnie zwiększyły swój potencjał redukujący w porównaniu do próbek zawierających czysty antyoksydant. Uzyskane wyniki sugerują, że cząstki węgla RF PACVD zawieszane w wodzie stwarzają korzystne środowisko dla aktywności związków redukujących z aktywną grupą hydroksylową (AA i BHA), ale nie dla związków z grupą sulfhydrylową (GSH).

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Polymorphism of insulin-like growth factor *IGF-1* in position 211 in national sheep breeds with carped wool compared to Polish Merino and European Mouflon (*Ovis aries musimon*)*

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Abstract: *Polymorphism of insulin-like growth factor IGF-1 in position 211 in national sheep breeds with carped wool compared to Polish Merino and European Mouflon (Ovis aries musimon).* Research was carried out on 1,684 sheep (1,093 ♀ and 591 ♂) originating from European Mouflon (*Ovis aries musimon*), its hybrids with Polish heat sheep and four sheep breeds that are characterized by a mixed wool compared to Polish Merino. All animals were subjected to gene identification factor insulin-IGF-1, in the assessment of allele C and T. In conclusion it should be noted that in the 7 examined breed groups sheep showed no polymorphism alleles and genotypes of insulin-like factor gene *IGF-1*, limiting its scope to determine the C allele and genotype CC. This result indicates the need for further research in this area in “culture” sheep imported and adapted to Polish conditions and the production environment.

Key words: sheep, *IGF-1*, distribution of alleles and genotypes

INTRODUCTION

Protein IGF-1 is one of the key components of the pathway of growth hormone (Franco et al. 2005). IGF-1 is produced in the liver and is responsible for cell growth and body treatments. It is believed that the effect of growth hormone occurs in the tissue in cooperation with local so-

matomedin e.g. *IGF-1* and stimulates the secretion of hypothalamic somatostatin inhibits secretion of growth hormone (Krzymowski et al. 1998). Insulin factor gene is exchanged among the conditions that allow the identification of races, as demonstrated in the Mediterranean countries (Pariset et al. 2006). This indicates the possibility of using the factors in the study of animal origin, what has signaled Heindleder et al. (2001). Accordingly, taking into consideration the fact that reported in the cited work conditions influence frequency of insuline-like factor *IGF-1*, decided to examine the distributions of the presence of domestic sheep breeds (PZO, 2013). Compared to the ancestor – European Mouflon (*Ovis aries musimon*) and Polish Merino, and the sheep breeds of mixed wool. In addition, conditioning it can assist in the work of research into the origins of the sheep (Heindleder et al. 2001), which also begins to raise more and more interest.

MATERIAL AND METHODS

The study Polish Merino sheep flock (two herds) of Wielkopolska voivodeship, sheep of mixed wool from

* Work done as part of the international project no 625/N-WĘGRY/2009/0.

Małopolska, Łódzkie i Podkarpackie (29 herds), and the European Mouflon (3 herds) i Muflonowrzosówek (2 herds) from Wielkopolskie and Lubuskie. Ewes and rams were aged 2 to 11 years (Table 1). Herds were randomly selected for sampling. From the jugular vein of animals blood was obtained into tubes containing EDTA, for the isolation of genomic DNA for the analysis of molecular genetics. The study was carried out assessment of genes and genotypes frequency of insulin-like gene factor *IGF-1*.

DNA was isolated from blood leucocytes using the conserved EDTA. In order to receive high quality DNA suitable after freezing and thawing of a reusable, blood is pretreated with the resulting

DNA modification by removal of heme compounds lysis of erythrocytes products. DNA is isolated from leukocytes by chromatography on mini-columns of silicate A&A Biotechnology. The fraction thus obtained was used as template DNA for amplification of polymorphic gene allele fragment. Genotyping of allele was performed with KASPar® system. This system (www.kbioscience.co.uk) method is to use a point SNP polymorphism using primers listed in Table 2.

Based on reading the DNA samples were genotyped within the ewes and rams shows distributions of alleles and genotypes frequency separately for each race. Allele and genotype frequencies were compared depending on the breed using SPSS v.21 with Chi² test was

TABLE 1. Summary of experimental material used in the study in 2009–2012

Breed	Sex		Herd sampling
	♀	♂	
European Mouflon	123	73	2010 – 2 ♂; 2011 – 3 ♀, 7 ♂; 2012 – 120 ♀, 64 ♂
Muflonowrzosówka	8	7	2010 – 4 ♀, 4 ♂; 2011 – 4 ♀, 3 ♂
Polish Heat Sheep	334	355	2009 – 114 ♀, 128 ♂; 2010 – 115 ♀, 113 ♂; 2011 – 105 ♀, 114 ♂
Swiniarka Sheep	109	33	2009 – 28 ♀, 16 ♂; 2010 – 81 ♀, 17 ♂
Polish Mountain Sheep – white	141	15	2010
Polish Mountain Sheep – coloured	168	13	2011
Polish Merino	210	95	2010 – 101 ♀, 40 ♂; 2011 – 94 ♀, 48 ♂; 2012 – 15 ♀, 7 ♂
Total within gender	1 093	591	×
Total	1 684		

TABLE 2. The primers and SNP genotyping of the locus of *IGF-1*

Locus	Name	Starters 3' to 5' (forward/reverse)	SNP	Localization
<i>IGF-1</i>	insuline-like factor	CACACACCTTGTTGCACTCC/ /GCTGAGTTGGTTGGATGCTCT	AY737509: 211 C > T ^a	Ekson 3

^aPariset et al. (2006).

assessed range of alleles and genotypes frequency between races, sexes, and the differences between the sexes in terms of individual alleles and genotypes. The results are presented in tables.

RESULTS AND DISCUSSION

Studies have shown that gene at position 211, factor *IGF-I* were found to have only the C allele. Analyses of 1,684 samples collected from the rated sheep did not show the presence of the T allele in any case. In comparison with the results of Pariset et al. (2006), mainly describing sheep found in the region of the Mediterranean, Black and Germany, sheep tested in Poland showed no polymorphism here. Due to the fact that many of the races comes from European Mouflon (Heindleder et al. 2001), it is expected that the distribution of the conditions occurring in sheep breeds reared in Poland (including European Mouflon) was characteristic of this part of the world and different from the observed trends. Perhaps it has to do with another course of life processes of growth and development, which wrote Krzymowski et al. (1998). In this situation, can be a useful series of studies on sheep imported into Polish, polymorphism alleles and genotypes of insulin-like factor gene subject to change due to processes of adaptation – which requires further work in this area.

Generally, it should be noted that the summing up of the 7 examined breed groups sheep showed no polymorphism alleles and genotypes of factor *IGF-I*, limiting its scope to determine is the C allele and genotype CC. This result indicates the need for further research in

this area in “culture” sheep imported and adapted to Polish conditions and the production environment.

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Streszczenie: Polimorfizm genu czynnika insulinopodobnego *IGF-I* w pozycji 211 u krajowych owiec o wlnie mieszanej w porównaniu do merynosa polskiego i muflona europejskiego (*Ovis aries musimon*). Badania przeprowadzono na materiale 1684 owiec (1093 ♀ i 591 ♂) pochodzących od muflona europejskiego (*Ovis aries musimon*), jego mieszańców z wrzosówką oraz czterech ras owiec charakteryzujących się okrywą wełnistą mieszaną porównywanych do merynosa polskiego. Wszystkie zwierzęta poddane były identyfikacji genu czynnika insulinopodobnego *IGF-I*, w zakresie oceny występowania alleli C i T. Podsumowując, należy stwierdzić, iż u badanych 7 grup rasowych owiec nie wykazano

polimorfizmu występowania alleli i genotypów genu czynnika insulinopodobnego *IGF-1*, ograniczając jego zakres do ustalenie jedynie do allelu C i genotypu CC. Wynik ten wskazuje na potrzeby przeprowadzenia dalszych badań z tego zakresu u owiec kulturalnych pochodzących z importu i adaptowanych w polskich warunkach środowiska produkcyjnego.

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Sensitivity of imago and larvae of the lesser mealworm *Alphitobius diaperinus* (Panzer 1797) in a sawdust litter to selected species and strains of Steinernematidae and Heterorhabditidae under laboratory conditions

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Abstract: *Sensitivity of imago and larvae of the lesser mealworm *Alphitobius diaperinus* (Panzer 1797) in a sawdust litter to selected species and strains of Steinernematidae and Heterorhabditidae under laboratory conditions.* Sensitivity of imagines and larvae of the lesser mealworm to selected species and strains of entomopathogenic nematodes was studied in a pine sawdust litter on which chicken were kept from one to six weeks according to their production cycle (Niemiec 1998). The following nematode species and strains were used: *S. feltiae* from bioinsecticides Ovinema and Nemaplus, *S. affinis*, *S. carpocapsae* and *H. bacteriophora* strain Brecon. *S. feltiae* from biopreparation Ovinema appeared most invasive to the larvae and imagines of *A. diaperinus*.

Key words: lesser mealworm, pine sawdust, entomopathogenic nematodes, biological control

INTRODUCTION

The lesser mealworm can be found worldwide in Europe, Asia North and South America, Australia and Africa. The insect inhabits dark, wet and warm places (Arshad and Khattack 1984, Awaknavar and Rajasekhar 1999) feeding on groats, flour, cereal grains (Lorenzo 1990) or milk powder (Basak et al. 1991). First information on the presence of the lesser mealworm in broiler houses appeared in the 1950s in the USA (Gould and Moses 1951, Harding and Biasell 1958).

The lesser mealworm is dangerous as a potential carrier of disease-causing organisms like: bacteria *Escherichia* sp. (McAllister et al. 1996), *Salmonella typhimurium*, *Bacillus*, *Streptococcus* (McAllister et al. 1994), viruses causing Marek's, Gumboro, Newcastle diseases and bird flu, parasites of the *Eimeria* sp., the larvae of tapeworms *Railietina* sp. and *Choanotaenia* sp. (Eidson et al. 1965, Wilson et al. 1986, Avancini and Ueta 1990, Goodwin and Waltman 1996, Steelman 1996). The pathogens are transmitted when birds eat infected insects. A broiler may eat 450 larvae a day. Moreover, the insects bring substantial economic losses. They destroy polyurethane isolation of farm houses during their migration and pupation (Despins 1991, Steelman 1996).

The control of the lesser mealworm with insecticides does not bring expected results, moreover, the chemicals have a long waiting periods and insects quickly become immune to insecticides. Therefore, entomopathogenic nematodes (EPNs) seem to be an alternative as bioinsecticides in the population control of this pest (Geden et al. 1985, 1987a and b, Geden and Axtell 1988, Szczepanik 2000, Szalanski et al. 2004).

MATERIAL AND METHODS

Steinernema feltiae from biopreparations Ovinema and Nemaplus, *S. affinis*, *S. carpocapsae*, and *H. bacteriophora* (Brecon) were selected for experiments on the sensitivity of growth stages of the lesser mealworm in straw and litter. These nematodes were grown in the laboratory of the Department of Zoology, Warsaw University of Life Sciences – SGGW with the following method. Ten caterpillars of *Galleria mellonella* were placed in a Petri dish of a diameter of 9 cm lined with filter paper and then 1 ml of a suspension of 500 larvae of appropriate nematode species was poured over the dish. Caterpillar mortality was controlled every day. Dead insects were transferred onto small sponges immersed in water. After 7 to 14 days invasive larvae of nematodes started to transfer into water. After two-week keeping in a fridge the invasive larvae of nematodes were ready for experiment.

Experiments with the larvae and beetles of the lesser mealworm were performed in plastic boxes 9.5 cm long, 7.5 cm wide and 6.0 cm high. The boxes were filled with pine sawdust from a broiler house to a height of 3.0 cm. Ten adult insects or 10 larvae of the lesser mealworm were placed in the box together with pine sawdust from substratum on which chicken were bred. Broiler production lasted 6 weeks.

Litter used in experiments was taken one, two, three and four weeks after the broiler house was stocked with chicken. Since no effect of nematode activity was found in three- and four-week litter, the experiments with older litter were neglected. The dose of nematodes used in

the experiment was $1 \cdot 10^6$ per m^2 in the variant with larvae and $1 \cdot 10^6$ per m^2 in that with beetles. Nematodes were introduced in 1 ml of water supplemented to 5 ml with distilled water. Experiments were carried out at 28°C in the SANYO chamber. Moisture was controlled every day. After 7 and 14 days insect mortality was counted and checked whether nematodes were the reason. Control variant consisted of insects in clean, moist pine sawdust sprayed with 5 ml of distilled water.

Tukey-Freeman test was used for statistical processing of results.

RESULTS AND DISCUSSION

As seen in Table 1 the highest efficiency in all types of substratum was found in *S. feltiae* from Ovinema biopreparation. The extensity of infection of *A. diaperinus* larvae was 60% in clean pine sawdust and decreased to 42% in one-week pine sawdust and to 34% in two-week sawdust. The older was the litter the more hen faeces and ammonia it contained. Therefore, nematodes lost their invasiveness to the lesser mealworm larvae in such a habitat. In three-week litter the effect of nematodes was the same as in the control (Table 1). Entomopathogenic nematodes are the animals very resistant to various unfavourable external conditions. For example, most pesticides are not harmful to invasive larvae of these nematodes (Kaya 1990, Kami-onek 1992) even at doses larger than the recommended. Some pesticides may even stimulate nematodes' movements (Vainio 1993). Only fungicides and herbicides, including ureal ones, decrease

TABLE 1. Comparison of the extensity of infection (%) of the lesser mealworm larvae after their contact with selected species/strains of Steinernematidae and Heterorhabditidae nematodes in substratum made of pine sawdust

Nematode species	Percentage of dead larvae of <i>A. diaperinus</i> in which nematodes were noted			
	Sawdust without faeces	One-week sawdust	Two-week sawdust	Three-week sawdust
<i>S. feltiae</i> (Ovinema)	60 d	42 c	34 bc	2 a
<i>S. feltiae</i> (Nemaplus)	46 c	36 c	20 b	2 a
<i>S. affinis</i>	32 bc	30 bc	20 b	4 a
<i>H. bacteriophora</i> (Brecon)	20 b	20 b	8 a	4 a
<i>S. carpocapsae</i>	32 bc	28 bc	16 a	2 a
Control	4 a	4 a	2 a	2 a

Different letters denote significant differences in the extensity at $p < 0.05$.

the pathogenicity of nematodes (Das and Divakar 1987, Kamionek 1992). The effect of organic fertilisation was studied by Bednarek and Gaugler (1997) who demonstrated the increased effectiveness of entomopathogenic nematodes after the contact with that fertiliser. Poultry faeces are also organic fertilisers but apart from N, P and K it contains large amounts of ammonia which is slowly released to the environment (Oudedag and Luesink 1998). This is probably the reason why most nematode species lose their pathogenic properties.

Mortality of adult insects of the lesser mealworm in various substrata is presented in Table 2. Most efficient were *S. feltiae* from Ovinema biopreparation. These nematodes caused 20% mortality in adult insects after two weeks of experiment, 24% mortality in one-week litter and 4% mortality in three-week litter. *S. affinis* caused 26% mortality in adult insects kept in clean sawdust and 20% mortality in one-week litter. Comparison of the Tables 1 and 2 shows that *S. feltiae* from Ovinema biopreparation were most

efficient against both growth stages of the insect. Geden et al. (1985) obtained 35.8% infection in larvae and 10.2% infection in adult forms of *A. diaperinus* with nematodes *S. feltiae* in a substratum composed of pine sawdust, food remains and faeces. Szalanski et al. (2004) obtained similar results using a pine-cedar substratum and nematode strains *S. carpocapsae* Mexican and *S. feltiae* Pye at a dose corresponding to 200 invasive larvae per insect. Pezowicz (2006) studied the invasiveness of entomopathogenic nematodes in straw and in litter from a broiler house and obtained lower extensity than in this study.

Not until recently attempts have been undertaken to improve entomopathogenic nematodes' ability of finding hosts, to increase their invasiveness and in general to increase their effectiveness in insect control. Gaugler (1987) was a promoter of studies on artificial selection, genetic engineering and strain hybridization. Nematodes from Ovinema biopreparation are an outcome of these studies (Tomalak 1994 and 1998).

TABLE 2. Comparison of the extensity of infection (%) of the lesser mealworm imagines after their contact with selected species/strains of Steinernematidae and Heterorhabditidae nematodes in substratum made of pine sawdust

Nematode species	Percentage of dead imagines of <i>A. diaperinus</i> in which nematodes were noted			
	Clean sawdust	One-week sawdust	Two-week sawdust	Three-week sawdust
<i>S. affinis</i>	26 c	20 bc	18 b	6 a
<i>S. feltiae</i> (Ovinema)	36 d	24 c	20 bc	4 a
<i>S. feltiae</i> (Nemaplus)	24 b	18 a	14 a	4 a
<i>H. bacteriophora</i> (Brecon)	28 bc	20 b	16 a	2 a
Control	4 a	2 a	2 a	4 a

Different letters denote significant differences in the extensity at $p < 0.05$.

CONCLUSIONS

1. The invasiveness of entomopathogenic nematodes decreases with increasing concentration of ammonia from chicken faeces.
2. *Steinernema feltiae* from the bio-preparation Ovinema appeared most effective in controlling larva and imagines of the lesser mealworm.
3. Pine sawdust is a better substratum than litter to control the lesser mealworm in broiler houses.

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- i szczepy nicieni Steinernematidae i Heterorhabditidae w warunkach laboratoryjnych.* Celem badań było określenie wpływu nicieni entomopatogenicznych na śmiertelność larw i postaci imaginalnych pleśniakowca lśniącego *Alphitobius diaperinus* (Panzer 1797) w ściółce z trocin sosnowych. Zbadano przeżywalność stadiów rozwojowych owadów w cyklu produkcyjnym hodowli kurcząt. Stwierdzono, że ze wzrostem stężenia amoniaku pochodzącym z odchodów kurcząt maleje inwazyjność nicieni entomopatogenicznych. Nicienie żyły tylko do trzeciego tygodnia cyklu produkcyjnego kurcząt. Najbardziej skutecznym gatunkiem w stosunku do larw i imago pleśniakowca okazał się *S. feltiae* z biopreparatu Ovinema. Trociny sosnowe są lepszym podłożem niż ściółka słomiana do zwalczania pleśniakowca lśniącego w brojlerniach.

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Streszczenie: Wrażliwość imago i larw pleśniakowca lśniącego *Alphitobius diaperinus* (Panzer 1797) w ściółce z trocin na wybrane gatunki

Comparison of calving course of Limousine purebreds and their crossbreeds with Polish Holstein-Friesian cows

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Abstract: *Comparison of calving course of Limousine purebreds and their crossbreeds with Polish Holstein-Friesian cows.* Calving course of 100 cows of Limousine breed covered by bulls of the same breed as well as 100 cows of Polish Holstein-Friesian breed inseminated with Limousine bull's semen was investigated. In purebred population the material was taken from the beef cattle recording scheme provided by the Polish Association of Beef Cattle Breeders and Producers. In case of commercial crossing the material was taken from the questionnaires collected by the technicians representing Mazovian Centre of Animal Breeding and Reproduction in Łowicz. The data base covered calving course degree and calf body weight at birth degree. It was proved, that the significantly higher ratio of deliveries when human help was needed was stated in the purebred Limousine population (14%), in comparison to crossbreeding where the above mentioned ratio was only 7%. The most difficult calvings were observed for the calves of the highest birth weight. The high ratio of assisted calvings in commercial crossing as well as in purebred population of Limousines suggest that all of the deliveries should be monitored by the breeders. It should be also stated that the ratio of difficult calvings in the commercial crossing is significantly lower than that observed in purebred PHF population. Because of so low ratio of assisted calvings in commercial crossing with Limousine bulls it should be recommended to use the semen of bulls which pedigree show high body weight at birth and muscularity.

Key words: Limousine, calving course, commercial crossing

INTRODUCTION

Beef cows are not milked, hence the economic results of this branch of beef production depend mainly on the number of healthy and vital calves born from cows without any negative influence on their health and future reproduction.

Among many factors influencing the proper calves development, beside of husbandry conditions, the calving course is mentioned by many authors (Nogalski 2004, Grodzki et al. 2009, Przysucha 2009).

Calving course depends on many factors like: breed, body weight and condition of cow, calving number, body weight and sex of calf at delivery. Calving difficulty, growth rate and calf muscularity are the main traits used to formulate the evaluation criteria of breeding indexes for beef cattle in many countries.

According to many authors (Philippson 1976, Philippson 1977, Burfening et al. 1978, Meijering 1984, Berger 1994, Nogalski and Klupeczyński 1999, Grodzki et al. 2010), calving course is influenced by many genetic and phenotypic factors. On the base of the wide literature review Nogalski (2004) and Przysucha (2009) agreed, that the main factors affecting calving course were: cow age (calving number), calf body

weight at birth, calf sex, cow caliber and its pelvis area, parents breed, pregnancy length, parents genotypes, cow condition and feeding during pregnancy, calf shape and its position at delivery. All the above mentioned factors are strictly connected one to the other.

Following the rules of the most breeding programs for beef breeds it is easy to recognize, that calving course, calf body weight at delivery, calf body shape, daily growth rate, feed conversion, cutting rate and carcass quality are the main items to the breeding value formula (Nogalski and Klupczyński 1999, Przysucha et al. 2005, Przysucha et al. 2007).

In the young Polish beef cattle production the purebred female population consists of fourteen beef breeds, but Limousine cows and heifers number amounts to about 70% and in commercial crossing to 89%. It means, that the Polish breeders have already made a decision about the most useful beef breed. Limousine breed is predisposed for the intensive fattening with the very high daily gain at the moderate feedstuffs use. Animals kept for slaughtering can be fattened to the high body weight without any risk of over fattening. The proper use of beef bulls (without regard of their breed) is crucial for the commercial crossing results. This type of crossbreeding is often identified with calving difficulties. Papers provided by many authors clearly proved, that the calving difficulties within specialized beef breeds are the same or even lower (in case of commercial crossing) than those in case of purebred dairy herd (Hanset 1981, Nix et al. 1998, Nogalski 2002, Przysucha and Grodzki, 2008, Przysucha et al. 2009).

The aim of the research was to determine the frequency of difficult calvings in Limousine cows and dairy cows inseminated by Limousine bulls semen in the commercial crossing.

MATERIAL AND METHODS

The calving course of 100 purebred Limousine (2–6 years old) cows inseminated by the same breed bulls as well as 100 Polish Holstein-Friesian (PHF) cows serviced by Limousine bulls semen (commercial crossbreeds) was monitored. Results of beef cattle recording scheme conducted by Polish Association of Beef Cattle Breeders and Producers constituted the material for investigation.

In case of commercial crossing (dairy cow \times Limousine bull) the calving course was examined by technicians from Mazovian Centre of Animal Breeding and Reproduction in Łowicz.

The data base covered calving course degree and calf body weight at birth degree. Calving course was evaluated as: normal, without any assistance (1), help of the breeder needed (2).

Depending on body weight at birth (kg) calves were divided into the following groups: up to 35 kg, 36–45 kg, >45 kg.

Statistical analysis of the calving difficulties frequencies was carried out by Chi-square test using SPSS 12.0 (Statistical Product and Service Solution 1998).

RESULTS AND DISCUSSION

The ratio of calving course examination for purebred and crossbred populations was presented in Table 1.

TABLE 1. The ratio of calving course examination for purebred and crossbred populations

Factor		Unit	Calving course		
			1	2	Total
Population	purebred	N	86	14	100
		%	86	14	100
	commercial crossing	N	93	7	100
		%	93	7	100

Obtained results show significantly higher ratio of difficult calvings in the purebred Limousine population in comparison to commercial crossing.

The calf body weight at birth had significant influence on calving course ratio. The highest ratio of difficult calvings were found, when calf body weight was the highest (Table 2). Obtained results are the same as those presented by practically all the authors dealing with the problem.

with Limousine bulls is much lower than those shown by the authors dealing with the purebred PHF (Fouz et al. 2013).

Because of the relatively small percentage of difficult deliveries in commercial crossing with Limousine bulls, this breed should be recommended to use for that purpose. The highest calving difficulty, compared with pure Holsteins was for crosses with Belgian Blue followed by Limousine and Galician Blonde (Fouz et al. 2013).

TABLE 2. The influence of calf body weight at birth on calving course

Population	Calf body weight at birth (kg)	Calving course (%)	
		1	2
Purebred	<35	100	0
	35–45	89	11
	>45	79	21
Commercial crossing	<35	100	0
	35–45	91	9
	>45	73	27

Relatively high percentage of difficult calvings (with assistance needed) in purebred Limousines and crossbreds from commercial crossing of PHF cows with Limousine bulls suggests, that all of the deliveries should be monitored by the breeder.

It should be stated, that the ratio of difficult calvings in commercial crossing

Irrespectively to calves genotype (purebreds or crossbreds) the frequencies of difficult calvings increase with the birth body weight of the calves (Table 2). Higher rate of dystocia were observed in Limousine crossbreds (38%) compared to purebreds (32%).

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Statistical product and service solution case version 8.0 for Windows, User's Guide, 1998, by SPSS Inc., USA.

Streszczenie: *Porównanie przebiegu porodu u krów rasy Limousine i jej mieszańców z bydłem rasy polskiej holsztyńsko-fryzyjskiej.* W pracy oceniono przebieg porodów 100 krów rasy Limousine krytych buhajami tej samej rasy oraz 100 krów rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej (PHF) krytych w krzyżowaniu towarowym z buhajami mięsnej rasy Limousine. W hodowli czystorasowej materiałem do badań były wyniki oceny użytkowości bydła mięsnego prowadzonej przez Polski Związek Hodowców i Producentów Bydła Mięsnego. W przypadku krzyżowania towarowego materiałem do badań były dane zawarte w „Kartach przebiegu ocielenia krowy” prowadzonych przez specjalistów Mazowieckiego Centrum Hodowli i Rozrodu Zwierząt Sp. z o.o. w Łowiczu. Dane dotyczące 100 porodów krów rasy Limousine inseminowanych nasieniem buhajów tej samej rasy oraz 100 porodów krów PHF inseminowanych nasieniem buhajów mięsnej rasy Limousine obejmowały ocenę przebiegu porodu oraz masę cielęcia przy urodzeniu. Uzyskane wyniki jednoznacznie pokazują, że znacznie większy i statystycznie istotny udział porodów wymagających pomocy człowie-

ka stwierdzono w populacji czystorasowej bydła Limousine (14%), w porównaniu z krzyżowaniem towarowym krów mlecznych z buhajami tej rasy, gdzie odsetek trudnych ocieień wyniósł zaledwie 7%. Analiza statystyczna wykazała istotny wpływ masy cielęcia na rozkład ocen przebiegu porodu w obu badanych populacjach. Najwięcej trudnych porodów zanotowano w grupie cieląt o największej masie przy urodzeniu. Stosunkowo duży odsetek porodów wymagających udziału człowieka zarówno w krzyżowaniu towarowym, jak i w populacji czystorasowej Limousine, sugeruje potrzebę monitorowania przez hodowcę wszystkich porodów. Należy podkreślić, że odsetek przypadków trudnych ocieień w krzyżowaniu towarowym z rasą Limousine jest znacznie mniejszy z ich częstością, jaką notuje się u bydła PHF utrzymywanego w czystości rasy.

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Number of piglets born and reared by sows with different number of mammary teats

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Abstract: *Number of piglets born and reared by sows with different number of mammary teats.* The purpose of the studies was to determine the number of piglets born (NPB) and reared until 21 day (NPR) by the sows of Polish Landrace and Polish Large White breeds, differing in the number of teats. For calculations, the results from breeding pig houses of the Mazovian region, collected during the years 2004–2009, were employed. Three groups of females were distinguished for the particular breeds: those ones, possessing 14, 15 or 16 teats. The number of records for Polish Large White was equal to 1,019 and for Polish Landrace, it was 1,732. The statistically confirmed differences were revealed in respect of NPB by the groups of females, possessing 14, 15 or 16 teats ($P \leq 0.01$) and NPR until the age of 3 weeks by the sows of the same breed, possessing 14 or 15 teats ($P \leq 0.05$). The differences in NPB and NPR in the sows which possessed 14, 15 or 16 teats were not statistically confirmed ($P \leq 0.05$). Any univocal relationships between the deaths of the piglets until 21 day of life and the number of teats in the sows of the examined breeds were not found; the level of losses varied from 10.60 to 12.65%. The obtained results indicate that the number of teats in the sows may affect the results of rearing the piglet until 21 day. The access to mother milk is not, however, the only one indicators of success in rearing of the progeny.

Key words: sows, number of teats, born piglets, reared piglets

INTRODUCTION

The number of teats in Suidae reveals considerable species and breed differentiation (Haley and Lee 1992, Komosińska and Podsiadło 2002 as cited by Paruch 2011). Heritability index (h^2) of the mentioned trait was determined for pigs of different breeds, *inter alia* for Polish Landrace and Polish Large White and their crossbreds as well as for Landrace, Yorkshire and Hampshire swine. The differences in its value depending on the breed (purebreds/hybrids) and the site of teats (front/rear) were found (McKay and Rahnefeld 1990, Haley and Lee 1992, as cited by Paruch 2011). The calculated values of h^2 for the number of teats amounted to 0.226–0.45 (McKay and Rahnefeld 1990, Lewczuk et al. 1991, Haley and Lee 1992, cited by Paruch 2011) and less than 0.1 (Lechowska and Ruda 2000). The studies confirmed also the incidence of specific relationship between the position in the group hierarchy, body weight of the piglets and the mother teats, suckled by her progeny (Surdacki and Józwiakowska-Rekiel 1988, Valros et al. 2002). Additionally, Lee and Wang (2001) found that the number of teats, their conformation and distribution were not only the traits

subject to control and selection but also revealing effect on development of the piglets.

The aim of the study was to determine the number of the piglets born (NPB) and reared until 21 day (NPR) by Polish Landrace and Polish Large White sows with different number of teats.

MATERIAL AND METHODS

The analysis employed the results, obtained in breeding pig houses of the Mazovian region, as collected in the years 2004–2009. The number of the records for Polish Large White breed was equal to 1,019 and for Polish Landrace – 1,732. The number of the piglets born and reared until 21st day was calculated separately for the sows of each breed; three groups of the females were distinguished i.e. those ones having 14, 15 or 16 teats. In the calculations (one-factor variance analysis), the package SPSS (2006) was applied.

RESULTS AND DISCUSSION

Statistically confirmed differences were revealed for NPB in the Polish Landrace sows possessing 14, 15 or 16 teats ($P \leq 0.01$) and for NPR up to the age of 3 weeks in the sows of the same breed possessing 14 or 15 teats ($P \leq 0.05$) – Table 1. The differences in NPB and NPR in Polish Large White sows who possessed 14, 15 or 16 teats have not been statistically confirmed ($P > 0.05$). Any univocal relationships between the deaths of the piglets until the 21st day of life and the number of teats in the sows of the

TABLE 1. Number of piglets born and reared by the PLW and PL sows with a differentiated number of teats (14–16)

Number of teats	Polish Large White				Polish Landrace					
	N	Number of piglets born		Number of piglets reared until 21 day		N	Number of piglets born		Number of piglets reared until 21 day	
		X	Se	X	Se		X	Se	X	Se
14	621	11.48	0.054	10.46	0.047	931	11.26 A	0.046	10.32 a	0.042
15	283	11.53	0.079	10.54	0.070	559	11.46 A	0.059	10.47 a	0.054
16	115	11.66	0.124	10.67	0.110	242	11.59 A	0.090	10.50	0.082
14–16	1019	11.56	0.052	10.56	0.046	1732	11.44	0.039	10.43	0.036

N – number of observations; X – mean number of piglets in the litter; SE – standard error, a, A – significance at $p \leq 0.05$; A, A – significance at $p \leq 0.01$ (in columns).

examined breeds were not found; the level of the losses varied from 10.60 to 12.65% (Table 2).

the number of teats of the sows, their correct conformation and distribution has a significant effect on development of the

TABLE 2. Losses of piglets in the litters until 21 day of rearing according to the number of teats of their mothers

Number of teats	Polish Large White		Polish Landrace	
	Losses in the litter			
	heads	%	heads	%
14	1.013	11.63	0.941	10.60
15	0.987	11.38	0.988	11.32
16	0.995	11.61	1.092	12.65

Mazaraki (1961) and Janiszewska et al. (1991), as cited by Paruch (2011), have not recorded any statistically significant effect of the number of teats in the sows on NPB and NPR. The results of own studies are confirmed by the results, obtained by Janiszewska et al. (1991) and Buczyński et al. (1996), as cited by Paruch (2011). They observed that when the sows possessed 15 teats and more, NPB and NPR revealed a growing tendency. They stated also that in case of the increased number of teats in the sows (≥ 15), the level of piglets' death during rearing by mothers was lower and the weight of the litter was higher; the differences were not, however, statistically significant. Jungst and Kuhlers (1983), as cited by Paruch (2011), studied the effect of all teats of Duroc and Landrace sows (situated in front of navel, anal teats and crater teats) on the number of piglets on 21st day of rearing and did not find any relations. Also, they did not find any effect of the sow's teats, having a correct conformation and of defective (crater) teats on weight of the litter on 21st and 42nd day of life. On the other hand, Lee and Wang (2001) stated that

piglets. They are important performance traits, being subject to control and selection, therefore the attempt to develop the effective models of selection, oriented towards the number of teats in breeding sows have been undertaken.

Buds of mammary teats are generated as early as during fetal life period. Morphogenesis of the mentioned glands may reveal a high interspecies as well as in-species, in-breed or in-line variability (Zaks 1969, as cited by Paruch 2011). In opinion of Hotchkiss et al. (2007), the increased expression of androgens in fetal period may bring about to disturbances in correct development of female, including inhibition of teats and reproduction system development. The changes in the level of the mentioned hormones affect the phenotypic modifications in adult females (masculinization and defeminization). Slob et al. (1980) and Warren et al. (1973), as cited by Paruch (2011), state a lack of evidence indicating the origin of androgens from developing ovaries in female fetuses. Most probably, placenta (Baum et al. 1991 and Houtsmuller et al. 1995, as cited by Paruch 2011) and adrenal glands (Stahl et al. 1991, as cited

by Paruch 2011) are the source of these hormones in female fetuses. Androgens may be also of maternal origin (vom Saal 1999, as cited by Paruch 2011) or come from male fetuses which are found in a defined intrauterine position (Ryan and Vandenberg 2002, as cited by Paruch 2011). The mentioned phenomenon supplements and explains one-direction flow of blood via uterus or fetal membranes. It is considered that effect of intrauterine position of on reproduction abilities in pigs is small. Proportion of gender of fetuses in uterus has a greater effect on the number of teats than their mutual distribution has (Clark et al. 1993 and Saala et al. 1999, as cited by Paruch 2011). Drickammer et al. (1999) and Ryan and Vandenberg (2002) report that the greater participation of males in the litter causes decrease of the number of teats in females (masculinization of females) as compared to females, originating from the litter with greater participation of females (feminization of females). The studies of Orzechowska et al. (2002), Górecki (2003) and Rekiel et al. (2012) confirm the relationship between the number of males in the litter from which the sow derived, and her reproduction results. Górecki (2003) indicates also to the increased number of teats in the females coming from the litters in which there was a domination of female individuals vs. males. In own studies, such relationships were not analyzed and only the effect of the number of teats in sows on NPB and NPR, what was confirmed in some cases.

The number of teats is hereditary similarly as quantitative traits; it reveals a high variability and is probably the ef-

fect of cooperation of many genes simultaneously, therefore, it is very difficult to choose the best selection method and the progress in respect of the trait is revealed at a very long time and to a minimal degree. High possibilities in this respect are found in BLUP model, e.g. in French model, the teats are considered (EPSPA 2007). It seems to be justified because – as it was shown in own studies (unpublished data) – their too small number in sows (13 and less) contributes to more than 6% increase of index of piglets' deaths during rearing period. The results of the studies of Kim et al. (2005) also indicate the justness of leaving the gilts with 14 teats and more for repairing of the herd. Their greater number causes the increase of the number of piglets born in the litter and of the piglets reared until 21st day. There is a possibility of regression of the discussed medium-heritable trait in the progeny in relation to the mean value of the trait in the population, therefore the control of mating and improvement of the trait are important in breeding work. In own studies, the sows, being evaluated in respect of NPB and NPR possessed the required number of teats but also, their number higher than minimum was connected with the improvement of the studied reproduction and rearing parameters what has been confirmed in literature (Lee and Wang 2001, Kim et al. 2005). The increase of breeding progress in respect of the traits connected with reproduction is possible owing to intensively developing molecular genetics. The traits differ in heritability; their expression may occur at later age or only in one gender. Control of the discussed traits is possible via study of

DNA polymorphism and mapping of genome, connected with the incidence of QTL – *loci* of economically significant quantitative traits, including the number of active teats (Bidanel and Rothschild 2002, Dekkers 2004, Rodriguez et al. 2005, Sato et al. 2006). Mc Kay and Rahnefeld (1990) determined the heritability of front and rear teats in purebred pigs of maternal and paternal breeds and their crossbreds and demonstrated that the heritability coefficient for front vs. rear teats was lower in purebred pigs (0.15–0.21 vs. 0.20–0.39) and crossbreds (0.03–0.18 vs. 0.08–0.29). The discussed traits, as affected by long-lasting selection, reveal a low genetic variability what causes that the heritability coefficient has low values; such values for the number of teats were also given by Lechowska and Ruda (2000).

CONCLUSIONS

The results of own studies indicate that the number of teats in sows may affect the results of rearing the piglets until 21st day of life. The access to and the utilization of mother's milk is not, however, the only one indicator of success in rearing of the progeny.

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- Streszczenie:** Liczba prosiąt urodzonych i odchowanych przez lochy o różnej liczbie gruczołów sutkowych. Celem badań było określenie liczby prosiąt urodzonych (LPU) i odchowanych do 21. dnia (LPO) przez lochy rasy PBZ i WBP, różniące się liczbą gruczołów sutkowych. Do obliczeń posłużyły wyniki z chlewni zarodowych rejonu mazowieckiego zgromadzone w latach 2004–2009. Wyróżniono dla ras po trzy grupy samic, tj. mających 14, 15 lub 16 sutków. Liczba rekordów dla rasy WBP wyniosła 1019, a dla rasy PBZ 1732. Różnice potwierdzone statystycznie wykazano w LPU przez grupy loch PBZ mające 14, 15 lub 16 sutków ($P \leq 0,01$) i w LPO do wieku 3 tygodni przez lochy tej rasy mające 14 lub 15 sutków ($P \leq 0,05$). Różnice w LPU i LPO przez lochy WBP, które miały 14, 15 lub 16 sutków nie zostały potwierdzone statystycznie ($P > 0,05$). Nie stwierdzono jednoznacznych zależności między upadkami prosiąt do 21. dnia życia a liczbą sutków u macior badanych ras; poziom strat wahał się od 10,60 do 12,65%. Uzyskane wyniki wskazują, że liczba sutków u loch może wpływać na wyniki odchovu prosiąt do 21. dnia. Dostęp do pokarmu matki nie jest jednak jedynym wyznacznikiem powodzenia w odchowie potomstwa.

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Assesment of slaughter value of three broiler chicken genotypes*

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Abstract: *Assesment of slaughter value of three broiler chicken genotypes.* The study was conducted to examine slaughter parameters of three genotypes of meat type chicken: F₁ crossbred derived from crossing of light hen of indigenous breed Greenleg Partridge with heavy meat type cocks, F₂ crossbred being an effect of re-crossing obtained crossbred C×GP with meat type males and medium growing Hubbard JA 957, designed for a longer, 9-week production. Chicken were reared till 63rd day of age. Examined parameters were: dressing percentage, breast and leg meat yield and fatness. F₁ crossbred (C×GP) were characterized by rather low body weight, typical for slow growing chicken and good musculature, especially breast. Re-crossing with meat type cocks affected significant ($P < 0.01$) improvement of the slaughter parameters. F₂ crossbred (C×(C×GP)) reached high body weight, typical for medium growing chicken. In comparison with Hubbard JA 957 F₂ crossbred had lower body weight, the same dressing percentage, better breast and worse leg musculature, and less abdominal fat. High breast meat percentage in carcass and less fatness suggested, that these chickens can be used in meat production, as a medium growing material designed for a longer fattening period (9 weeks).

Key words: meat chicken, genotype, carcass traits

INTRODUCTION

The European market for live poultry production is dominated by the broiler production using fast growing genetic material. But actually the demand for alternative product increases. Consumer are more interesting in chicken meat from longer fattening system, characterized by better physical and chemical properties and better taste, as compared to the classic broiler. These birds are often kept in outdoor system with the access to pasture, what increases their attractiveness to the consumers. For the longer, 9-week, fattening alternative genetic material is used, characterized by slower growing rate and better adaptation to different environmental conditions and feeding them diets, which improve meat quality and taste and health-promoting qualities (Fanatico et al. 2005, Chen et al. 2013).

On the other hand increased interest in using an indigenous breeds or their hybrids, which are often better adapted to local climatic and/or consumer requirements is observed (Jaturasitha et al. 2008, Zanetti et al. 2010, Zhao et al. 2011, Dal Bosco et al. 2012, Wang et al. 2013). Also in Poland scientists are interested in possibility of using local heritage breeds in organic or free range meat production (Brodacki et al. 2011, Brodacki i Batkowska 2011, Gornowicz

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2011, Muchacka et al. 2011). In Poland actually the popularity of Greenleg Partridge breed grows. It is an indigenous breed, designed for outdoor laying production, because of its low weight and lightweight construction. However the some of the Polish farms successfully use males of Greenleg Partridge to produce capons (<http://kaplony.pl>; <http://www.kaplony.eu>). It suggests the possibility to use this breed as a maternal material to produce hybrids suitable for the meat production. These crossbreds obtained by single or double crossing with other chicken breeds or lines can be a good material for the longer fattening.

The purpose of this study was to examine slaughter analysis parameters of crossbred chicken $C \times (C \times GP)$ derived from double crossing of hen of Polish indigenous breed Greenleg Partridge with heavy meat type cocks and to compare them with the maternal breed $C \times GP$ and with commercial medium growing Hubbard JA 957 chicken.

MATERIAL AND METHODS

Hens of the Polish indigenous Greenleg Partridge (GP) light breed were crossed with the heavy meat type cocks (C) and the obtained F_1 crossbreds were examined for slaughter analysis parameters. Hens from this crossing were used as a maternal material in next crossing, again with the heavy meat type cocks (C). The chicken from the second generation (F_2) of crossbred ($C \times (C \times GP)$) were also examined for slaughter analysis parameters and compared with commercial medium growing Hubbard

JA 957 broilers, designed for a longer, 9-week production.

Total 3,300 meat type chicken derived from three genotypes: F_1 crossbred derived from crossing Greenleg Partridge hen with meat type cocks; F_2 crossbred, an effect of re-crossing F_1 crossbred with meat type males; commercial medium growing Hubbard JA 957, were used in the study. One-day chickens were placed to 30 pens (110 birds per pen), 10 pens of each genotype. Chicken were kept in the same chicken house on litter under controlled environment conditions till 63rd day of age. All birds were provided the same diets included Starter – till 14th day, Grower 1–15–28th days, Grower 2 – 29–35th day, and Finisher – from 36th day. Access to feed and water was freely available. At the 63rd day, 96 males and 96 females from each genotype were taken according to mean body weight for the group and sex. After 4 hour fastening chicken were weighted individually and slaughtered in a professional slaughterhouse. Obtained chilled carcasses were dissected after 12 hour storage in 4°C. Carcass dissection was carried out by removing of breast, thigh and drumstick meat and abdominal fat pad. Carcass dressing percentage was expressed as percentage of live body weight. Abdominal fat and the breast and leg muscles were removed and weighed to determine their percentage share in the carcass.

The obtained results were analysed statistically by one-way analysis of variance as least square means (GLM procedure, SPSS 14.0 PL Software for Windows), separately for each sex.

RESULTS

The F_1 generation of crossbred (C×GP) characterized by worse slaughter parameters than other birds (Table 1). Chickens had much lower body weight and muscle weight and also lower dressing percentage, what limited their usefulness for meat production. Live body weight and carcass weight C×GP chickens were similar to medium growing Hubbard ISA Red JA at the age of 56 days (Aksoy et al. 2010) and to slow growing chickens at 81st day of age (Fanatico et al. 2005), and the dressing percentage – likewise slow growing and medium growing chickens from Fanatico et al. (2005) and Wang et al. (2009) studies. However meat content in carcass of F_1 crossbred was equal to Hubbard JA 957 commercial chicken. Obtained results allow to consider C×GP crossbred as slow growing chickens. They can be used as maternal breed in commercial crossing rather than as chickens for broiler production. The usefulness this crossbreds hens in a broiler parent stock was confirmed by the results obtained in F_2 generation.

In comparison with maternal breed C×GP, double crossbred C×(C×GP) had higher body weight ($P < 0.01$), and consistently much higher carcass weight and were better muscled ($P < 0.01$). There was improved dressing percentage, increased breast meat percentage in the carcass ($P < 0.01$). In the contrary leg meat percentage was lower ($P < 0.01$), although leg weight was much higher ($P < 0.01$). It indicates positive effect of re-crossing with meat type cocks. A similar characteristic both crossbred types was much worse musculature of legs than breast (Table 1).

Slaughter results show, that C×(C×GP) crossbred F_2 are typical medium growing chicken characterized by high body weight, and good musculature of breast and leg. They can be suitable, like Hubbard JA 957, for meat production in 9-week fattening system.

Data presented in Table 1 indicate significant ($P < 0.01$) differences among genotypes in most of slaughter traits. F_2 crossbred was characterized by lower body weight and, in effect, also lower carcass weight. However, regardless of these differences, both Hubbard JA 957 and crossbred C×(C×GP) chicken had the same dressing percentage, similar to six-weeks Cobb 500 broilers (Beg et al. 2011). Higher breast meat weight and breast meat yield was found in the crossbred ($P < 0.01$). Similar high value (25%) was obtained in fast growing broilers at 56th day of age in Aksoy et al. (2010) study, and also in Arbo Acres chicken at the age of 42 days in Liu et al. (2011) study. Romero et al. (2009) suggested maternal effect on a chicken breast meat yield. On the contrary, leg meat weight and leg meat yield were significantly lower than in Hubbard JA 957 chickens. C×(C×GP) crossbred chicken had lower abdominal fat weight and abdominal fat percentage.

DISCUSSION

Studies of Fanatico et al. (2005) and Aksoy et al. (2010) indicated genotype effect on dressing percentage – fast growing chickens has higher dressing percentage than slow growing and medium growing ones. Body weight of fast growing broilers Cobb (Aksoy et al. 2010) at the age of

TABLE 1. Comparison of slaughter analysis of the crossbred chickens C×(C×GP) with maternal breed C×GP and with Hubbard JA 957 commercial chicken

Trait	Sex	Crossbred F ₁ C×GP			Crossbred F ₂ C×(C×GP)			Hubbard JA 957			P ₁	P ₂	P ₃
		N	LSM	SE	N	LSM	SE	N	LSM	SE			
Live body weight, g	males	96	2 142	6	96	3 131	21	96	3 346	16	0.001	0.001	0.001
	females	96	1 689	5	96	2 437	10	96	2 700	6	0.001	0.001	0.001
Carcass weight, g	males	96	1 494	6	96	2 304	16	96	2 472	12	0.001	0.001	0.001
	females	96	1 184	5	96	1 819	10	96	2 014	8	0.001	0.001	0.001
Breast meat, g	males	96	319	2.6	96	588	7.0	96	538	4.8	0.001	0.001	0.020
	females	96	267	2.5	96	476	4.2	96	459	3.8	0.001	0.001	0.036
Leg meat, g	males	96	305	2.2	96	455	5.7	96	510	4.1	0.001	0.001	0.001
	females	96	226	1.6	96	330	2.9	96	385	3.4	0.001	0.001	0.001
Abdominal fat, g	males	96	45.6	1.13	96	45.0	2.28	96	75.3	2.30	0.824	0.001	0.001
	females	96	45.3	1.12	96	52.6	2.02	96	84.9	2.79	0.002	0.001	0.001
Dressing percentage, %	males	96	69.7	0.19	96	73.6	0.26	96	73.9	0.27	0.001	0.001	0.402
	females	96	70.1	0.22	96	74.6	0.23	96	74.6	0.30	0.001	0.001	0.790
Breast meat, % of carcass	males	96	21.3	0.15	96	25.5	0.21	96	21.8	0.31	0.001	0.239	0.001
	females	96	22.5	0.18	96	26.2	0.18	96	22.8	0.21	0.001	0.404	0.001
Leg meat, % of carcass	males	96	20.4	0.14	96	19.7	0.17	96	20.6	0.18	0.001	0.590	0.001
	females	96	19.1	0.12	96	18.2	0.13	96	19.1	0.21	0.001	0.699	0.001
Abdominal fat, % of carcass	males	96	2.1	0.05	96	1.6	0.04	96	3.0	0.09	0.001	0.001	0.001
	females	96	2.7	0.06	96	2.3	0.05	96	4.2	0.13	0.001	0.001	0.001

P₁ – significance of differences between crossbreds of F₁ and F₂ generations, P₂ – significance of differences between crossbreds of F₁ generations and Hubbard JA 957, P₃ – significance of differences between crossbreds of F₂ generations and Hubbard JA 957.

56 days amounted 3.4 kg, while the slow growing Hubbard ISA Red JA weighted only 2.2 kg. Body weight of chicken in Fanatico et al. (2005) study ranged from 2.1–2.2 kg in medium growing chicken at the age of 81 day to 2.4–2.5 kg in fast growing ones at the age of 53 day. Also Wang et al. (2013) comparing slaughter yield and meat quality of fast growing and slow growing chickens found, that genotype had the large effect on these features. Gornowicz et al. (2009) pay attention to significant differences in the quality of carcass and meat among Cobb 500, Hybro G+ and Ross 308 broiler chickens, while Janisch et al. (2011) did not found significant differences in body weight or meat yield of Ross 308, Ross 708 and Cobb 700 broilers.

A very good breast meat yield characterized fast growing broilers Cobb 308, whose muscles at 56 day of age weighed 648 g (Aksoy et al. 2010). In the same experiment breast muscles of Hubbard ISA JA weighted only 288 g. Breast muscles of Ross 308 chicken weight was 450–500 g, breast meat percent in carcass amounted to 22–23% (Berri et al. 2008, Janish et al. 2011), and leg meat percent – 26% (Janish et al. 2011). Arbo Acres chickens slaughtered at 42nd day of age characterized very good musculature, both of breast 25–26% and 27.7%, and leg 23–24% and 20.6%, respectively in Liu et al. (2011) and Wu et al. (2011) studies.

Guo et al. (2011) indicates genotype and selection direct impact on fatness. Fat weight in Arbo Acres chickens differed significantly according to line – carcasses of chicken selected on lower-

ing of fat deposition contained 20 g of fat while carcasses from fatty line – 115 g. Also Dal Bosco et al. (2012) found that chicken fatness is related to their genotype and is higher in fast growing broilers, because of the combination of age, low kinetic activity, and the high feed intake. Fat content in carcasses of slow growing chicken at the age of 112 days ranged from 3 to 6.5% according to different raising system (Wang et al. 2009). Fat percentage in six-week Arbo Acres broilers amounted to 1.2–1.5% (Liu et al. 2011, Wu et al. 2011), Hubbard 1.3–1.8% (Mahmood et al. 2007), Cobb 500 from 1.2–2.2% (Beg et al. 2011) to 2–2.5% (Hajati et al. 2009).

CONCLUSION

Crossbred of F₁ generation derived from Greenleg Partridge had much lower body weight, compared to commercial broilers, and consistently also lower carcass and meat. But slaughter analysis results were not different so much from values obtained for most slow growing and medium growing chickens. Hens from this breed can be used as maternal breed in a broiler parent stock.

Crossbred of F₂ generation (C×(C×GP)), obtained after re-crossing F₁ with meat type males, were characterized by very good slaughter results, especially high percentage of breast meat and low content of abdominal fat in carcass. It suggests the possibility to use these chickens for longer fattening period (9 weeks) to produce lighter, but good muscled carcasses.

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- Streszczenie:** Ocena wartości rzeźnej kurcząt z trzech materiałów genetycznych. W badaniach porównano kurczęta z trzech grup genetycznych: mieszańce F₁ powstałe ze skrzyżowania lekkich kur rasy Zielononózka kuropatwiana z ciężkimi kogutami typu mięsnego, mieszańce F₂, efekt powtórnego krzyżowania powstałego mieszańca Cobb×Zk z kogutami typu mięsnego oraz Hubbard JA 957, przeznaczone do dłuższego chowu (9 tygodni). Kurczęta odchowywano do wieku 63 dni. Oceniano wydajność rzeźną, umięśnienie i otluszczenie tuszek. Mieszańce F₁ (C×Zk) charakteryzowała dość mała masa ciała, typowa dla kurcząt wolno rosnących, oraz dobre umięśnienie, zwłaszcza piersi. Ponowne skrzyżowanie z kogutami typu mięsnego wpłynęło na istotną (P < 0,01) poprawę parametrów oceny poubojowej. Mieszańce F₂ (C×(C×Zk)) uzyskały dużą masę ciała, typową dla kurcząt średniorosnących. W porównaniu do kurcząt Hubbard JA 957 mieszańce F₂ miały małą masę ciała, taką samą wydajność rzeźną, lepsze umięśnienie piersi, gorsze nogi i mniejsze otluszczenie. Duży udział mięśni piersiowych w tuszce oraz małe otluszczenie wskazują na przydatność tych kurcząt w użytkowaniu mięsnym jako materiał średniorosnący, przeznaczony do dłuższego, 9-tygodniowego chowu.

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Semen quality parameters in outbred male mice from four different selected lines

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Abstract: *Semen quality parameters in outbred male mice from four different selected lines.*

Breeding of (outbred) selective lines of laboratory mouse was initiated in Warsaw University of Life Sciences about 40 years ago. It bred Heavy (C) and Light (L) mice selected opposite for body weight at weaning (21st day of life), S mice line selected for higher testes weight, and control (K) mice without selection. All lines have identical genetic background, but different directions of selections caused diversification of specific phenotypic traits between them. The purpose of this study was to compare semen quantity and quality parameters in outbred C, K, L and S male mice in the context of measurements of average body and testes weight for each line.

Research materials were seminal fluids squeezed out of the vas deferens from 20 outbred C, K, L and S male mice (5 males per group). Animals had been euthanized, and necropsy was performed. Body and testes weight was measured. Also sperm concentration, viability (by Eosin test), cytoplasmic membrane integrity degree (HOS test), sperm head morphology and maturity were estimated.

It was shown that S male mice, which have much higher testes weight, also have a significant increase of viable spermatozoa according to control line. Moreover, sperm concentration from S males is at least two times higher than in other selective lines.

Key words: semen quality parameters, HOS, selective lines, Eosin test, mouse

INTRODUCTION

Breeding of selected lines of laboratory mouse was initiated in The Department of Genetics and Animal Breeding, Warsaw University of Life Sciences 43 years ago (Sławiński 1974). It bred Heavy (C) and Light (L) mice selected opposite for body weight, S mice line selected for higher testes weight and control (K) mice without selection. Next, lines were bred also in The Department of Genetics, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw, where it started getting inbred stocks from them, but until now none of selective lines is homozygotic inbred strain (G8-G13, depending on line).

All lines have identical genetic background, but different directions of selections caused diversification of specific phenotypic traits between them (Wirth-Dzięciołowska et al. 2005). It was showed differences in reproduction traits such as the ovulation rate, prenatal mortality and embryo number (Wirth-Dzięciołowska 1973). It was also correlation between body weight and lifetime reproduction

rate as well as time of maturation (Wirth-Dzięciołowska et al. 1996). Moreover L mice lived longer than C mice (Wirth-Dzięciołowska and Czumińska 2000).

MATERIAL AND METHODS

The purpose of this study was to compare sperm quantity and quality parameters in outbred C, K, L and S male mice in the context of measurements of average body and weight for each line according to previous studies (Wirth-Dzięciołowska 1973).

Tests were carried out in The Department of Genetics, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw.

Animals

Five adult (3–6 months old) males from each of the C, K, L and S lines were used in the study. Animals were maintained in a barrier facility (constant light cycle – 12L/12D, room temperature about 23°C, water and food (Labofeed H) were available *ad libitum*). It were mice post-selected, obtained from the herd.

Necropsy and morphometrical parameters

Male mice been euthanized by cervical dislocation, and necropsy was performed. Body and testes weight was measured ($\pm 0,001$ g). Research materials were seminal fluids squeezed out of the vas deferens into 100 μ l of M2 Medium – common medium used for *in vitro* culture of preimplantation stage embryos. It can be used for collecting viable gametes outside a CO₂ incubator and it also extend sperm fluids.

Sperm quality parameters

Tests were performed per 200 spermatozoa (Krzanowska 1962), and analyzed under light microscope – Olympus, type B091 and B201. Percentage of sperm without cytoplasmic droplet (mature spermatozoa), sperm with proximal (immature spermatozoa) and distal droplet (during maturation, but normal) were counted from fresh semen suspension (400 \times magnification).

First portion of suspension (10 μ l) was diluted (1 : 5, v/v) with sterile water (50 μ l) and integrity of cytoplasmic membrane of sperm tails was carried out by Hypoosmotic Swelling Test (HOS). Percentage of spermatozoa with integral membrane (swollen tails) and without integral cytoplasmic membrane (broken and straight tails) was counted (400 \times magnification).

Second portion of semen suspension (10 μ l) and 0,2% eosin Y (10 μ l) was mixed (1 : 1, v/v) and incubated for 10 min. Sperm viability was elucidated (Eosin Test), by counting of percentage of dead (red) and alive (green) sperm head (400 \times magnification).

Next, 10 μ l of the sperm suspension was extended (1 : 5, v/v) with PBS (50 μ l) and sperm number was evaluated per at least 5 large squares and at least 200 spermatozoa were counted in a haemocytometer.

Sperm head morphology was analyzed under 1,000 \times magnification. Smears of sperm suspension was fixed in a mixture of 99.8% ethanol and acetic acid (3 : 1, v/v) and stained with 1% eosin Y for 15 min. Percentage of normal sperm head, and abnormal spermatozoa was counted with classification of sperm head abnormalities by Krzanowska (1976).

The statistical significance of differences between the analyzed lines were calculated by ANOVA (IBM SPSS Statistics 21).

RESULTS AND DISCUSSION

Figure 1 shows the mean C, K, L, S male mice body weight. It was noted significant differences between average body weight of all lines. C (43.87 g) and S (35.96 g) lines were characterized by the highest body weight as well as L line (25.45 g) had the lowest body weight.

The highest testes weight (Fig. 2) was recorded in S males (0.56 g) in the main direction of this line selection, which was more than 2 times higher than in C line and more than 3 times higher than in the K and L male mice.

S male mice was characterized by 4 times higher sperm concentration $162.19 \cdot 10^6/\text{ml}$ (Fig. 3) in comparison with C ($37.86 \cdot 10^6/\text{ml}$) and K line ($39.55 \cdot 10^6/\text{ml}$). Level of this parameter in L mice was about 1.5 times higher than in control and C males ($68.31 \cdot 10^6/\text{ml}$), but it was more than 2 times lower in relation to S males.

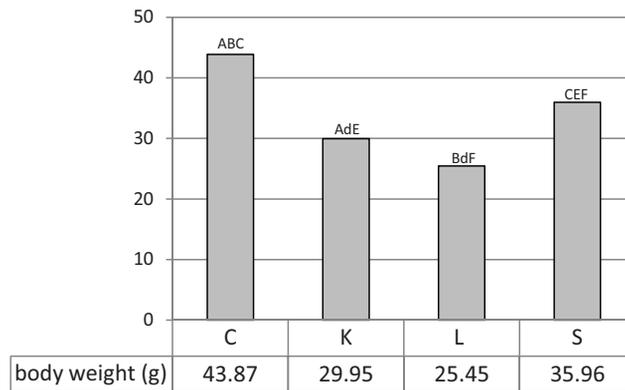


FIGURE 1. Average body weight (g) of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). ABCEF at $P \leq 0.01$, d at $P \leq 0.05$

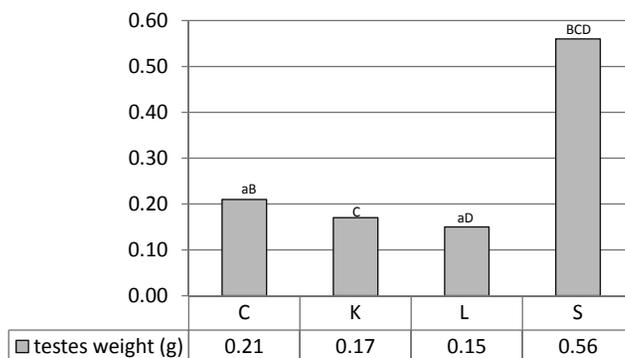


FIGURE 2. Average testes weight (g) of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). BCD at $P \leq 0.01$, a at $P \leq 0.05$

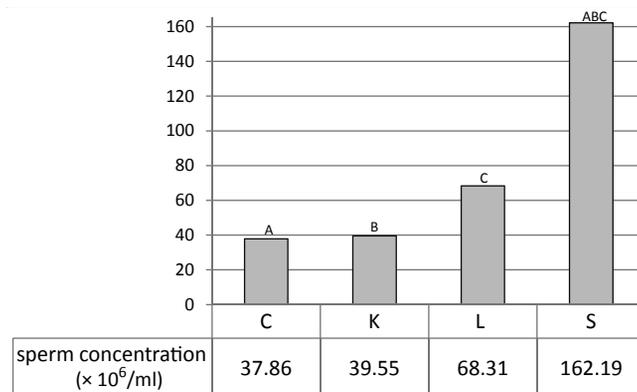


FIGURE 3. Sperm concentration [$\times 10^6/\text{ml}$] of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). ABC at $P \leq 0.01$

The study of sperm head morphology (Fig. 4) shows the highest percentage of morphologically normal S male mice sperm heads (89%). This parameter in C, K and L lines oscillated between 79.9–83.3%. Higher completely mature sperm (Fig. 4) percentage was detected mainly in the semen fluids of K and S male mice (60.9–62.35%), than in L and C male (respectively 47.07 and 47.9%), but observed differences were not statistically significant.

The results of HOS test and Eosin test (Fig. 5) showed that the largest sperm viability (alive, swollen tails) were observed in S male mice (alive – 59.70%, swollen tails – 71.70%). The differences among other lines, C (55.15%; 66.65%), K (50.85%; 61.20%) and L (55.90%; 71.70%), were smaller and not statistically significant.

Reproduction is one of the most important functions of the free-living and breeding organisms. Thus regular assessment of fertility parameters in

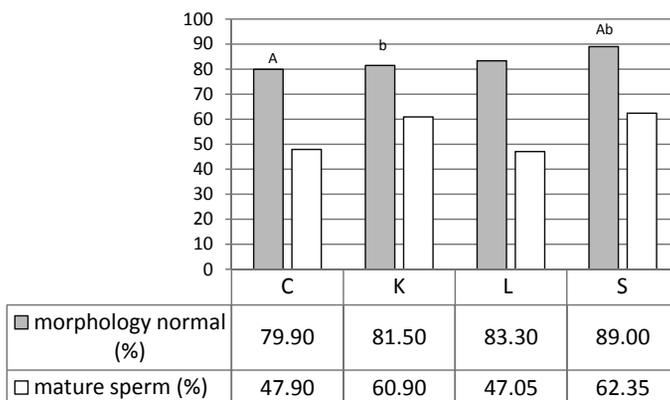


FIGURE 4. Sperm head morphology and tail membrane integrity of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). A at $P \leq 0.01$, b at $P \leq 0.05$

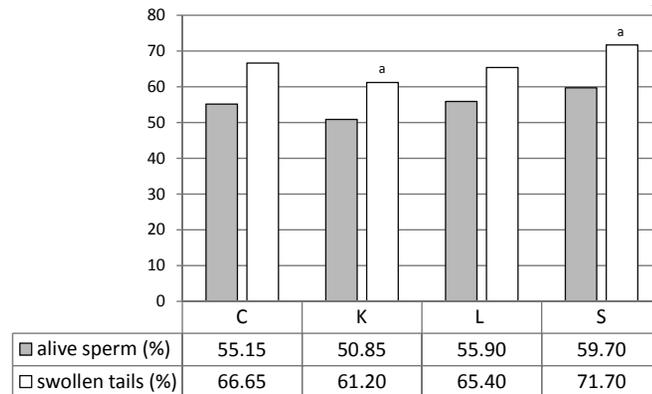


FIGURE 5. Sperm viability (Eosin test and HOS test) of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). a at $P \leq 0.05$

animals seems to be necessary to maintain high-quality reproductive herd (Bakker et al. 1978). Assessing reproductive parameters is also of utmost importance in genetic control of outbred herds.

Sperm quality parameters were analyzed to determine viability, motility and maturity of spermatozoa, cytoplasmic membrane integrity of tails (Krzanowska 1962, Gołas et al. 2011), sperm head morphology (Krzanowska 1976) as well as sperm concentration was counted.

Our study reveals that K and C male mice have rather “basal” level of sperm parameters, nearing inbred strains parameters (Gołas et al. 2011). L males fertility parameters are gently higher in relation with C and K results, which can make them effective males in reproduction. S male mice have the highest sperm quality parameters and sperm concentration.

Testes size may affects sperm production, so in bigger testes much higher number of spermatozoa is produced (Le Roy et al. 2001, Gołas et al. 2011). Our tests shows that in S males, which are selected for high testes weight, at least

2.5 times more spermatozoa is produced, which is also alive and motile with normal morphology. It should also be noted that testis weight could be correlated ($r = 0.3$) with a body weight of lines analyzed. However, in other publications there found no correlation between these morphologically parameters (Hill et al. 1990, Le Roy et al. 2001). C and L line, which is selected for high and low body weight, respectively have also higher and lower testes weight, but probably these results are only the line effect and false correlation is caused by small number of animals in analyzed groups.

Probably long-term selection (more than 100 generations) can not affect sperm quality directly (Wirth-Dzięciołowska 1992). L males have smaller testes than C and K males, but L male mice semen quality parameters are higher than C and K average level.

Nevertheless, S males selected for high testes weight have also higher body weight and fertility levels which can be close to the maximum. Significantly better sperm parameters of S males are the effect of an increase of selection for

testes weight, what was confirmed by Hill et al. (1990) and Le Roy et al. (2001).

CONCLUSIONS

Increase of testes weight causes much higher sperm concentration and increase semen quality parameters in S males in comparison with C, K and L mice. According to our investigations, long-term selection for body weight seems not affect directly qualitative and quantitative parameters of semen in male mice.

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Streszczenie: Parametry jakości plemników samców myszy z czterech outbredowych linii selekcyjnych. Celem przeprowadzonych badań było dokonanie oceny jakości parametrów plemników nasieniowodowych, pobranych od 20 samców (po 5 samców z linii) myszy z linii selekcyjowanych przez wiele pokoleń: przeciwstawnie na masę ciała (C i L), masę jąder (S) oraz samców stanowiących linię kontrolną (K), w kontekście pomiarów średnich mas ciała i jąder dla poszczególnych linii. Materiał badawczy stanowiły

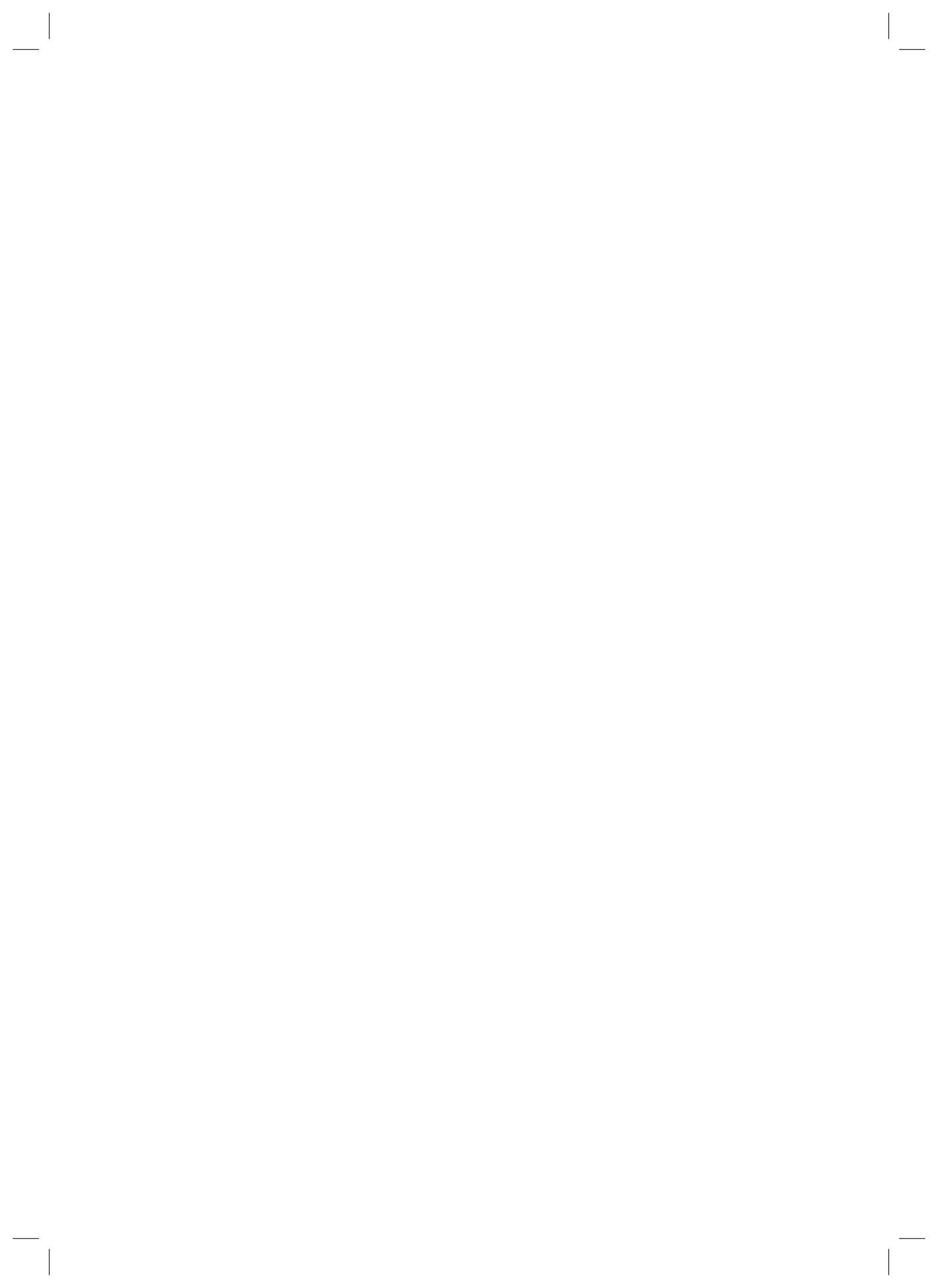
plemniky pobrane z nasieniowodów od zwierząt poselekcyjnych. Oszacowano liczbę plemników w 1 ml pożywki (M2). Dokonano analizy parametrów jakości plemników, wykonując test oceny żywotności plemników, test położenia kropli cytoplazmatycznej, który jest miarą dojrzałości plemników oraz test hipoosmotyczny (HOS) do oceny integralności błony cytoplazmatycznej witek plemników. Ponadto dokonano oceny morfologii główek plemników. Wykazano, że samce linii S w porównaniu z osobnikami z linii kontrolnej K oraz linii ciężkiej (C) i lekkiej (L) mają istotnie większą masę jąder, większy odsetek dojrzałych i żywotnych plemników, a także 2–4-krotnie większą koncentrację plemników liczoną w 1 ml medium.

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The influence of nanodiamond particles on rat health status

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Abstract: *The influence of nanodiamond particles on rat health status.* The objective of the present investigation was to evaluate the effect of nanodiamond (ND) particles on rats health status. 1 mg/kg b.w. of nanodiamond particles was administered intravenously and intraperitoneum. The presence of an adverse impact was examined. The results show significant changes in biochemical (glucose and total protein level decrease) and hematological (elevated platelets count) parameters, only in case of intravenous injection.

Key words: nanodiamond particles, health status, biochemical and hematological parameters, rats

INTRODUCTION

The recent large interest in carbon nanomaterials (fullerenes, nanotubes, nanodiamonds) is a consequence of their unique mechanical, electrical and thermal features. Numerous biological applications suggested by several investigators include their potential application as the drug delivery agents controlling the release of genes, proteins or other molecules (Bondar and Puzyr 2004). Nanodiamonds present the high effectiveness in drug delivering chemotherapy, presenting the low negative effects associated with the known drug-delivery agents. It was proposed to apply nanodiamonds as a biochip or biosensor for a medical use (Puzyr et al. 2007). As

the biofunctional agent, nanodiamond should reveal the biocompatible characteristics. It should exhibit the lowest toxic properties and it should remain neutral for the organism parameters (Bakowicz-Mitura et al. 2007). According to the recent studies, nanodiamonds present the high biological tolerance and they exhibit biocompatibility with the blood components (Mitura et al. 2006). However, we observe the insufficiency of the *in vivo* studies characterizing the systemic organism response to diamond nanoparticles. The hematologic and biochemical parameters of rats are published by Exotic Animal Companion Medicine Handbook for Veterinarians (Johnson-Delaney 1996).

Therefore, the objective of the study was to evaluate the effect of intravenous and intraperitoneal administration of nanodiamond on biochemical and hematological parameters. Properly interpreted results of the blood chemistry values may provide the precise picture of the animal health status at the time of its sampling (i.e., nutritional status, disease condition and stress after environmental changes). Therefore, using these data for diagnostic purposes we compared the obtained results with reference or normal values.

MATERIAL AND METHODS

Diamond nanoparticles (ND) was produced by the method described by Danilenko (2003) with modification of ampoule-free synthesis in the explosion chamber instead of ampoule synthesis. Graphite was placed directly into a cylindrical charge consisting of a TNT-hexogen mixture TG40. The charge was enveloped in a water jacket to suppress graphitization and reduce the unloading rate of the synthesized diamond. Shape and size of ultrananocrystalline diamond (2–10 nm) were inspected by transmission (TEM) and scanning (SEM) electron microscope (Bakowicz 2003, Czerniak-Reczulska et al. 2010). Figure 1 shows that the grains of nanoparticles forming conglomerates (HR TEM). Figure 2 shows nanodiamond clusters (SEM).

ND at the concentration of 500 mg/l was suspended in deionized water then the mixture was sterilized and sonificated. 21 male Wistar rats were divided into three equal groups and kept in indi-

vidual cages for 10 days under standard conditions: temperature 22°C, humidity 50–70%, light/dark cycle 12/12h. The animals had free access to water and feed. Diet was formulated in compliance with NRC requirements (1995). Rats were administrated by intravenous (group 1) and intraperitoneal injection (group 2) of 0.5 ml of ND colloid (1 mg/kg body weight of ND particles). Clear deionized water was administrated by intravenously into the animals of control group (group 3). At the end of the experiment the rats were fasted for 12 h and then sedated by intramuscular ketamine (Ketamini hydrochloricum 5%, Narkamon, SPOFA, Praha, Czech Republic). Blood was sampled from the heart into heparinized tubes and cooled to 4°C. The animals were euthanized by ketamine overdoses. The samples were collected for further analysis. Blood morphology (red blood cell (RBC) count, hematocrit, mean cell volume (MCV), hemoglobin concentration, white blood cell (WBC), neutrophils, lymphocytes and platelets

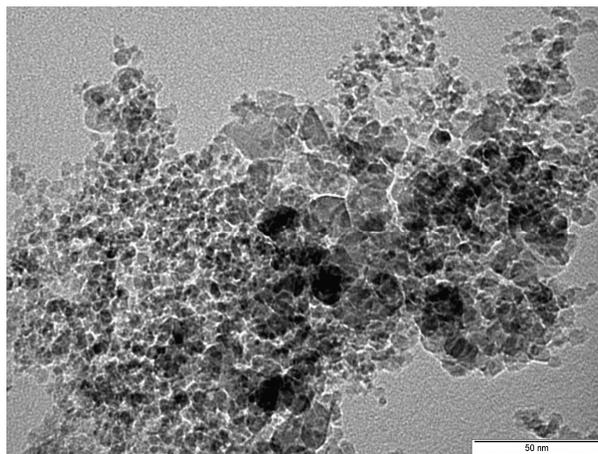


FIGURE 1. Nanodiamond particles manufactured by detonation method — HR TEM. Reprinted with permission from Bakowicz (2003)

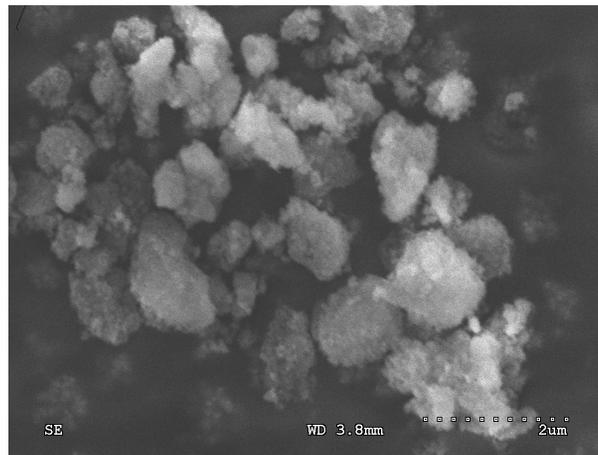


FIGURE 2. View SEM of nanodiamond powder manufactured by detonation method. Reprinted with permission from Czerniak-Reczulska et al. (2010)

count was determined using standard methods and a Danam-510 analyser (France). The following were assayed in serum: total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, triglycerides using a Vitros DTII analyzer (Johnson and Johnson).

RESULTS AND DISCUSSION

The haematological parameters indicate that hydrocolloid of detonation diamond nanoparticles, regardless of the route of administration results in the elevation in platelet number in the rats, compared to the control group. Other morphological indicatives remained unchanged (Table 1). Additionally, hydrocolloidal ND intravenously injection decreased glucose and total protein concentration in the animal serum. Other biochemical parameters did not differentiate the experimental groups and remained within the reference values (Table 2).

Platelets are assisting and modulating inflammatory reactions and immune responses. This is achieved by the regulated expression of the adhesive and immune receptors on the platelet surface and also by the release of a multitude of secretory products including inflammatory mediators and cytokines, which can mediate the interaction with leukocytes (Hundelshausen and Weber 2007). The primary regulator of platelet production is thrombopoietin (THPO). After binding to the cell receptor, it affects all stages of development of megakaryocytes. THPO is synthesized mainly in the liver and it is stimulated by IL-6 (Śliwińska-Staczyk 2005). Based on the presented results, we hypothesize that the ND stimulates immune system and it leads to the expression of inflammatory mediators (IL-6), which had consequences in the increased production of blood platelets. However non-specific immune and hematologic indicators (Niemiec et al. 2011), do not prove any inflammatory activity of ND. However, the presented blood cell

TABLE 1. Hematological parameters in peripheral blood of control and experimental rats

Parameter	Reference values*	Control	Injection		SE pooled	P-value
			intravenous	intraperitoneal		
RBC** (10 ¹² /l)	6.76–975	8.1	7.7	8.0	0.242	0.5561
Hematocrit (l/l)	37.6–50.6	41.8	41.9	42.6	0.99	0.207
MCV** (fl)	50–80	52.0	54.0	53.0	0.88	0.207
Hemoglobin (g/dl)	11.5–16.1	14.0	14.0	14.2	0.26	0.861
WBC** (10 ⁹ /l)	6.6–12.6	8.6	8.3	7.6	1.197	0.8245
Neutrophils(%)	3–42	18.0	12.0	11.0	3.42	0.276
Lymphocytes (%)	50–95	81.0	88.0	88.5	3.46	0.285
Platelets (10 ⁹ /l)	150–460	487.3 b	645.5 a	715.2 a	45.70	0.009

*Exotic Animal Companion Medicine Handbook for Veterinarians, Johnson-Delaney 1996.

** Abbreviations: RBC = red blood cells, MCV = mean corpuscular volume, WBC = white blood cells. a, b – significant difference at $P < 0.05$.

TABLE 2. The level of blood serum parameter in control and experimental rats

Parameter	Reference values*	Control	Injection		SE pooled	P-value
			intravenous	intraperitoneal		
Glucose (mmol/l)	2.8–7.56	9.01 b	7.58 a	9.24 b	0.236	0.0002
Triglycerides (mmol/l)	0.35–1.4	1.1	0.97	1.39	0.119	0.0685
AST** (U/l)	39–111	124.6	119.4	112.0	8.33	0.573
ALT** (U/l)	20–61	57.7	54.28	51.42	5.414	0.7178
Albumin (g/l)	38–48	34.4	29.7	36.7	2.34	0.126
Total protein (g/l)	53–69	66.3 b	54.0 a	67.7 b	3.58	0.027

*Veterinary Reference Guide, Clinical Diagnostic Division Eastman Kodak Company.

** Abbreviations: AST = aspartate aminotransferase, ALT = alanine aminotransferase.

a, b – significant difference at $P < 0.05$.

parameters did not differ groups in the experiment. Puzyr et al. (2002) showed that ND damages blood cells including erythrocytes by following mechanisms: (1) direct binding of NDs to the cell membrane proteins, which may cause irreversible inhibition of their functions and (2) a imbalance of electrolytic and osmotic equilibrium caused by adsorption of blood plasma protein components to ND particles (Puzyr et al. 2002). Degradation of blood cells depends on the concentration and ND surface properties. In

our study we demonstrated no significant changes in the quantity of white and red blood cells. Histological studies also did not confirm the negative impact of ND on kidneys and liver cell structures (Niemiec et al. 2010). According to Puzyr et al. (2007), the modified diamond nanocolloid administered for 6 months, did not affect the mortality of the tested mice. There were no abnormalities observed in the process of organ growth and weight, however the increased the level of leukocytes in the blood was shown. ND

administered subcutaneously did not cause any inflammation nor damage of the cells adjacent to applied nanoparticles. On one hand, we expect certain level of degradation of the treated cells after diamond nanocolloid applied *in vitro* versus *in vivo*. This may be related to the numerous of extracellular factors (protein, lipoprotein, sugars) involved in the nanoparticles interactions. On the other hand, diamond nanoparticles can affect the large number of the cells after *in vivo* injection. Puzyr et al. (2007) observed no changes in the blood morphological parameters after intravenous injection of ND. However, the results of that work were not significant and indicate the increase in the number of platelets after nanocolloid injection.

The values of biochemical markers of health in all experimental groups remained within the reference ranges. Glucose and total protein concentration was significantly lower in animals after intravenous injection of ND, compared to the control group. Puzyr et al. (2007) has documented that diamond nanoparticles modulate concentration of biomarker of liver injury (bilirubin/transaminases) and lipid metabolism (cholesterol, low density lipoprotein, triglycerides) indicators in the rabbit serum. We assumed that ND affects the excretion of urine glucose and protein by mechanical damage of renal corpuscles. Nephrotoxic compounds were shown to be a reason for pathological changes such as proteinuria and glycosuria (Ascioglu et al. 2000). However, Niemiec et al. (2008) has reported that histopathological examination of kidney and liver showed normal architecture, suggesting no morphological disturbances in rats treated by intravenously injection

of nanodiamond particles. However, the histological assessment cannot rule out the presence of microdamages in the examined organs. This was confirmed by Yu et al. (2005) who presented the cytotoxicity of the diamond powder on the kidney cells.

CONCLUSIONS

The nanoscale diamond particles have become an interesting innovative pharmaceutical material. Due to particular size and surface structure, nanoparticles are well-suited for different biological applications. Most of the toxicology investigations carried out on *in vitro* models reveals positive results. The recent study presents wide biological benefits of nanodiamond particles obtained by detonation methods such as low cytotoxicity and biocompatible (Bondar et al. 2005, Schrand et al. 2006, Bakowicz-Mitura et al. 2007). We have recently showed that intravenous and intraperitoneal injection of 1 mg/kg b.w. detonation diamond powder increased platelet counts on rats. Additionally, the intravenous injection of nanoparticles decreased serum level of glucose and total protein. It is hypothesized that ND stimulates the TPO synthesis pathway through the activation of pro-inflammatory cytokines. We also speculate that exposure to diamond nanoparticles may lead to the nephrotoxicity characterized by hematuria and proteinuria together with the reduced serum total protein and glucose level. Our results show the necessity of the determination of the minimum toxic dose and the evaluation of the ND biological properties, especially in the experiments conducted *in vivo*.

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Streszczenie: *Wpływ koloidu nanodiamentu na parametry stanu zdrowia szczurów.* Celem badań była ocena wpływu pozajelitowego wlewu hydrokoloidu nanodiamentu otrzymanego metodą detonacyjną na parametry hematologiczne i biochemiczne krwi u szczurów. Uzyskane wyniki wskaźników hematologicznych wskazują, że hydrokoloid nanodiamentowy, niezależnie od drogi podania, wpłynął na zwiększenie się liczby płytek krwi u szczurów w porównaniu z grupą kontrolną.

Ponadto wlew dożylny hydrokoloиду ND wpłynął na zmniejszenie koncentracji glukozy i białka całkowitego we krwi badanych zwierząt. Analiza danych doświadczenia dowodzi pilnej potrzeby ustalenia minimalnej dawki toksycznej nanocząstek ND oraz szczegółowej oceny biologicznych właściwości nanodiamentów otrzymywanych metodą detonacyjną w badaniach na zwierzętach.

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Occurrence of entomopathogenic fungi in soil of Santiago and Fogo islands (Republic of Cape Verde)

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Abstract: *Occurrence of entomopathogenic fungi in soil of Santiago and Fogo islands (Republic of Cape Verde).* The soil is a habitat for many entomopathogenic fungi (EPF) all over the world. The Galleria bait method has been chosen to isolate the EPF from soil samples. The common occurrence of EPF in agricultural land was confirmed by these studies, which concluded, that the soil of Cape Verde is a habitat for EPF, such as *Beauveria bassiana* and *Metarhizium anisopliae*. The presence of *B. bassiana* was recorded in the cultivable land of the island of Fogo, while *M. anisopliae* occurred in the soil of Fogo as well as Santiago. There were no records of EPF in woodlands. Statistically, more soil samples containing *B. bassiana* were derived from perennial crops. The amount of sand in the soil determined the presence of the fungus. On the other hand, *M. anisopliae* was present on less inclined grounds. *Fusarium* spp. was present in half of the examined soil samples. The isolation of native EPF and their subsequent application in developing countries may significantly contribute to reduce pest populations in agriculture effectively.

Key words: entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, *Fusarium* spp., soil

INTRODUCTION

The advantages of using biological methods to control crop pests were largely discussed and documented in different countries (Cock et al. 2009, Hussein et al. 2010). In the last few years there has been an increasing interest in native

biological control agents. In Europe, in the first decade of the twenty-first century seven exotic species used in biological control were successfully replaced by native species. In this period, a total of 18 native species compared with six exotic ones were commercialized. The situation was different in the African continent, where from a total of 26 species commercialized as natural enemies, 25 were originate from material collected and initially bred in other continents (Cock et al. 2009).

The designing an effective biological control in developing countries with tropical and subtropical climates is a priority. In the regions, where government programmes (e.g. Programme of the Government of Cape Verde 2011–2016, 2010) predict a growth in agricultural output, an effective plant protection should be provided. What is more, the abuse of synthetic pesticides has been declared as being one of the environmental problems in these countries (Programme strategy of Cape Verde 2009–2012, 2010).

The Cape Verde Islands are of volcanic origin. The climate of this sub-sahelian archipel is dry and semidry (de Faria 1970). The intensive rainfall between August and October is responsible for erosion, especially of steeply declined regions (between 25 and 45%).

The islands of Santiago and Fogo both provide the climatic and ecological conditions for the development of agriculture.

The agriculture of irrigated grounds is showing a growing tendency, each year increasing the areas where drop by drop irrigation is used instead of the still dominating traditional flooding irrigation. The most popular on irrigated grounds are orchards (*Musa* spp., *Mangifera* spp., citruses) and vineyards (*Vitis* sp.)

Dryland farming, with its main crops being corn and legumes such as *Cajanus cajan*, still occupy 95% of all arable grounds of Cape Verde (National Adaptation Programme of Action on Climate Change 2008–2012, 2007).

Worldwide research indicates the effectiveness of microorganisms in the pest control, in case of permanent tropical crops, such as citruses, bananas, coconut palms, mango, guava, papaya and pineapple (Dolinski and Lacey 2007).

The significance of entomopathogenic fungi (EPF) was mainly documented as a key element of an integral defence of plants against pests. There are 1,200 described species of EPF. 12–15 of these species are used throughout the world in pest control (Kowalska and Pruszyński 2007), in the form of around 120 registered biopesticides (Tkaczuk 2008).

Boczek and Lipa (1978) pointed out, that entomopathogenic representatives of Ascomycetes occur mostly in tropical and subtropical regions.

It has been proven, that the place of origin predicts the behaviour of the fungi tested afterwards in the biological pest control (Bidochka et al. 2001). That is why the usage of local strains may con-

tribute to a more effective pest control, especially in cases, where exotic organisms have not yield any satisfying results.

In these studies, the presence of EPF in soil samples collected in agricultural and reforested areas of Santiago and Fogo was analyzed.

MATERIAL AND METHODS

Soil samples

The soil samples were collected in November and December 2011 from two islands of the Cape Verde archipelago. Seven localities were visited on the islands of Santiago (63.6%) and four on Fogo (36.4%).

44 soil samples were collected (four samples in each location). In each location, 2 kg of soil was collected at four points around the plant and mixed to obtain homogeneous sample. The samples were collected from 15 cm below the surface, previously having removed the plant waste.

The soil was sampled in three different types of habitat: reforested areas (27.3% of localities), irrigated agricultural land (54.5%) and rainfed agriculture (18.2%).

Reforested areas are highlands with a steep decline. The highlands of Santiago are mainly covered in *Jacaranda mimosifolia*, *Lantana camara* and eucalyptuses. In Fogo, however, *Cupressus* sp., *Acacia* sp., *Pinus* sp. and *Eucalyptus* sp. stand out.

Irrigated agricultural land is covered with perennial crops such as *Musa* spp., *Mangifera* sp., *Citrus* sp., *Vitis* sp.

Rainfed areas represent agricultural land with perennial crop of pigeon pea *Cajanus cajan*.

The soil samples were transported in clear plastic bags to the laboratory of the National Institute for Research and Agricultural Development (INIDA) located in São Jorge dos Órgãos (Santiago island) where they were sieved through a 3 mm mesh.

The percentage of sand in the soil samples was determined by the Analytical Laboratory of Soil, Water and Plants (LASAP) located in São Jorge dos Órgãos.

Isolation and identification of EPF

The soil samples were studied in the laboratory for the presence of EPF using the Galleria bait method (Zimmermann 1986). The last larval instar of *Galleria mellonella* (L., 1758) was used.

The larvae of *G. mellonella* (Lepidoptera: Pyralidae) were originated in laboratory in INIDA. They were produced based on an artificial diet according to the recommendations of Dr. Charles Waturu Nderito (2010).

A total of 455 larvae of *G. mellonella* was buried in 52 sterile closed plastic containers. Each box carried 400 g of soil and four larvae or 1,200 g of soil and 12 larvae. The containers were incubated at 25°C ± 1°C for 30 days.

The mortality of larvae was recorded three times a week. The dead larvae were sterilized on the surface with 5% sodium hypochlorite and 70% alcohol followed by two washes in sterile, distilled water and kept separately for four days in moist chambers at 25°C. The fungi developed on the cadavers were subsequently cultivated for seven days in PDA medium

in the dark and identified microscopically using taxonomic keys described by Barnett and Hunter (1987) and Watanabe (2009).

In order to statistically analyze the results, the SPSS programme was used.

RESULTS AND DISCUSSION

The mortality of larvae of *G. mellonella* introduced into soil samples reached 40.5%.

EPF were found in soil originate from Cape Verde. 57.8% of the collected soil samples contained EPF.

Besides EPF, in 37.7% of samples the presence of other potential entomopathogenic organisms such as rhabditid nematodes was noted. In 4.5% of soil samples no potential natural enemies were found.

EPF were represented by isolates of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae). Each species was isolated from 34.6% of soil samples (Fig. 1). The occurrence of *Beauveria* and *Metarhizium* in the soil from the islands of Santiago and Fogo confirms the cosmopolitan character of the aforementioned species of fungi (Meyling and Eilenberg 2007, Sánchez-Peña et al. 2011).

In addition, in 50.0% of samples *Fusarium* spp. was found. 19.2% of soil samples contained fungi with unsporulated mycelium (Fig. 1).

From some of the samples, two or three different species of fungi were isolated. 7.7% of samples contained both aforementioned species of EPF.

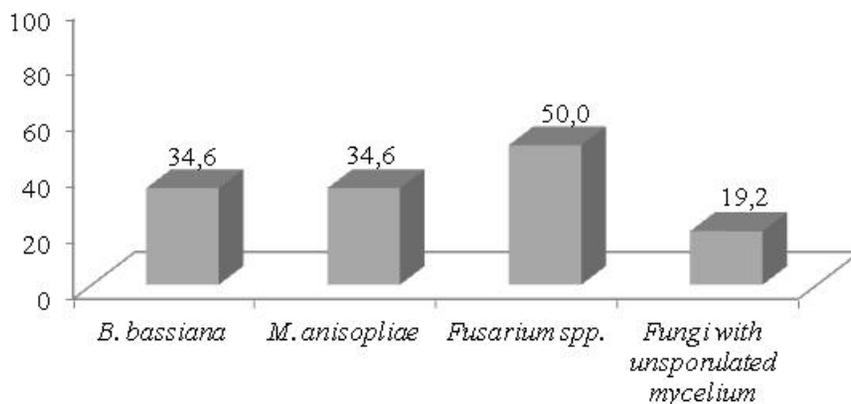


FIGURE 1. Percent of soil samples from Cape Verde containing fungi (November and December 2011)

The fungus *B. bassiana* was found on the island of Fogo (Table 1), in soil samples taken from perennial crops, represented by vineyards (75.0% of samples) and pigeon pea *C. cajan* cultivated in rainfed areas (85.7%).

of adult weevils *Cosmopolites sordidus* (Coleoptera: Curculionidae) found on banana plantations in Santiago were infected by *B. bassiana* (Nascimento, not published). This fact may indicate the presence of endophyte strains of

TABLE 1. Distribution of fungi in soil of different habitats on the Santiago and Fogo islands in Cape Verde, 2011

Fungal species	Percent of samples containing fungi				
	Reforestation		Irrigated agricultural land		Rainfed areas (Fogo)
	Santiago	Fogo	Santiago	Fogo	
<i>Beauveria bassiana</i>	0.0 a	0.0 a	0.0 a	75.0 b	85.7 b
<i>Metarhizium anisopliae</i>	0.0 a	0.0 a	40.0 ab	100.0 b	14.3 a
<i>Fusarium spp.</i>	50.0 a	66.7 a	40.0 a	50.0 a	57.1 a
Fungi with unsporulated mycelium	50.0 a	33.3 a	30.0 a	0.0 a	0.0 a

Values in lines followed by the same letters are not significantly different.

According to Tkaczuk (2008), cultivating plants from family *Fabaceae* favours the occurrence of EPF. But Tkaczuk's results obtained in Poland indicated a dominance of *M. anisopliae* in soils, where *Fabaceae* were cultivated.

There was no recorded presence of *B. bassiana* in the soil of Santiago. Earlier studies, however, showed that 5%

B. bassiana associated with banana crops (Dolinski and Lacey 2007).

A significant Pearson's correlation was shown between the presence of *B. bassiana* in soil samples and the presence of sand ($r = 0.647$; $p < 0.01$). On average, there was more sand in samples originate from Fogo than there was in samples from Santiago. Marjańska-

-Cichoń et al. (2005) pointed out, that sandy soil is richer in entomopathogenic fungi than argillaceous soil. But in sandy soil from Poland *M. anisopliae* was dominating.

The presence of the species *Metarhizium* was confirmed in all soil samples collected on the island of Fogo on the vine plantation and in 14.3% of soil planted with pigeon pea. On the island of Santiago the presence of *M. anisopliae* (40.0%) was related to banana plantations. All of these terrains had an inclination of no more than 25%.

A significant Pearson's correlation ($r = -0.714$; $p < 0.01$) was found between the presence of *M. anisopliae* and the inclination of the terrain.

The EPF were isolated from soil samples collected from lots occupied by orchards, vine and *C. cajan*. These perennial crops create beneficial circumstances for the survival of EPF, more so given the stability of the environmental conditions and, consequently, the presence of potential hosts, as mentioned by Chandler et al. (1997) and Tkaczuk (2008).

There were no EPF in the soil samples collected in reforested areas in Santiago and Fogo (Table 1). The reforested areas of Cape Verde are located in steeply inclined terrains and suffer from strong water erosion during the rainy season. For the record, these studies were conducted at the end of the rainy season.

The results of the studies are consistent with the results of Sánchez-Peña et al. (2011), who showed, that wooded areas are not always a source of natural enemies. On the other hand, Quesada-Moraga et al. (2007) isolated both *M. anisopliae* and *B. bassiana* from soil

originate from wooded areas in Spain. In Poland, in the soil from forested habitats the *B. bassiana* was definitely dominating (Tkaczuk 2008).

The presence of a *Fusarium* species was frequently noted (40.0–66.7%) in soil samples collected on both islands (Table 1).

The common practice of isolating *Fusarium* from soil samples using the Galleria bait method should be brought to attention. As Wenda-Piesik (2011) points out, *Fusarium* is an example of a phytopathogenic fungus also able to induce lethal reaction in insects. The dualistic properties of the various species of *Fusarium* are the subject of studies in the biological control of mosquitoes and nematodes *Radopholus similis*. Dolinski and Lacey (2007) assessed the potential of *Fusarium* spp. in the biological control of *C. sordidus*.

It is to be noted, that the survival and pathogenicity of EPF depends on various abiotic factors. The conidia of some of the isolates of *B. bassiana* lose their pathogenicity after three hours of insolation (Kowalska and Pruszyński 2007). Therefore, the usage of native entomopathogens in the biological control of different pests may be profitable. Native isolates are adapted to the local climatic conditions, as well as to the local entomofauna (Tangchitsomkid and Sontirat 1998, Dolinski and Moino Jr. 2006), which is why they are more effective against pests than introduced isolates.

The EPF are used, on a large scale, all over the world in the biological control of various pests of crops, such as aphids, locusts, trips, whiteflies (Cavalcanti 2006). The development of plant protection in the Cape Verde should follow the

example and investigate the influence of isolated strains of *B. bassiana* and *M. anisopliae* on pests occurring on the islands.

CONCLUSIONS

The occurrence of *B. bassiana* and *M. anisopliae* in agricultural land of Cape Verde was confirmed. The presence of *B. bassiana* was recorded in the soil with higher sand content, while *M. anisopliae* occurred on less inclined fields.

The frequent presence of *Fusarium* spp. drew attention and should be studied more.

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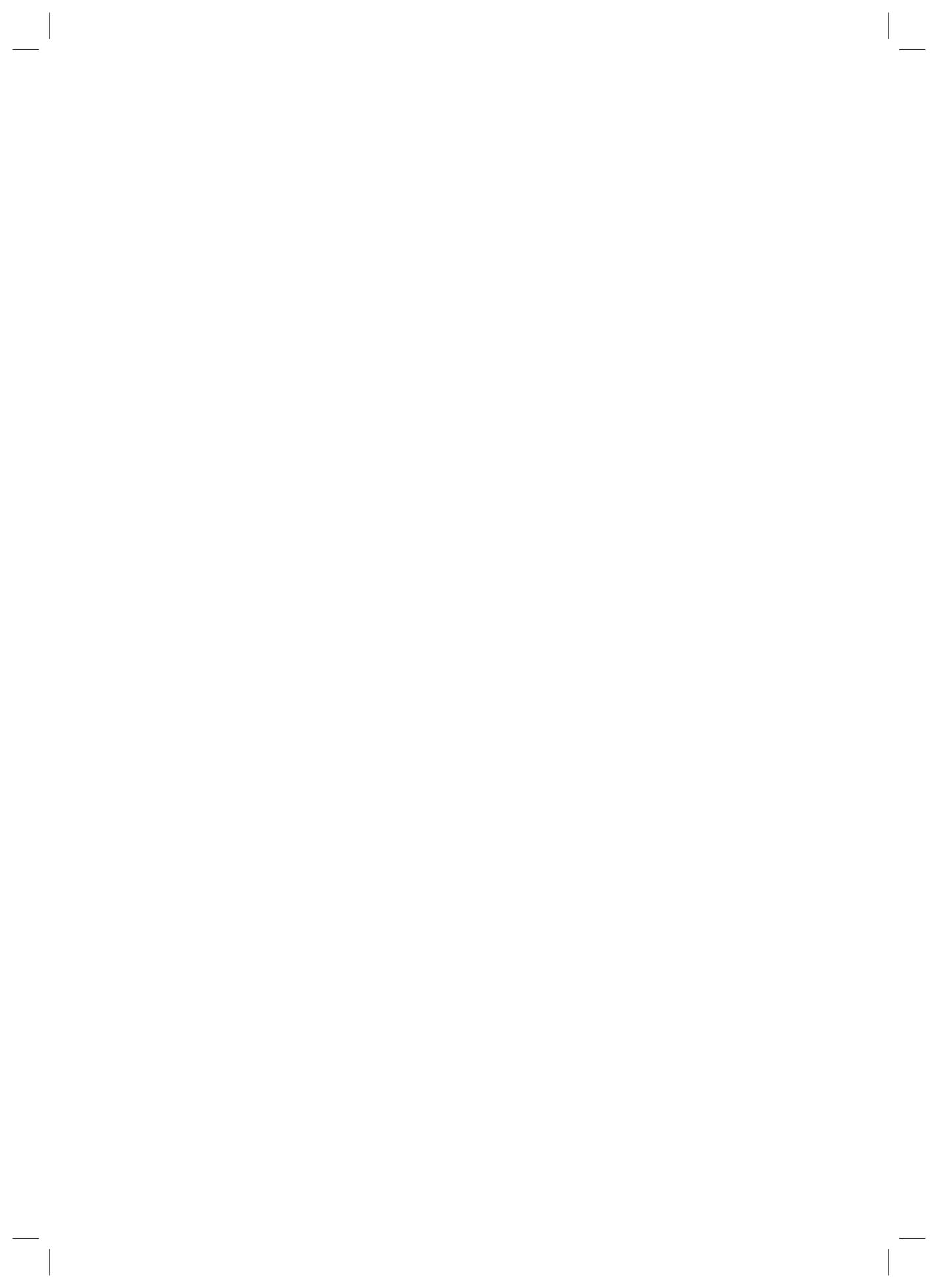
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Streszczenie: Występowanie grzybów owadobójczych w glebach wysp Santiago i Fogo (Republika Zielonego Przylądka). Gleba jest siedliskiem wielu grzybów owadobójczych (EPF) na całym świecie. Do izolowania EPF z prób glebowych zastosowano metodę owadów pułapkowych. Powszechność występowania EPF na terenach rolnych została potwierdzona niniejszymi badaniami, w wyniku których stwierdzono, że gleby Republiki Zielonego Przylądka są siedliskiem EPF, takich jak *Beauveria bassiana* i *Metarhizium anisopliae*. Obecność *B. bassiana* zarejestrowano w glebie wyspy Fogo a występowanie *M. anisopliae* zarówno na wyspie Fogo, jak i Santiago. Nie stwierdzono EPF na terenach zalesionych. Statystycznie więcej prób glebowych zawierających *B. bassiana* pochodziło z terenów pokrytych uprawami roślin wieloletnich, przy czym istotnym czynnikiem wpływającym na obecność grzyba była zawartość piasku w glebie. *M. anisopliae* był natomiast obecny na terenach o mniejszym nachyleniu terenu. *Fusarium* spp. był obecny w połowie przebadanych prób glebowych. Izolowanie EPF rodzimych z późniejszym ich zastosowaniem w krajach rozwijających się może w istotny sposób przyczynić się do skutecznego ograniczenia liczebności populacji szkodników w uprawach.

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Effect of rearing system and gender on histological profile of chicken breast and leg muscles in hybrid (Cobb×Zk)

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Abstract: *Effect of rearing system and gender on histological profile of chicken breast and leg muscles in hybrid (Cobb×Zk).* The experiment was conducted on 930 slow-growing chickens from the crossing of a Cobb male and Greenleg Partridge female. The chicks were randomly assigned to two groups: control (BW) which did not have access to a free run and the experimental group (W) using the grassy runs from 4 weeks of age. The experiment showed a statistical effect of sex on breast ($P \leq 0.01$) and leg ($P < 0.05$) muscle fiber diameter in Cobb×Zk hybrid roosters. There were no significant gender-dependent differences in the surface area of the muscles tested. No effect was either reported of the rearing system on the histological picture of breast and leg muscles.

Key words: rearing system, broiler chickens, muscle fiber diameter

INTRODUCTION

Animal products are an essential component of a man diet. They are a source of balanced protein, they contain low levels of cholesterol and fat, which is at the same time rich in unsaturated fatty acids (Wangang et al. 2010). Poultry meat surpasses meat of slaughter animals in terms of nutritional value, because of a valuable source of balanced animal protein that is highly digestible and easy to assimilate. According to PN-65/A-82000,

meat constitutes “skeletal muscles and adherent adipose, connective and bone tissues, derived from carcasses, half carcasses or quarter carcasses of particular types of animals for slaughter”. The main components of skeletal muscle tissue are muscle fibers, which are highly specialized. The number, size and type of muscle fibers, their biochemical, physiological and histological characteristics may lead to changes in meat quality and sensory evaluation (Damez and Clerjon 2008). The chemical composition of skeletal muscle is 75% water, 19% protein, 0.5–8% lipids and 1% glycogen (Lefaucheur 2010). However, their main mass is formed by muscle fibers, which diameters fall within the range of 10 to 100 μm , and largely depend on the type of muscle fibers (Tumova and Teimouri 2009, Lefaucheur 2010).

Histological structure of muscles slightly varies depending on their functions (Klont et al. 1998). According to Remignon et al. (1995), the diameter of muscle fibers depends on their type and to great extent on genetic background. Other factors important in this respect include sex (Ozawa et al. 2000), age (Candek-Potokar et al. 1998), breed (Ryu

et al. 2008), physical activity (Karlsson et al. 1999) and rearing system (Castellini et al. 2002a and 2002b, Branciarri et al. 2009).

Nowadays consumers are becoming more aware of their needs and begin to pay attention to the welfare of the animals, making it one of the criteria when choosing products of animal origin. The research carried out on the British population clearly demonstrated that animal welfare was treated as a “very important” or “important” factor, which indirectly influences the suitability and sustainability of food (DEFERA 2011). For the most part it refers to both the health and food safety. According to Sundrum (2001), attention is increasingly drawn to the rearing system of birds, including the density per m² and access to free runs.

The aim of this experiment was to determine the effect of the rearing system and gender on the histological profile of breast and leg muscles in slow-growing Cobb×Zk chickens.

MATERIALS AND METHODS

The study was conducted at the Warsaw University of Life Sciences – SGGW, experimental field station in Wilanów-Obory. Experimental procedures were approved by the Ethics Commission (approval no. 27/2009 dated 16 April 2009). The study was conducted on 930 chickens from the cross of Cobb male and Greenleg Partridge female (Zk) reared for 63 days. Day-old chicks were randomly divided into two groups of 465 chicks each: BW (control) and W (experimental) in five repetitions each of 93 chicks. A differentiating factor was

the possibility to use grassy free runs from 4 weeks of age by the experimental group. In each group the runs were 3×5 m in size. Half of the run area was canopied. The run area was dry, sunny, with permeable soil, covered with perennial ryegrass *Lolium perenne* L. (40%), red fescue *Festuca rubra* L. (50%) and Kentucky bluegrass *Poa pratensis* L. (10%). The free run had an area with sand where birds could use sand baths. A four-phase feeding system was applied during the rearing with the use of starter, grower 1, grower 2 and finisher feed mixtures (Table 1). Birds had unrestricted access to both feed and water.

During the experiment after each week an individual body weight, food intake and death rate of chickens was checked. On the 63rd day of rearing, 24 females and 24 males were selected from each group with body weight about average for each sex in a group and specimens of breast and leg muscles were collected for histology. Samples in size of 0.5×0.5×1 cm were collected within 15 min since slaughter after appropriate exsanguination of the chickens and subsequently subjected to 24-hours fixation. The samples were then washed in ethanol to remove the fixing agent and dehydrated by a series of increasing ethyl alcohol concentrations. Dehydrated samples were saturated with paraffin. Paraffin saturation was carried out in the incubator at the melting point of paraffin. Saturation duration was adapted to muscle samples collected and amounted to a few hours. Paraffin blocks were formed after completion of the saturation process. Microtome Leica RM 2265 (Leica Microsystems, Nussloch, Germany) was used to cut paraffin sections. Muscle

TABLE 1. Feed mixture composition and nutritional value according to producer's

Specification	Starter (1–11)	Grower I (12–24)	Grower 2 (25–37)	Finisher (38–63)
Content (%)				
Corn	10.00	11.40	10	10.00
Wheat	53.00	55.00	59.60	60.80
Soybean meal	30.60	27.40	23.20	21.60
Feeding limestone	1.19	1.20	1.11	0.97
Sodium bicarbonate	0.20	0.14	0.14	0.16
NaCl	0.24	0.28	0.28	0.26
Stimulator	0.01	0.01	0.01	0.01
Dicalcium phosphate	1.18	0.78	0.70	0.64
Soybean oil	2.10	2.40	3.60	4.40
Methionine 84% calcium salt	0.48	0.42	0.36	0.28
Lysine	0.36	0.34	0.36	0.28
Threonine	0.14	0.13	0.14	0.10
Premix C196 PX05802 0.5%	0.50	0.50	0.50	0.50
Nutritional value				
ME (kcal)	2990.20	3047.19	3125.72	3217.10
Fat	3.67	4.00	5.14	5.92
Protein	21.99	20.78	19.26	18.51
Fiber	3.60	2.55	2.45	2.41
Ash	5.83	5.35	4.96	4.67
Lysine	1.38	1.28	1.19	0.97
Methionine + cystine	1.08	1.01	0.92	0.76
Available phosphorus	0.45	0.38	0.36	0.35

provided per kilogram of diet: STARTER: vitamin A 11.00 K UL; organic phosphorus 0.59%; calcium 0.98%; phosphorus available 0.45%; calcium chloride 0.24%; sodium 0.15%; chlorine 0.27%; potassium 0.90%; magnesium 0.17%; manganese 142.32 mg; copper 31.59 mg; selenium 0.41 mg; iron 191.51 mg; sulfur 0.34%; zinc 116.80 mg; lysin1.36%; methionine 0.31%; GROWER I vitamin A 11.00 K UL; organic phosphorus 0.51%; calcium 0.87%; phosphorus available 0.38%; calcium chloride 0.28%; sodium 0.15%; chlorine 0.29%; potassium 0.85%; magnesium 0.16%; manganese 141.84 mg; copper 30.82 mg; selenium 0.41 mg; iron 174.55 mg; sulfur 0.32%; zinc 115.03 mg; lysin1.26%; methionine 0.30%; GROWER II vitamin A 11.00 K UL; organic phosphorus 0.48%; calcium 0.81%; phosphorus available 0.36%; calcium chloride 0.28%; sodium 0.15%; chlorine 0.30%; potassium 0.77%; magnesium 0.16%; manganese 141.40 mg; copper 30.21 mg; selenium 0.40 mg; iron 165.98 mg; sulfur 0.30%; zinc 113.79 mg; lysin1.17%; methionine 0.28%; FINISHER vitamin A 11.00 K UL; vitamin D3 3.00 K UL; vitamin E 40.00 mg; organic phosphorus 0.73%; calcium 0.35%; calcium chloride 0.26%; sodium 0.15%; chlorine 0.27%; potassium 0.74%; magnesium 0.15%; manganese 140.80 mg; copper 29.92 mg; selenium 0.40 mg; iron 159.92 mg; sulfur 0.28%; zinc 113.14 mg; lysin1.06%; methionine 0.27%;

cross sections had the thickness of 5 μm . Standard H&E staining was performed. The diameter and area of 200 muscle fibers was measured in each slide using a Nikon Ellipse E200 light microscope equipped with a Nikon DS-Fi2 camera and COOL view 2.7 software.

The results were analyzed statistically using the analysis of variance, calculated by least squares method with statistical software SPSS 19.0 GB (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Results of the analysis of breast muscle microstructure in chickens are shown in Tables 2 and 3. The diverse diameters of muscle fibers of breast muscles of the birds were to a greater extent correlated with gender than rearing system. The diameter of muscle fibers increases with the age of birds (Branciari et al. 2009). According to Sobolewska et al. (2011), in broiler chickens reared to the 35th day, the most intense increase in muscle fiber diameter was between the 8th and 21st day of rearing. Breast muscle fibers fully developed not before the 56th day. According to Papinaho et al. (1996) and Geyukouku et al. (2005), the average fiber thickness of *m. pectoralis major*, *m. biceps femoris*, *m. extensor hallucis longus*, and *m. gastrocnemius* is 60.0, 51.6, 59.8 and 60.45 μm , respectively. Fast-growing chickens have a much greater diameter of muscle fibers as compared to the slow-growing chickens (Khoshooi et al. 2013).

In the current experiment, the majority of muscle fibers in the cross sections had a clear shelving structure, and

TABLE 2. The influence of gender on the diameter and area of chickens muscle fibers in hybrid (Cobb×Zk)

Gender	Diameter (μm)		Area (μm^2)	
	mp	mn	mp	mn
Roosters	63.02 ^A	50.82 ^A	1401.54	1142.09
Hens	48.05 ^B	45.69 ^B	955.29	808.77
SE	3.44	3.66	119.71	113.93

mp – breast muscle, mn – leg muscle; ^{A,B}Means with the different subscripts differ significantly at $P \leq 0.01$.

TABLE 3. The influence of rearing system on the diameter and area of chickens muscle in hybrid (Cobb×Zk)

Rearing system	Diameter (μm)		Area (μm^2)	
	mp	mn	mp	mn
BW	54.09	52.32	1265.32	1100.50
W	56.98	44.18	1091.51	850.35
SE	4.76	3.38	150.92	123.70

mp – breast muscle, mn – leg muscle.

individual giant fibers could be distinguished, the diameter of which, according to Dransfield and Sosnicki (1999), is generally about three times greater than of the normal fibers. The hypertrophy of muscle fibers affects mainly fast-growing chickens and results from intense selection (Brocka et al. 1998, Guernec et al. 2003, Lefaucheur et al. 2010). Study by Miraglia et al. (2006) showed that the proportion of giant fibers in the *pectoralis major* in slow-growing chickens was 0.56%, in the fast-growing 3.17%, while in *ileotibialis lateralis* it was 1.70 and 3.18%, respectively. Intense muscle growth is associated with an increase in thickness and the surface of muscle fibers, rather than their number.

Our experiment showed a statistical effect of gender on breast ($P \leq 0.01$) and leg ($P < 0.05$) muscle fiber diameter in male of Cobb×Zk hybrid (Table 2). They had a larger diameter in both breast and leg muscles (63.02 and 50.82 μm , respectively) compared to female (48.05 and 45.69 μm). Khoshoo et al. (2013) studied the effect of gender on the diameter of breast muscle fibers and demonstrated that both slow-growing and fast-growing male had a larger diameter of muscle fibers. Biesiada-Drzazga et al. (2006) demonstrated in geese that both breast and leg muscles of females had a greater diameter of muscle fibers. However Mobini (2013) showed no effect of gender on the diameter of the muscle fibers.

There were no significant gender-dependent differences in the surface area of the muscles tested. However males had a greater surface area of muscle fibers in both breast and leg muscles (1,401.54 and 1,142.09 μm^2 , respectively) compared to females (955.29 and 808.77 μm^2). The largest area was observed in breast muscles of males and the smallest in females leg muscles. The study by Scheuermann et al. (2004) clearly showed that gender had an influence on the surface area of the breast muscle. This trend continued on 7th, 21st and 35th day of rearing at a significance level of $P < 0.05$. According to Choi and Kim (2008), differences in fiber number and size are primarily under the control of sex hormones. Differences in fibers of males and females can arise from hormonal action if differences in androgen hormones are at a sufficiently high level during periods of prenatal fiber formation.

There was no significant effect observed of the rearing system on the histo-

logical picture of breast and leg muscles (Table 3). However birds that could use free runs had a larger diameter of breast muscles (56.98 μm) and a lesser diameter of leg muscles (44.18 μm) compared to birds that did not benefit from grassy free runs (54.09 and 52.32 μm , respectively). Sowińska (2002) either did not identify the influence of the rearing system on skeletal muscle morphology. Polak et al. (2010) compared *m. pectoralis* and *m. gastrocnemius* in 215 Anak Titan and Isa chickens and showed that fiber size was affected by the rearing system. In general, in birds of both lines, the fiber diameter of *m. pectoralis* was smaller in groups with outdoor access ($P < 0.01$).

Birds from the control group had a greater surface area of muscle fibers in both breast and leg muscles (1,265.32 and 1,100.50 μm^2 , respectively) compared to chickens from the experimental group (955.29 and 808.77 μm^2). The opposite trend was shown in the study by Branciaro et al. (2009) where they compared an impact of the rearing system and genotype on muscle fiber area. These authors showed that the fast-growing chickens (Ross) reared under conventional conditions had the largest surface area of muscle fibers. The opposite trend was observed in the case of slow-growing chickens where the fiber surface area was larger than in the conducted experiment. In the conventional rearing it ranged from 955 to 1,610 μm^2 and from 1,272 to 2,396 μm^2 , for breast and leg muscles, respectively. Whereas in the organic rearing system, the fiber surface area ranged from 908 to 1,699 μm^2 and from 1,758 to 3,006 μm^2 for breast and leg muscles, respectively.

CONCLUSIONS

The experiment showed a statistical effect of gender on breast ($P \leq 0.01$) and leg ($P < 0.05$) muscle fiber diameter in Cobb×Zk hybrid roosters. There were no significant gender-dependent differences in the surface area of the muscles tested. No effect was either confirmed of the rearing system on the histological picture of breast and leg muscles. The increase in thickness of the muscle fibers could have influence on tenderness and juiciness.

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Streszczenie: *Wpływ systemu utrzymania oraz płci na profil histologiczny mięśni piersiowych i nóg kurcząt mieszańca Cobb×Zk.* Doświadczenie przeprowadzono na 930 kurczętach wolno rosnących pochodzących z krzyżowania koguta Cobb oraz kury zielononóżki kuropatwianej. Pisklęta losowo przydzielono do dwóch grup: kontrolnej (BW) niemającej dostępu do wybiegu oraz grupy doświadczalnej (W), korzystającej z trawiastych wybiegów począwszy od 4. tygodnia życia. W przeprowadzonym doświadczeniu wykazano statystyczny wpływ płci na średnicę włókien mięśni piersiowych ($P \leq 0,01$) oraz nóg ($P < 0,05$)

kogutów mieszańca Cobb×Zk. Nie stwierdzono istotnych różnic dla wartości pola powierzchni badanych mięśni w zależności od płci. Nie wykazano wpływu systemu utrzymania na wyniki obrazu histologicznego mięśni piersiowych i nóg.

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Influence of the system of rearing on cholesterol level and its fraction in blood serum of slow-growing chickens

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Abstract: *Influence of the system of rearing on cholesterol level and its fraction in blood serum of slow-growing chickens.* The experiment was carried out on 936 slow-growing chickens from the crossbreed of Cobb cock with Greenleg Partridge hen. Chicks were randomly divided into two groups: controlled group (BW) with no access to the field and experimental group (W) with access to grass fields since their fourth week of life. In blood serum overall cholesterol, lipoproteins with high density (HDL), triacylglycerols (TG) level was marked. The concentration of lipoproteins with low density was calculated while using the Friedewald et al. formula (1972). No statistically significant influence of sex and system of breeding on cholesterol and its fractions level in blood serum of crossbreed chickens was observed.

Key words: broiler chickens, slow-growing chicken, blood

INTRODUCTION

In Poland most poultry meat production is based on fast growing chickens kept in an intensive system (Polak et al. 2010). The fast growth rate while the whole body develops at different rates very often results in numerous dysfunctions such as: syndrome of sudden heart death, ascites, limb dysfunctions – which, consequently, have a bad influence on chicken health and most of all – according to consumers – on the meat quality (Julian 2005, Olkowski et al. 2008). Society is

more and more interested in conditions in which domestic animals are kept. In highly developed countries for many years a rising interest in a healthy diet has been noticed, with paying special attention to comfort of life of domestic animals and high standards of animal welfare in order to achieve high quality products (Sundrum 2001). Morphological and biochemical blood tests are of great importance in animal treatment, it is one of basic measures used in condition assessment, for example, of a cat or a dog (Kliszcz 2010). While looking at birds, unfortunately, there are only some physiological norms available in the literature, insufficient to form a complete picture of biochemical and hematological indicators or the amount of lipid compounds in blood serum of birds (Krasnodebska-Depta and Koncicki 2000, Mazurkiewicz 2005, Gryzińska et al. 2010).

A lot of various factors may influence levels of biochemical and hematological indicators in blood, among other things: sex, age, species, race, diet, physiological condition and rearing system (Mazurkiewicz 2005). Blood plays a distinct role in a proper function of animal organisms. It takes part in transporting nutrients, thermoregulation, homeostasis,

defense reactions (Elagib et al. 2008). In blood, besides morphological elements, we can find a lot of other chemical compounds which take part in a proper body function. Cholesterol and triacylglycerol's are transported in body fluids as lipoprotein particles which are classified according to their density, chylomikrons, lipoproteins with very low density (VLDL), lipoproteins with intermediate density (IDL), lipoproteins with low density (LDL), lipoproteins with very high density (HDL). The basic function of lipoproteins is transporting lipids from the place of their origin to their destination.

The purpose of the conducted research was to establish the influence of breeding system on the level of cholesterol and its fractions in a blood serum of slowly growing chickens.

MATERIAL AND METHODS

The research was conducted on an experimental farm of University of Life Sciences RZD Wilanów-Obory in 2011. Procedures used in the research were in accordance with the ones established by the ethical commission 27/2009 on 16 April 2009. The research was conducted on 936 chickens from crossbreeding of Cobb male with Greenleg Partridge kept until their 63rd day of life. One-day chickens were randomly divided into two groups: BW (controlled) and W (experimental) in five repetitions each. An indicator which diversified the groups was the ability to use grass fields since the 4th week of life: the experimental group (W). The fields were 3 m wide and 5 m long. Half of the field area was under roof. The field was dry with per-

meable soil, full of sunlight, with plants: *Lolium perenne* L. (40%), *Festuca rubra* L. (50%) and *Poa pratensis* L. (10%). There was also a sandy spot in the field for the birds to take sand baths. During the period of breeding a four-step system of feeding was implemented while using food blends starter, grower 1, grower 2 and finisher (Table 1). Birds had an unlimited access to food and water.

During the experiment after each week an individual body weight, food intake and death rate of chickens was checked. On the 63rd day of breeding there were chosen from each group 24 cocks and 24 hens with body weight about average for each sex in a group. Blood samples were taken from wing veins using sterilized syringes and needles. After 30–60 min since blood taking, the blood samples were spun for 10 min with 3,000 of spins per min. In the obtained blood serum the concentration of overall cholesterol, lipoproteins of high density (HDL), triacylglycerol's (TG) was marked. The concentration of lipoproteins with low density was calculated while using the Friedewald et al. formula (1972).

$$LDL = \frac{CHOL - TG}{2.2 - HDL} \text{ (mmol/l)}$$

For marking overall cholesterol, HDL, triglycerides commercial sets by CORMAY Company were used.

The obtained results were worked out statistically while using variance analysis of the smallest squares method in a statistic program SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Values of the examined traits as given in the tables and standard errors of the means (SEM).

TABLE 1. Feed mixture composition and nutritional value according to producer's

Specification	Starter (1–11)	Grower I (12–24)	Grower 2 (25–37)	Finisher (38–63)
Content (%)				
Corn	10.00	11.40	10.00	10.00
Wheat	53.00	55.00	59.60	60.80
Soybean meal	30.60	27.40	23.20	21.60
Feeding limestone	1.19	1.20	1.11	0.97
Sodium bicarbonate	0.20	0.14	0.14	0.16
NaCl	0.24	0.28	0.28	0.26
Stimulator	0.01	0.01	0.01	0.01
Dicalcium phosphate	1.18	0.78	0.70	0.64
Soybean oil	2.10	2.40	3.60	4.40
Methionine 84% calcium salt	0.48	0.42	0.36	0.28
Lysine	0.36	0.34	0.36	0.28
Threonine	0.14	0.13	0.14	0.10
Premix C196 PX05802 0.5%	0.50	0.50	0.50	0.50
Nutritional value				
ME (kcal)	2 990.20	3 047.19	3 125.72	3 217.10
Fat	3.67	4.00	5.14	5.92
Protein	21.99	20.78	19.26	18.51
Fiber	3.60	2.55	2.45	2.41
Ash	5.83	5.35	4.96	4.67
Lysine	1.38	1.28	1.19	0.97
Methionine + cystine	1.08	1.01	0.92	0.76
Available phosphorus	0.45	0.38	0.36	0.35

Provided per kilogram of diet: STARTER: vitamin A 11.00 K UL; organic phosphorus 0.59%; calcium 0.98%; phosphorus available 0.45%; calcium chloride 0.24%; sodium 0.15%; chlorine 0.27%; potassium 0.90%; magnesium 0.17%; manganese 142.32 mg; copper 31.59 mg; selenium 0.41 mg; iron 191.51 mg; sulfur 0.34%; zinc 116.80 mg; lysin 1.36%; methionine 0.31%; GROWER I vitamin A 11.00 K UL; organic phosphorus 0.51%; calcium 0.87%; phosphorus available 0.38%; calcium chloride 0.28%; sodium 0.15%; chlorine 0.29%; potassium 0.85%; magnesium 0.16%; manganese 141.84 mg; copper 30.82 mg; selenium 0.41 mg; iron 174.55 mg; sulfur 0.32%; zinc 115.03 mg; lysin 1.26%; methionine 0.30%; GROWER II vitamin A 11.00 K UL; organic phosphorus 0.48%; calcium 0.81%; phosphorus available 0.36%; calcium chloride 0.28%; sodium 0.15%; chlorine 0.30%; potassium 0.77%; magnesium 0.16%; manganese 141.40 mg; copper 30.21 mg; selenium 0.40 mg; iron 165.98 mg; sulfur 0.30%; zinc 113.79 mg; lysin 1.17%; methionine 0.28%; FINISHER vitamin A 11.00 K UL; vitamin D3 3.00 K UL; vitamin E 40.00 mg; organic phosphorus 0.73%; calcium 0.35%; calcium chloride 0.26%; sodium 0.15%; chlorine 0.27%; potassium 0.74%; magnesium 0.15%; manganese 140.80 mg; copper 29.92 mg; selenium 0.40 mg; iron 159.92 mg; sulfur 0.28%; zinc 113.14 mg; lysin 1.06%; methionine 0.27%.

RESULTS AND DISCUSSION

Production results in both groups during 63-day period of breeding are presented in Table 2. The influence of system rearing (with outdoor access) on the final body weight of chickens was observed. Rooster's with access to the fields had a distinctly larger ($P < 0.01$) body weight when compared to rooster's the from the controlled group. On the 63rd day of breeding cocks weighed on average 2,134 g and hens 1,684 g. The conducted statistic analysis did not show any significant differences in food intake or death rate. However, higher intake of food and higher death rate was noticed in the experimental group (W) – having the ability to use the fields (Table 2). Similar results were obtained in research by Castellini et al. (2002), Nielsen et al. (2003) and Fanatico et al. (2005).

The analysis of all known in literature biochemical tests of blood serum established that birds in comparison to other animal species have different values for those indicators. Only some physiological norms available in the literature are insufficient to form a complete picture of both biochemical and hematological in-

dicators. Moreover, we must pay attention to individual differences in single bird populations. In some cases an individual bird may be still placed within reference values even if it is sick (Piasecki and Krasoń 2012).

No statistically significant influence of sex or breeding system on cholesterol and its fractions level was observed in blood serum of slowly growing chickens (Table 3). The highest concentration of overall cholesterol was noticed in a blood serum of cocks with the ability to use the field (2.317 mmol/l). The growth tendency was observed for chickens from the experimental group (W) depending on their sex. The highest level of triacylglycerol's (0.867 mmol/l) was observed in the controlled group of chickens (BW). Brodacki et al. (2006) in their research did not observe any influence of a breeding system on overall cholesterol and triacylglycerol's level in a blood serum of turkeys. Kirkpınar et al. (2011) for broiler chickens and Gryzińska et al. (2010) in a blood serum of polar hens observed the influence of sex on cholesterol and level. Mostly cholesterol and its fractions level is influenced by various food additives. Research conducted by Dehkordi et al. (2010) proved significant differences in overall cholesterol and triacylglycerol's level in a group of chickens fed with a standard food blend. Toghyani et al. (2011) observed the influence of cinnamon, Traesel et al. (2011) the mixture of ethereal oils form oregano, salvia, rosemary and chili. Research conducted by Gryzińska et al. (2010) proved that with age overall cholesterol, triacylglycerol's and lipoproteins with low density (LDL) level decreases in blood serum. Just the opposite is for lipoproteins with

TABLE 2. Influence of the system of rearing on production results of broiler slow-growing chicken

Specification	Sex	BW (g)	FCR (%)	Mortality (%)
W	♂	2 171 ^A	2.34	3.52
	♀	1 688		
BW	♂	2 098 ^B	2.30	3.50
	♀	1 680		

BW – body weight; FCR – feed conversion ratio; A,B – means with the different subscripts differ significantly at $P \leq 0.01$.

TABLE 3. Cholesterol and its fractions and triglycerides in the blood serum of chickens slow-growing based on gender and rearing system (mmol/l)

Specification	Sex	CHOL	SEM	TG	SEM	HDL	SEM	LDL	SEM
W	♂	2.317	0.214	0.780	0.060	1.627	0.165	0.653	0.071
BW		2.140		0.867		1.433		0.667	
W	♀	2.307		0.737		1.607		0.667	
BW		2.037		0.747		1.393		0.610	

CHOL – cholesterol; TG – triglycerides; HDL – high-density lipoprotein; LDL – low-density lipoprotein.

high density (HDL) which concentration is the highest in blood of the youngest birds. Krasnodębska-Depta and Konwicki (2002) studied the influence of a short-term heat stress on some chosen biochemical indicators in turkey blood. They observed that after 6, 26 and 50 h of heat stress in a group of turkeys, a statistically significant increase of triacylglycerol's appeared. There was not observed any influence on overall cholesterol level. Wójcik et al. (2011) and Czech et al. (2012) proved that a longer before-slaughter transport has a significant influence on overall cholesterol concentration in blood serum of chickens.

CONCLUSIONS

The influence of system rearing (with outdoor access) on the final body weight of chickens was observed. In the conducted research no statistically significant influence of sex and system of breeding on cholesterol and its fractions level in blood serum of crossbreed chickens was observed.

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Streszczenie: *Wpływ systemu utrzymania na zawartość cholesterolu i jego frakcji w surowicy krwi kurcząt wolno rosnących.* Doświadczenie przeprowadzono na 936 kurczętach wolno rosnących pochodzących z krzyżowania koguta Cobb oraz kury Zielononóżki kuropatwianej. Pisklęta losowo przydzielono do dwóch grup: kontrolnej

(BW) niemającej dostępu do wybiegu oraz grupy doświadczalnej (W), korzystającej z trawia-
stych wybiegów począwszy od 4. tygodnia życia.
W surowicy oznaczono stężenie cholesterolu cał-
kowitego, lipoprotein o dużej gęstości (HDL),
triacyllogliceroli (TG). Zawartość lipoprotein
o małej gęstości (LDL) wyliczono na podstawie
wzoru Friedewald et al. 1972. Nie wykazano sta-
tystycznie istotnego wpływu płci oraz systemu
utrzymania na zawartość cholesterolu i jego frak-
cji w surowicy krwi kurcząt mieszańca.

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Studies on the potential use of entomopathogenic nematodes for biological control of animals in the stables

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Abstract: *Studies on the potential use of entomopathogenic nematodes for biological control of animals in the stables.* The study was aimed at selecting species and strains of entomopathogenic nematodes to be used in practical control of the housefly in stables, which should provide welfare of bred animals. Test insect, the housefly, and entomopathogenic nematodes of the family Steinernematidae and Heterorhabditidae were used in experiments. Laboratory strains of nematodes and those commercially available in Poland and in Europe were used in performed tests. Larvae, pupae and imagines of *M. domestica* were cultivated in the Institute of Organic Industry in Warsaw. Four groups were created for each nematode species. Not all nematode species and strains were equally pathogenic to houseflies.

Key words: biopreparations, entomopathogenic nematodes, housefly, animal welfare

INTRODUCTION

Excessive agricultural chemicalization leads to the degradation of the natural environment. Insecticides accumulate in plants, soil and ground waters and their remains are ingested by a consumer. They may pose a risk to human and animal life and health. Consequences after the contact with pesticides include the disturbances of nervous, respiratory and alimentary systems, skin wounds and even death.

The appearance of resistant pest races is an unfavourable effect of insecticide application. Insects often develop the resistance to more than one type of insecticides. Overuse of insecticides and application of substances from the same chemical group decreases their effectiveness from year to year. A disadvantage of chemical means for insect control is their broad spectrum of activity. They kill both harmful and beneficial organisms (Malinowski 2003).

Integrated method does not completely eliminate synthetic preparations. The term IPM was first introduced in 1959 by Stern. Chemical means should be applied only if the need arises (Stern et al. 1959).

The housefly is a synanthropic insect which makes problems in farm houses. The invasion of this insect stresses animals and causes economic losses. The housefly is a vector of viruses, bacteria, protozoans and fungi. Up to 6 million microorganisms may be found on its body. Its most intensive invasion takes place in the summer month (Ignatowicz 2000). Preventing invasions of this insect is troublesome, particularly when the interior of farm houses is permanently open to the external habitat. Therefore, breeders are advised to use several methods

of housefly control in order to obtain expected effects. Housefly control may be performed in several ways using physical, chemical and biological methods (Wojciechowska and Kamionek 2012). In this study entomopathogenic nematodes were used for this purpose.

Nematodes are used in the biological control of the populations of harmful insects. Control group consisted of housefly larvae with distilled water. Mortality and the extensity of infection of housefly larvae by various species and strains of Steinernematidae and Heterorhabditidae nematodes were determined.

MATERIAL AND METHODS

Study material

Insects (*M. domestica*)

Studies were carried out on the housefly *M. domestica*, a species common in farm houses (stables). Experimental insects were cultivated in the Institute of Organic Industry in Warsaw. Patented substratum prepared in the Institute was used to breed houseflies.

Entomopathogenic nematodes (EPNs) *Steinernema affinis*, *S. carpocapsae*, *S. feltiae*, *Heterorhabditis megidis*: Is, Ic WNZ /2009, biopreparations based on nematodes *S. feltiae*: Owinema (Oviplant), Entonem (Koppert), Nemaplus (E-nema) and biopreparations based on nematodes *Heterorhabditis bacteriophora*: Larvanem (Koppert) Nematop (E-nema) were used in this experiment.

Methods

Laboratory tests

Eleven species and strains of nematodes, including five commercial biopreparations were used in experiments on the housefly control in stables. Tests were made on Petri dishes 10 cm in diameter lined with a double layer of filter paper. Twenty housefly larvae were placed onto each dish and 5, 10, 20 or 50 invasive larvae of appropriate nematode species were instilled onto dish. Tests were made in three repetitions. Mortality was checked every 24 h for 5 days. The control consisted of larvae with 100 ml of water added instead of nematode suspension. After infection dead and live insects were counted and the former were dissected to check whether nematodes were the cause of their death (Figures 1 and 2).



FIGURE 1. Infected larvae of the housefly (Wojciechowska 2013)

STATISTICAL ANALYSIS

In the development of the results of the experiments used a using multivariate analysis of variance (ANOVA). It allows the inference the significance of



FIGURE 2. Nematodes visible in a dead larva (Wojciechowska 2013)

the impact factor of the test to the test parameter (extensiveness of infestation, the number of outlets). When we found a significant effect of a particular factor on a particular trait, with LSD test was evaluated, between which there are sig-

nificant differences in average ($p < 0.05$). Calculations made using the computer program Statgraphic.

RESULTS AND DISCUSSION

The highest mortality in performed tests was noted with the use of *S. feltiae* (Owinema, Nemaplus) and *S. carpocapsae*. The lowest mortality was noted after application of *S. riobrave*, *H. bacteriophora* (Larvanem and Nematop) and *H. megidis* Ic and Is. Most extensive infection was recorded for *S. carpocapsae*, *S. feltiae* (Owinema) and *S. feltiae* (Nemaplus). The lowest extensity was characteristic for *S. affine*, *S. intermedia*, *S. riobrave* and *H. megidis* Ic and Is (Tables 1 and 2, Fig. 3). The reasons

TABLE 1. Mortality and the extensity of infection of four-day larvae of *M. domestica* after application of various species and strains of Steinernematidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection	
		%	SD
<i>S. affine</i>	57 ^b	30 ^{bc}	3.67
<i>S. intermedia</i>	65 ^c	37 ^c	3.82
<i>S. carpocapsae</i>	80 ^e	70 ^{ef}	6.18
<i>S. riobrave</i>	55 ^b	42 ^{bd}	2.53
<i>S. feltiae</i> (Owinema)	90 ^g	75 ^g	6.58
<i>S. feltiae</i> (Nemaplus)	85 ^f	73 ^{fh}	6.26
<i>S. feltiae</i> (Entonem)	73 ^d	57 ^{de}	4.15

Different letters in columns denote significant differences at $p < 0.05$.

TABLE 2. Mortality and the extensity of infection of four-day larvae of *M. domestica* after application of various species and strains of Heterorhabditidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
<i>H. megidis</i> Ic	55 ^b	24 ^b	2.53
<i>H. megidis</i> Is	47 ^a	21 ^{ab}	1.17
<i>H. bacteriophora</i> (Nematop)	44 ^a	18 ^a	1.13
<i>H. bacteriophora</i> (Larvanem)	45 ^a	15 ^a	1.16

Different letters in columns denote significant differences at $p < 0.05$.

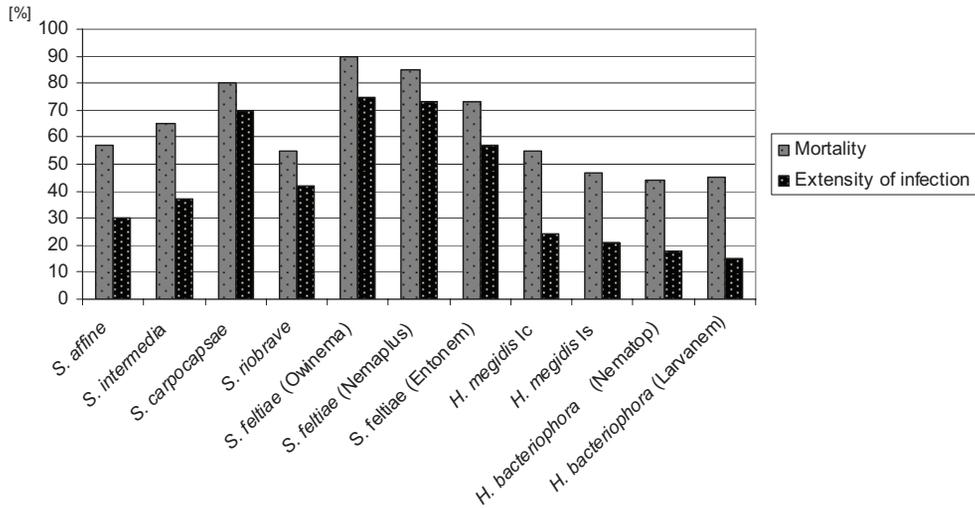


FIGURE 3. Mortality and the extensity of infection of four-day larvae of *Musca domestica*

of a higher extensity of infection in older larvae should be sought in the anatomy of natural openings, through which invasive larvae penetrate their body (Brzeski and Sandner 1974).

The highest mortality was noted after application of *S. feltiae* (Owinema, Nemaplus) and *S. carpocapsae*, the lowest – with *S. riobrave*, *H. bacteriophora* (Larvanem and Nematop) and *H. megidis* Ic and Is (Tables 3 and 4, Fig. 4).

The highest mortality was found in the case of *S. feltiae* (Owinema, Nemaplus) and *S. carpocapsae*, the lowest was obtained after application of *S. riobrave*, *H. bacteriophora* (Larvanem and Nematop) and *H. megidis*. Mortality and the extensity of infection were significantly lower in pupae than in housefly larvae. The former are immobile, which was probably the reason of their low mortality and extensity of infection (Tables 5 and 6, Fig. 5).

TABLE 3. Mortality and the extensity of infection of two-day larvae of *M. domestica* after application of various species and strains of Steinernematidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
<i>S. affine</i>	47 ^b	29 ^b	2.67
<i>S. intermedia</i>	39 ^c	25 ^c	2.82
<i>S. carpocapsae</i>	55 ^e	45 ^e	4.18
<i>S. riobrave</i>	45 ^b	24 ^b	2.53
<i>S. feltiae</i> (Owinema)	65 ^f	53 ^f	4.58
<i>S. feltiae</i> (Nemaplus)	60 ^d	49 ^{de}	4.26
<i>S. feltiae</i> (Entonem)	59 ^c	40 ^{cd}	3.15

Different letters in columns denote significant differences at $p < 0.05$.

TABLE 4. Mortality and the extensity of infection of two-day larvae of *M. domestica* after application of various species and strains of Heterorhabditidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
<i>H. megidis</i> 1c	20 ^b	15 ^b	2.53
<i>H. megidis</i> 1s	16 ^a	12 ^a	1.17
<i>H. bacteriophora</i> (Nematop)	12 ^a	10 ^{ab}	1.13
<i>H. bacteriophora</i> (Larvanem)	10 ^a	7 ^a	1.16

Different letters in columns denote significant differences at $p < 0.05$.

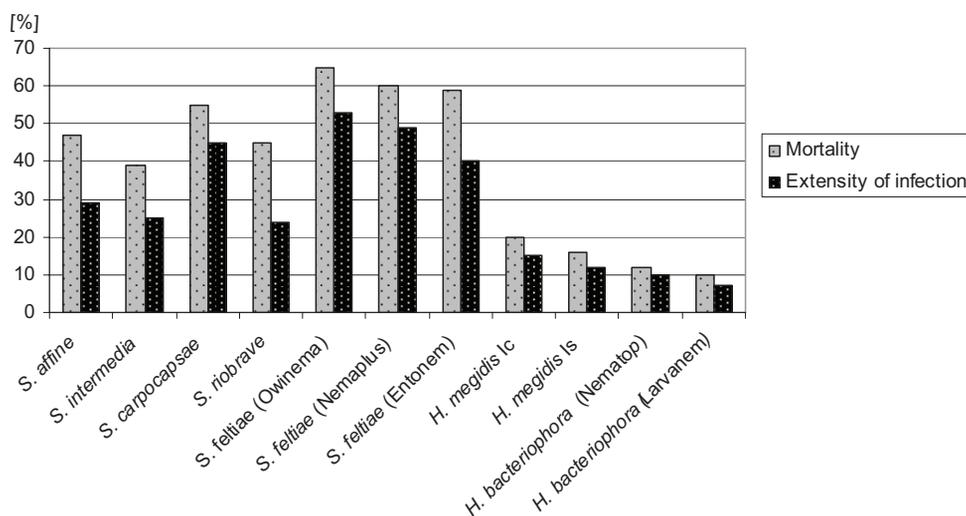


FIGURE 4. Mortality and the extensity of infection (%) of two-day larvae of *M. domestica*

TABLE 5. Mortality and the extensity of infection of *M. domestica* pupae after application of various species and strains of Steinernematidae (%). Initial dose of nematodes for insect – 50

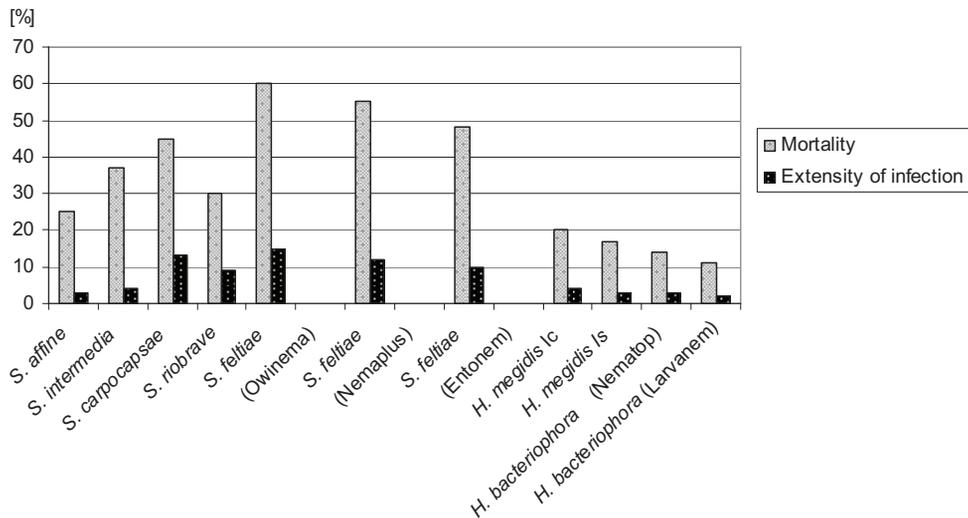
Nematode species	Mortality (%)	Extensity of infection (%)	SD
<i>S. affine</i>	25 ^b	3 ^{ab}	1.01
<i>S. intermedia</i>	37 ^c	4 ^{bc}	1.08
<i>S. carpocapsae</i>	45 ^d	13 ^{de}	1.16
<i>S. riobrave</i>	30 ^c	9 ^c	1.14
<i>S. feltiae</i> (Owinema)	60 ^e	15 ^e	2.63
<i>S. feltiae</i> (Nemaplus)	55 ^e	12 ^{de}	2.53
<i>S. feltiae</i> (Entonem)	48 ^d	10 ^d	1.17

Different letters in columns denote significant differences at $p < 0.05$.

TABLE 6. Mortality and the extensity of infection of *M. domestica* pupae after application of various species and strains of Heterorhabditidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
<i>H. megidis</i> 1c	20 ^b	4 ^b	1.01
<i>H. megidis</i> 1s	17 ^a	3 ^a	0.95
<i>H. bacteriophora</i> (Nematop)	14 ^a	3 ^a	0.95
<i>H. bacteriophora</i> (Larvanem)	11 ^a	2 ^a	0.95

Different letters in columns denote significant differences at $p < 0.05$.

FIGURE 5. Mortality and the extensity of infection of *M. domestica* pupae

The highest mortality in performed tests was demonstrated after application of *S. feltiae* (Owinema and Nemaplus) and *S. carpocapsae* and the lowest with the use of *S. riobrave*, *H. bacteriophora* (Larvanem and Nematop) and *H. megidis* (Tables 7 and 8, Fig. 6).

Nematodes of the families Steinernematidae and Heterorhabditidae are effective in controlling many species of harmful insects. Studies have long been performed in many countries on nematodes as a means used in biological methods for pest control. They found practical application in controlling dipterans

of the families Sciaridae and Phoridae in mushroom-growing cellars. The highest extensity of infection of *Lycoriella solani* Winnertz was found after application of *S. affinis*, *S. feltiae* (Nemaplus) and *S. feltiae* (Owinema). In the case of *Megaselia halterata* Wood the highest extensity was obtained with the use of *S. affinis*, *S. feltiae* (Nemaplus) and *S. feltiae* (Owinema) (Szynek-Basalyga 2002).

Commercial preparations based on entomopathogenic nematodes are produced in many countries. In Poland the production of biopreparation Owinema

TABLE 7. Mortality and the extensity of infection of *M. domestica* imagines after application of various species and strains of Steinernematidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
<i>S. affine</i>	8 ^b	0 ^{ab}	0
<i>S. intermedia</i>	9 ^c	0 ^{ac}	0
<i>S. carpocapsae</i>	12 ^d	1 ^{bd}	0.55
<i>S. riobrave</i>	4 ^a	0 ^a	0
<i>S. feltiae</i> (Owinema)	13 ^d	2 ^{bd}	0.75
<i>S. feltiae</i> (Nemaplus)	11 ^d	2 ^{bd}	0.71
<i>S. feltiae</i> (Entonem)	10 ^c	1 ^{bc}	0.48

Different letters in columns denote significant differences at $p < 0.05$.

TABLE 8. Mortality and the extensity of infection of *M. domestica* imagines after application of various species and strains of Heterorhabditidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
<i>H. megidis</i> Ic	7 ^b	0 ^{ab}	0
<i>H. megidis</i> Is	5 ^a	0 ^a	0
<i>H. bacteriophora</i> (Nematop)	8 ^b	0 ^{ab}	0
<i>H. bacteriophora</i> (Larvanem)	3 ^a	0 ^a	0

Different letters in columns denote significant differences at $p < 0.05$.

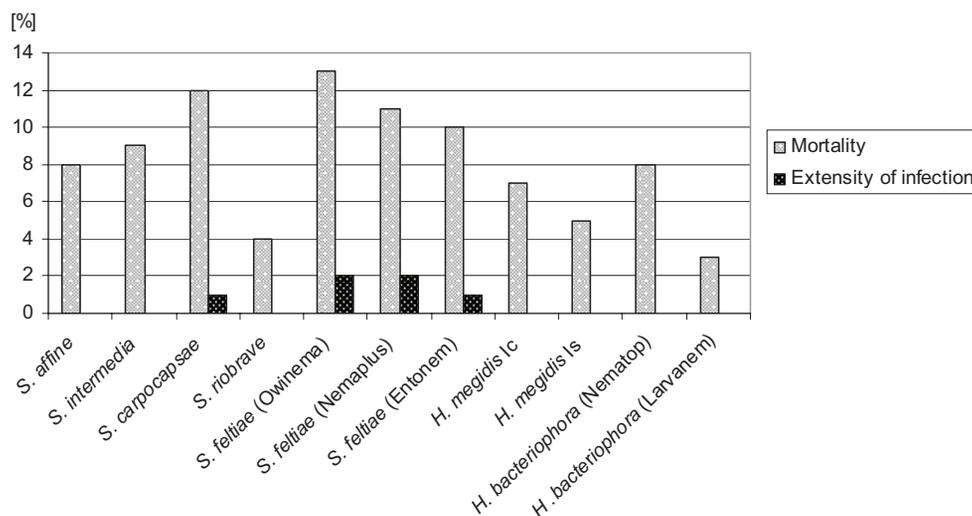


FIGURE 4. Mortality and the extensity of infection of *M. domestica* imagines

is based on *S. feltiae*. Biopreparations are considered safe in controlling pests in agriculture and animal breeding (Poinar 1979, Pezowicz 2005).

Unruh and Lacey (2001) used *S. carpocapsae* to control a fruit fly (*Carpocapsa pomonella* L.). Tomalak (2004) used *H. megidis* and *S. feltiae* against tree pests: the alder leaf beetle (*Agelastica alni* L.) and the oak flea beetle (*Haltica quercetorum* Foudras). This study showed that prepupae and pupae are sensitive to nematode infection, and that *H. megidis* was more effective than *S. feltiae*. The western flower thrips (*Frankliniella occidentalis*, Pergande) may also be controlled by nematodes (Borgemeister et al. 2002). In this case *Heterorhabditis* spp. was more pathogenic than *Steinernema* spp. Bednarek et al. (2002) controlled grubs of a cockchafer from the family Melolonthinae in forests. Also the pea leaf weevil (*Sitona lineatus* L.) is sensitive to nematodes (Jaworska and Ropek 1998). Mortality of these insects was 70–80%.

Insect species of different orders show differentiated susceptibility to nematodes. The easiest infected are the butterfly caterpillars. Beetles are resistant and the least mortality was found in dipteran populations (Dutky 1959).

M. domestica is an undesirable insect in stables since it harasses animals and carries diseases and parasites. Poland as a member of the EU should obey zoohygienic standards and norms. This includes also the control of houseflies.

In presented study, the tested nematode species and strains were more effective for four-day larvae of *M. domestica* (55–90% mortality) than for small, two-day larvae (39–65% mortality).

Pezowicz (2005) in her studies on the control of the lesser mealworm (*Alphitobius diaperinus* Panzer) showed that nematodes infected both young and older larvae. The extensity of infection of older larvae was, however, significantly higher. Higher extensity of infection of older larvae is an effect of natural openings, of the size of stigmas, through which the invasive larvae penetrate insect's body (Koppenhöfer et al. 2000). My studies revealed the same regularity – nematodes infected two-day larvae to a very small extent. According to Pezowicz (2005), nematodes penetrated all growth stages of the insect.

Dipteran larvae were controlled more effectively by nematodes of the family Steinernematidae than by those from Heterorhabditidae. The highest extensity of infection in laboratory tests was obtained after application of two nematode species *S. carpocapsae* and *S. feltiae* (Ovinema and Nemaplus). *S. feltiae* is also most effective in the control of other dipterans of the family Sciaridae in contrast with *S. carpocapsae* which showed much lower pathogenicity (Szyk-Basalyga 2002). My studies do not confirm this observation since the extensity of infection was 70%.

S. affinis oraz *H. megidis* exerted the weakest effect on *M. domestica*.

Performed studies demonstrated that *S. feltiae* is the most effective nematode species in controlling the number of the housefly. Ovinema and Nemaplus are the biopreparations adapted to be applied in practise. It is unfortunate that in Poland abandoned production Ovinema.

CONCLUSIONS

1. Most sensitive to nematodes were the larvae of *M. domestica*. Four-day larvae were more sensitive than two-day ones.
2. Pupae and imagines were more resistant to nematode infection.
3. Larvae of the housefly were more effectively killed by nematodes of the family Steinernematidae than by those of the family Heterorhabditiidae.
4. The housefly was most effectively killed by nematodes *S. feltiae* from biopreparations Owinema and Nema-plus and by *S. carpocapsae*.

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Streszczenie: *Biologiczne metody zwalczania muchy domowej w stajniach.* Doświadczenia miały na celu wybór gatunków i szczepów nicieni owadobójczych, które zostaną wykorzystane w praktycznym zwalczaniu muchy domowej w stajni, co powinno zapewnić dobrostan zwierzętom hodowlanym. Owad testowy – mucha i owadobójcze nicienie z rodziny Steinernematidae i Heterorhabditidae zostały wykorzystane w eksperymentach. Laboratoryjne szczepy nicieni i w Polsce i w Europie tych dostępnych na rynku zostały wykorzystane w przeprowadzonych testach. Larwy, poczwarki i owady dorosłe *M. domestica* były hodowane w Instytucie Przemysłu Organicznego w Warszawie. Cztery grupy zostały stworzone dla każdego gatunku nicieni. Nie wszystkie gatunki nicieni i szczepy były równie zjadliwe dla much.

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Genotype – factor influencing performance of chicken production

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Abstract: *Genotype – factor influencing performance of chicken production.* The aim of this study was to establish the effect of the genotype on performance of chickens. The experimental material included 1,320 slow-growing chickens with two genotypes Hubbard JA 957 – available on the Polish market hybrid with a declared slow growth and non-commercial hybrid C×GP, for creation of which a light native breed Greenleg Partridge hens were used. Chickens were maintained up to 63 days of age. During the experiment body weight, feed intake and health of the birds were under control. Based on the observations the growth rate and the feed conversion ratio (FCR) were determined. It was found, that chickens C×GP were characterized by lower body weight, slower rate of growth and higher feed conversion ratio compared with the chickens Hubbard JA 957, but chickens C×GP had a higher health.

INTRODUCTION

Many people choose poultry as main and preferred brand of meat. However consumers frequently declare their willingness to purchase the products obtained from chickens with less intensive farming, which provides a higher animal welfare (Pavlovski et al. 2009). This trend is particularly evident among Western European and the United States societies, where consumers are interested in the welfare standards and have a concern about the way the animals are kept in intensive poultry production (Vanhonacker et al. 2009). Raising chickens in

alternative methods consumers associate also with the environment protection (Bogosavljević-Bošković et al. 2012). Latter-Dubois (2000) defined the reasons, why consumers choose alternative poultry housing systems as major contributors to better taste, higher nutritional value, higher welfare of the birds and greater safety of such food.

In Poland, poultry meat from birds kept in unconventional systems constitutes a small part of the market and is available mainly as organic product. According to the regulations of the European Union (EC 1804/99) for organic production, it is recommended to choose the right genetic material for such a system of farming. Currently in our country, the organic production is based on the fast-growing chickens, commercial hybrids used in typical, intensive production.

The only commercially available material of slow-growing chickens is Hubbard JA 957. Using of birds of slow growth, better adapting to changing environmental conditions, showing a higher health status is particularly important due to the introduction of a number of limitations in respect of organic production. In addition, rearing chickens in the alternative system should be reared longer and be willing to prey (Branciarri et al. 2009). Moreover the birds in non-conventional system should reach slaughter weight in

a period no longer than required for organic production, which is 81st day.

The aim of this study was to evaluate the results of production of hybrids with different genotypes in terms of their usefulness in non-conventional farming systems.

MATERIAL AND METHODS

The experiment was conducted in 2011 in spring (March–May) at the experimental station of the Warsaw University of Life Sciences (RZD Wilanów-Obory). Experimental procedures were approved by the Ethical Commission (approval no. 27/2009 of the 16 April 2009). The experimental material consisted of 1,320 slow-growing chickens of two genotypes

Hubbard JA 957 (660 birds in six replication containing 110 birds in pen) and non-commercial hybrid C×GP (660 birds in six replication containing 110 birds in pen). Day-old chicks have been marked by chicken stamps and maintained up to 63 days of age in compliance with the Regulation of the Minister of Agriculture and Rural Development dated 15 February, 2010 on the requirements and manner of maintaining livestock species, for which protection standards are provisions of the European Union. Stock density in the poultry house was 11 birds per m². In the experiment, 4-stage feeding was applied: the starter (0–14 days), grower I (15–35 days), grower II (36–56 days) and finisher (57–63 days). The composition of components and the nutritional value of compound shown in Table 1.

TABLE 1. Nutritive value of basal diet in broiler feeding

Specification	Starter	Grower I	Grower II	Finisher
CONTENT (%)				
Maize	10	11.4	10	10
Wheat	53	55	59.6	60.8
Soybean meal	30.6	27.4	23.2	21.6
Limestone Ca39	1.165	1.175	1.085	0.945
Sodium bicarbonate	0.2	0.14	0.14	0.16
NaCl	0.24	0.28	0.28	0.26
Stimulator	0.01	0.01	0.01	0.01
Dicalcium phosphate	1.18	0.78	0.7	0.64
Soybean oil	2.1	2.4	3.6	4.4
Methionine	0.48	0.42	0.36	0.28
Lysine	0.36	0.34	0.36	0.28
Threonine	0.14	0.13	0.14	0.1
Premix	0.525	0.525	0.525	0.525
NUTRITIVE VALUE				
EM (MJ)	12.52	12.76	13.20	13.47
Total protein (%)	21.99	20.78	19.26	18.51
Crude fat (%)	3.67	4	5.14	5.92
Crude ash (%)	5.83	5.35	4.96	4.67

During the experiment, the chickens were individually weighed at weekly intervals. Feed intake and health of chickens were also controlled. Based on the results of observations the growth rate, feed conversion ratio and birds' mortality as a percentage of dead and culled to inserted were determined.

The results were statistically analyzed by one-way analysis of variance using SPSS 21.0 PL for Windows software.

RESULTS AND DISCUSSION

In the experiment the body weight of chickens Hubbard JA 957, and C×GP were monitored at weekly intervals. Based on the obtained data growth rate of birds was determined. Figure 1 shows the body weight changes between compound and rate of growth of chickens.

Significant differences in body weight were already noticed at the insertion, Hubbard JA 957 chickens were heavier than chicken C×GP ($P < 0.001$). The differences observed at the beginning were

also evident in the following weeks, chickens Hubbard JA 957 throughout the rearing period had significantly higher body weight compared with the chickens C×GP ($P < 0.001$).

The growth rate of chickens Hubbard JA 957 at the beginning of rearing was high and amounted to almost 120%. C×GP chickens had a lower rate of increase during this period (94%). It should be noted that in chickens C×GP growth rate declined steadily without clearly marked periods and a sudden drop in the final period of rearing (8 and 9 weeks) was higher than in Hubbard. However, in chickens Hubbard JA 957, upon a high initial rate, its sudden decline and a very low level in the last week of life were observed.

Rachwał (2008) reports that in one week old commercial hybrids growth rate is about 140%, i.e. much higher than the results obtained in chickens C×GP and slightly higher than that observed in Hubbard JA 957. Chickens C×GP characterized by low body weight; the 63 day old birds had a weight comparable

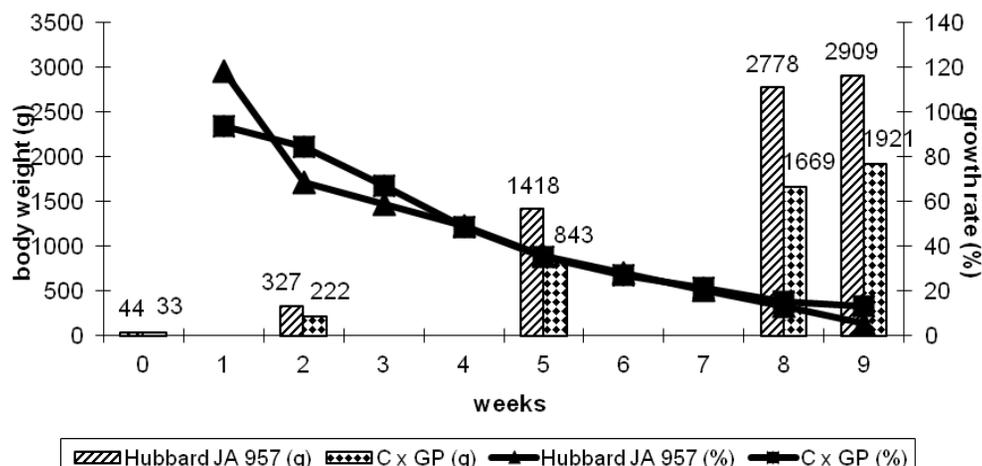


FIGURE 1. Body weight (g) and growth rate (%) in chickens depending of the genotype

to that achieved by commercial broilers at 35 day (Aviagen 2012). It should be noted, that the main purpose for creating hybrid C×GP was not to maximize production results, but to produce the material adapted to less intensive farming conditions.

Hubbard JA 957 chickens had significantly higher body weight, which oscillated between 2,900 g. It is worth to note, that in poultry production the value of 2,000–2,500 g is given as a standard live weight of chickens for slaughter, then a carcass can get the weight mostly desired by consumers – about 1,300–1600 g (Fanatico et al. 2005). So Hubbard chicks reached too high body weight at 63 day of age, therefore slaughter of these birds should have been carried out appropriately earlier. If the aim was to determine the genotype of birds, that could be used in alternative housing systems with access to range, it is worth noting that the chickens with a slower rate of growth better adapt to such conditions. On the basis of Branciaro et al. (2009) it can be concluded, that the fast growing commercial hybrids behave very similarly in intensive production and organic farming. Chickens do not have the desire to prey and do not display the typical behavioral poultry burying behavior. The birds are unwilling to use runs and a large part of time were staying in livestock buildings.

On the paddock practically do not move, spend most of their time lying down or standing. The slow growing birds show totally different behavior. They more likely have a run, significantly increase their activity, much less time are lying standing and eating, and significantly more move and bury.

Based on the measurements of body weight and growth rate a preliminary hypothesis, that chickens C×GP is the genetic material better adapting to the slow-range farming than Hubbard JA 957. However, it appears necessary to conduct a distinct behavioral research and to determine the basis of their behavior in unconventional rearing system.

Table 2 shows feed consumption (kg/kg) and mortality (%) in chickens depending on the genotype.

Basing on these results, it is clear, that Hubbard JA 957 chickens use less feed per 1 kg of body weight gain compared with the chickens C×GP. Such result can be explained by a significantly higher body weight of Hubbard chicks at slaughter. Feed consumption of 2.27 kg/kg (for Hubbard) and 2.84 (for C×GP) obtained in the experiment was significantly higher than that achieved in intensive production. Commercial broilers slaughtered at the age of 42 days use about 1.7 kg of feed to gain 1 kg of body weight (Feddes et al. 2002). However, with increas-

TABLE 2. Feed conversion ratio and mortality in chicken depending on the genotype

Specification	Hubbard JA 957	C×GP
Feed conversion ratio FCR (kg/kg)	2.27	2.84
Energy conversion (MJ/kg)	29.48	36.89
Protein conversion (kg/kg)	0,47	0,56
Mortality (%)	2.72A	0.30B

A, B – difference significant at $P \leq 0.01$.

ing time of rearing the birds an increase of this parameter should be expected; this is due to lowering rate of growth and increasing feed consumption. Castellini et al. (2002) maintaining the chickens up to 81 days of age received even higher feed consumption (3.0 kg/kg).

Mortality of chickens in the experiment was generally low and amounted to 2.72% for Hubbard JA 957 chickens and 0.36% for C×GP. Particularly low mortality for hybrid C×GP can prove the high health of these birds, which is especially important when raising chickens are reared in the conditions ensuring the access to open air.

Fanatico et al. (2008) pay special attention to the genotype in shaping this production factor. Slow-growing chickens have a lower mortality rate compared to the fast-growing chickens. This is mainly due to a lower share of diseases associated with the fast pace of growth, among which tibial dyschondroplasia occurs. Castellini et al. (2002) found, however, that the main cause of falls in fast-growing chickens is ascites and sudden death syndrome, which in slow-growing birds are much more rarer.

Birds' genotype may be particularly important in shaping mortality in free range or organic rearing conditions. Branciarri et al. (2009) observed, that fast growing chickens characterized by relatively low mortality when the rearing is carried out in a closed breeding conditions. Mortality in a flock of broilers considerably increase, when the birds use the free run. However, exactly the opposite situation can be observed in medium and slow-growing poultry, ac-

cess to open-air significantly reduces mortality among them. These results further confirm the necessity of obtaining slowly growing hybrids in the alternative housing systems.

The results obtained in this experiment as well as the ones of other authors (Castellini et al. 2002, Fanatico et al. 2008, Branciarri et al. 2009) indicate the need to adjust the growth rate of birds to the chosen farming system. It should be noted, that due to the production results, which are worse in comparison with commercial broilers (lower body weight, lower growth rate, higher consumption of feed) producers of poultry kept in the alternative housing systems must compensate for the loss resulting in a correspondingly higher price for the poultry product.

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Streszczenie: *Genotyp – czynnik warunkujący wyniki produkcyjne w stadach kurcząt rzeźnych.* Celem badania było określenie wpływu genotypu na wyniki produkcyjne kurcząt. Badania przeprowadzono na 1320 kurczętach o dwóch genotypach Hubbard JA 957 – dostępnym na rynku polskim mieszańcem o deklarowanym wolniejszym tempie wzrostu oraz C×Zk – mieszańcem, do którego wytworzenia wykorzystano kury rasy lekkiej zielononóżki kuropatwianej. Kurczęta utrzymywano do 63. dnia życia. W czasie odchowu kontrolowano masę ciała, spożycie paszy i zdrowotność ptaków. Na podstawie prowadzonych obserwacji określono tempo wzrostu oraz wskaźnik zużycia paszy. Stwierdzono, że kurczęta C×Zk charakteryzowała mniejsza masa ciała w porównaniu z kurczętami Hubbard JA 957 oraz wolniejsze tempo wzrostu. Mniejsze wykorzystanie paszy na przyrost masy ciała stwierdzono dla kurcząt Hubbard JA 957. Kurczęta C×Zk charakteryzowała natomiast wyższa zdrowotność.

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Comparative behaviour analysis of some colubrids with reference to suitability of captive bred snakes for reintroduction to natural habitat

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Abstract: *Comparative behaviour analysis of some colubrids with reference to suitability of captive bred snakes for reintroduction to natural habitat.* A sample of newly hatched American colubrids, divided according to their level of domestication, understood herewith as the generation history in captivity, was tested in an open field test (OFT) for degree of activity and confidence in open terrain and in confrontation with individuals of such species as *Pantherophis guttatus* and *Lampropeltis getula splendida* as well as *L. g. californiae*, in order to compare their antagonistic and feeding behaviour reactions. The cluster comparison of defence reactions revealed no effect of domestication (many generation in captivity) in the face of potential danger. Even albino phase Kingsnakes, considered to be the form of this species most affected by captivity (altered genome), maintained their natural feeding response. The degree of activity in OFT conditions was seen to decrease with the snakes' age. Thus it was demonstrated that captive specimens bred in terrariums may be considered a gene bank for *ex situ* conservation strategy. However it is recommended that specimens as young as possible should be used in reintroduction to natural habitat, as these will respond most faithfully to the pressure of natural selection, based on their behavioural variation not suppressed by apathy caused by captivity.

Key words: *Lampropeltis*, *Pantherophis*, antagonistic and feeding reactions, captive breeding, *ex situ* conservation

INTRODUCTION

Herpetofauna belongs to that most vulnerable group of animals and those most endangered by anthropogenic pressure to their natural habitat. There have been many attempts of *in situ* conservation and some have proved quite effective – for more information on this, see the Materials of VI World Congress of Herpetology – Manaus 2008. Despite these efforts, more and more trials are undertaken for *ex situ* strategies. Traditionally this activity is the domain of universities or ZOOs, which maintain certain species as gene banks – potential sources of material for reintroduction. In the case of snakes, there are quite a few examples of attempts in this field. During the years 1980–1986, some 34 specimens of captive bred Rocky Python *Python sebae* were released into the Great Fish River Nature Reserve (Eastern Cape), but there was no follow-up (Alexander and Marais 2007, Mattison 2013 – personal communication). In the USA, five AZA facilities have created a consortium “Conservation

Centre for Species Survival” undertaking preparatory work for reintroduction programs for various taxons, including snakes. The species of choice are to be the San Francisco garter snake *Thamnophis sirtalis tetrataenia* and the Louisiana Pine Snake *Pituophis ruthveni* (Mattison 1995, Conway 2011, Mattison 2013 – personal communication). Godwin et al. in 2008 presented a comprehensive report on the captive propagation of the critically endangered Eastern Indigo Snake *Drymarchon couperi*, which included suggestions concerning other snake species. An initial scenario was developed for supplementing the local population of Grey Banded Kingsnake *Lampropeltis alterna* by captive animals (Spanowicz and Życzyński 2002). Similar efforts were undertaken by Łódź University, in cooperation with Łódź ZOO, for a domestic (Polish) species – Smooth Snake *Coronella austriaca* (Zieliński and Stanisławski 2001, Stanisławski 2003). All these attempts led to the release of captive snakes into the wild, but were not continued after the first trial.

Another attempt worth mentioning was the successful reintroduction of the Antigua Racer *Alsophis antiguae* (Daltry 1999 and 2006). In this project, the reintroduced snakes were field collected on Great Bird Island, tagged and released on small Rabbit Island. On the contrary the results of reintroduction of Woma Python *Aspidites ramsayi* in Arid Recovery Reserve in northern South Australia did not meet the short-term and medium-term success criteria mostly due to predator pressure (Moseby et al. 2011).

Potential support for such work may come from private enthusiasts and keepers. This is a significant new idea. Al-

though as early as 1975, John Coborn organized the symposium “Conservation and Captive Reptiles and Amphibians”, where the case was made by many herpetologists for and against captivity as an aid to the preservation of wild species, there is still much work to be done in this field. In any case, it must be admitted that the level of knowledge of herpetofauna husbandry is constantly growing and additionally, the pure breeding strategy according to locality of origin has become the ethical rule among the most advanced keepers. As a result, in many cases the numbers of certain species in captivity exceed those ever to be seen in the wild and these can be treated as pure gene banks. Their active population, with a quite large effective number (N_e) is much greater than could be assured by official institutions limited by state budget conditions. But if we are to treat captive animals as candidates for restocking, we must be sure that these fulfil the demands of gene bank purity, as well as the demands with regard to sanitarian status, in order to prevent introduction of exotic pathogens to the natural habitat. The captive material must also exhibit the natural degree of fitness that can be subjected to natural selection.

The aim of the present study is a comparative behavioural analysis of captive bred snakes, which can be differentiated according to the level of their domestication (understood herewith as the multi-generation history in terrariums’ life).

MATERIALS AND METHODS

The snakes used in this study were born in Laboratory of Department of Genetics and Animal Breeding of WULS

– SGGW. They belonged to the genera *Lampropeltis* and *Pantherophis*. Authors chose these taxons in view of their popularity among keepers. Compared to other genera, most of these snakes have a quite long generation history in captivity and thus constitute interesting material for an evaluation of the level of natural behaviour preserved. These may serve as a model for conclusions concerning other, potentially truly endangered species, which are currently not stocked in collections.

The material was divided into the following groups:

1. *Ls* × *Lc* – inter subspecies cross between Sinaloan Milk Snake *Lampropeltis triangulum sinaloe* and Pueblan Milk Snake *Lampropeltis triangulum campbelli* – (parents from multigenerational captive breeding) – here – captive (domesticated) type – 10 specimens;
2. *Lpp* × *Lpk* – inter subspecies cross between Arizona Mountain Kingsnake *Lampropeltis pyromelana pyromelana* and Chihuahua Mountain Kingsnake *Lampropeltis pyromelana knoblochi* – second generation in captivity – intermediate type – 3 specimens;
3. *Ege* – Great Plains Rat Snake *Pantherophis emoryi* – from field collected parents – wild type – 8 specimens;
4. *Ege* × *Egg* – interspecies cross between Great Plain Rat Snake *Pantherophis emoryi* (field collected) and Corn Snake *Pantherophis guttatus* (amelanistic form – fixed mutation in multigenerational captivity) – intermediate type – 6 specimens;
5. *Eggalb* × *Eggan* – *Pantherophis guttatus* – a crossbreed of two fixed mutations – snow albinism and anerythristic (black albinism) – “domestic” type – 7 specimens.

The snakes were subjected to open field test – OFT (Markowska 1979) scoring points for movement activity within the field. Animals were placed within a ring 90 × 90 cm, fenced with wooden walls. The surface of the ring consisted of squares 30 × 30 cm forming three rows. The scores for activity were counted as follows: snake was getting one point when slithered along one square adjacent to the walls and 3 points for distancing from the ring walls and entering the square in the centre of the field. After 5 min of observation the points were added up.

Later the snakes were confronted with a young Corn Snake *Pantherophis guttatus* – a species preferring rodents as the natural diet, and a Desert Kingsnake *Lampropeltis getula splendida* – living (as the whole genus *Lampropeltis*) on lizards and other snakes as its most typical food. The points scored and body weights of each snake tested were subjected to one-way ANOVA. When necessary the F-test was followed by D-test to verify the significance of differences among the groups. Additional analysis of covariance with body weight as an operand variable was done to check whether the sizes of snakes could affect their confidence and thus the results of OFT test.

During confrontations (lasting 5 min each) with another snake, the following reactions were noted on a Yes or No basis: lack of any reaction, strong tail rattling (loud and lasting longer than 5 s), weak tail rattling (lasting less than 5 s), tossing movements, sudden retreat, avoidance and attack.

In authors' experience, the chosen/described reactions reflect well the emotional status of the snake during the test. The probabilities of each reaction in two

separate trials, for confrontations with Corn Snake *P. guttata* and Desert King-snake *L. g. splendida* and combined, were subjected to cluster analysis (Nei 1972) permitting to build dendrograms of behavioural distance.

In an additional analysis (not included in all experiments), authors used Sinaloan Milk Snake *Lampropeltis triangulum sinaloe* – 4 specimens, Grey-banded Kingsnake *Lampropeltis alterna* – 2 specimens, and California Kingsnake *Lampropeltis getula californiae* (albino) – 2 specimens. All of these were born a year later. These snakes were confronted with each other within their own species and with albino Kingsnakes.

RESULTS

The points scored in OFT in both observations met the criteria of normal distribution with mean values 7.7 and standard deviation 5.4 and mean value 8.5 with standard deviation 5.5 for I and II trial respectively.

The differences between groups are significant $P \leq 0.05$ for the I observation

only. As the snakes aged, the differences between groups disappeared.

The score in the II observation is generally smaller – *vide* Table 1. This can be explained by increased apathy caused by captive conditions.

The ANOVA results for snakes body weights were significant $P \leq 0.05$. As the activity in OFT could be affected by snakes' sizes we made the additional analyses, correcting the analysis for points scored in the first observation by deviation from regression line for body weights.

The deviations from regression line (body weight as an operand variable) are highly significant ($P \leq 0.01$) and confirm that the significant differences ($P \leq 0.05$) in points scored in OFT (I observation – Table 1), were not caused by differences in the sizes of specimens, but in reality depended on group classification.

Activity in the field test was lower during the II observation except in the case of the group 5. Growth was different in each group – in the *pyromelana* group, this was even a negative value.

The results for cluster analysis are shown in the dendrograms in Figures 1, 2, 3, 4 and 5.

TABLE 1. Points scored in OFT and measurements of body weights during the I and II observations

Groups	N	Scores in OFT		Body weights [g]	
		I Observation	II Observation	1st Observation	2nd Observation
		$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$
<i>Ls</i> × <i>Lc</i>	10	7.9 ± 0.9 ^a	7.3 ± 3.2	35.2 ± 3.1 ^{A,E}	36.4 ± 3.3
<i>Lpp</i> × <i>Lpk</i>	3	3.3 ± 1.8 ^{b,d}	1.7 ± 2.1	27.7 ± 1.0 ^{B,F}	26.3 ± 3.3
<i>Ege</i>	8	10.9 ± 2.5 ^{d,e}	8.0 ± 5.4	174.9 ± 34.7 ^{A,B,C,D}	185.1 ± 37.0
<i>Ege</i> × <i>Eggam</i>	6	13.2 ± 1.9 ^{a,b,c}	10.3 ± 6.4	112.0 ± 21.1 ^{C,E,F,G,H}	141.9 ± 21.3
<i>Eggan</i> × <i>Eggalb</i>	7	4.7 ± 1.3 ^{c,e}	7.6 ± 1.9	40.5 ± 2.9 ^{D,H}	47.9 ± 4.0

Letters (in pairs): a–e point out the significant differences between means of groups for points scored during the first trial – Duncan test; $P < 0.05$;

Letters (in pairs): A–H show the significant differences between means of groups for body weights during the first trial – Duncan test; $P < 0.05$.

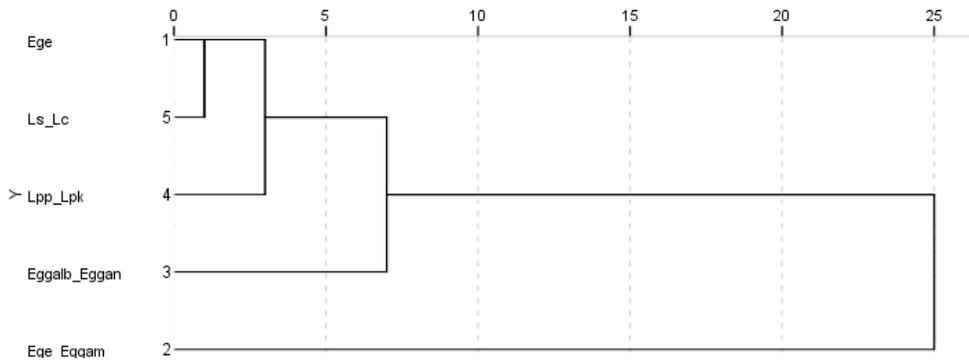


FIGURE 1. Comparison of group reactions during the I observation – confrontation with *Pantherophis guttatus*

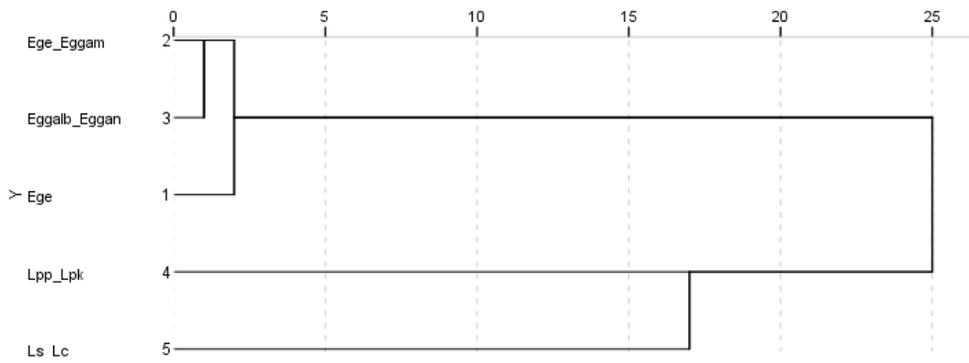


FIGURE 2. Comparison of group reactions during the II observation – confrontation with *Pantherophis guttatus*

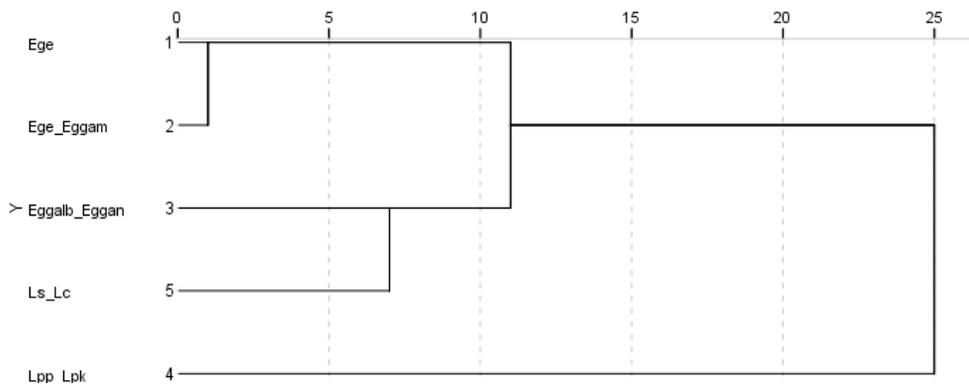


FIGURE 3. Comparison of group reactions during the I observation – confrontation with *Lampropeltis getula splendida*

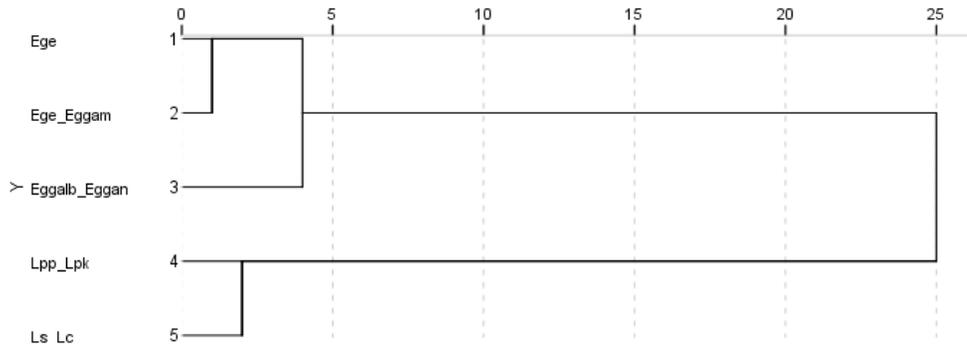


FIGURE 4. Comparison of group reactions during the II observation – confrontation with *Lampropeltis getula splendida*

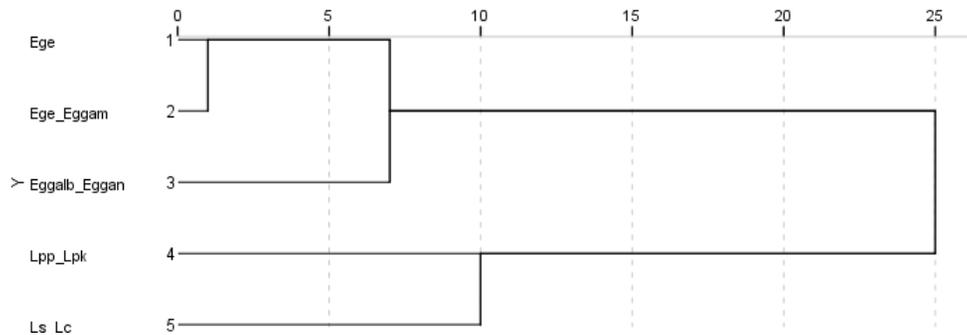


FIGURE 5. Comparison of group reactions – both types of confrontation grouped together

Confrontation with the relatively harmless Corn Snake *Pantherophis guttatus* has not revealed any differences in reactions between groups, which could be attributed to either the level of domestication or the taxonomic position.

In contrast to the I observation, during the II observation, taxonomy played an important part in sorting the snake groups within clusters. This tendency remained constant during subsequent trials – *vide* Figures 3–5.

Figures 3–5 show dendrograms that are practically identical with Figure 2. There is no noticeable influence of domestication or the addition of “wild

blood” as a factor differentiating the groups.

During confrontation with their own species (Greybanded Kingsnakes, Milk Snakes and California Kingsnakes), authors observed some slight excitation (avoidance, tail rattling) in the tested snakes. Confrontation with the albino Kingsnake resulted in an attack by the latter showing the typical feeding response. The Kingsnakes themselves did not react to each other.

Following the attack, the trials were immediately halted, with no harm being caused to the snakes by the testing procedure.

DISCUSSION

Analysis of the snakes' activity in OFT showed significant differences only during the I observation (Table 1). Comparison within genus *Pantherophis* revealed that groups 3 and 4 did not differ significantly from each other but both deviate from group 5 (the smallest specimen – Table 1) designated the most domesticated – the longest generation history in captivity. Correction on regression line for body weight showed that points scored in OFT do not depend on body weight. This result accords with the fact that during the II observation, differences between groups in terms of body weight increased, but differences in activity (points scored in OFT) disappeared (*vide* Table 1).

Based on these findings, we can conclude that snakes kept for generations in captivity behave less actively than those field collected or those with the addition of "wild blood". We may ask whether decreased activity reflexes lower fitness ability. Each group of reptiles has its own survival strategy. Research carried on turtles (Mrosovsky and Gogfrey 1995) has shown that the more active young (1YO) specimens have a lower survival rate than those less mobile, whereas the higher mobility of young lizards (Van Damme et al. 1992) provides them a greater success rate in hunting and a greater ability to avoid predators. If greater activity and courage in young snakes protects them in the wild, researchers may conclude that a longer generation stage in captivity debilitates adaptation and fitness. This is true for the first stages of life only, as during the II observation all groups behaved similarly

– probably due to apathy caused by captive conditions.

Another source of information is provided by analysis of the dendrograms. The I confrontation, with the relatively harmless Corn Snake (Fig. 1), showed a considerable variance in reactions between groups, which could not be attributed to either taxonomy or level of domestication. The II confrontation, with a Corn Snake and the effect of exposing the tested specimens to more dangerous stimuli i.e. Desert Kingsnake *L. g. splendida*, (Fig. 2–5) provided uniform dendrograms arranged according to taxonomy and not the captivity status.

Albino Kingsnakes tolerated each other well, but in the presence of another species, manifested an immediate feeding response by attempting to attack.

Rodriguez-Robles and De Jesus-Escobar (1999) studied Colubridae evolutionary relationships by analyzing the mt DNA variability and adjusting their conclusions to the evolution of feeding customs. According to the authors, a diet based on reptiles (lizards, snakes) is more primitive than one based on rodents, which developed much later. They presented their results in the form of dendrograms, showing both the genetic relations between genera of American colubrids and the hypothetical evolution of feeding behaviour.

The results presented earlier correspond with those quoted. Divergence in behaviour during confrontations with predators phylogenetically approximates feeding behaviour and differs by definition from inner aggression.

The divergences noted above are very important from the point of view defined in the title of this study. During the first

trials, we observed diversity in both the fitness of the specimens and their reactions during confrontation. These differences were reduced as the snakes got older. So any decision to reintroduce snakes should only consider very young specimens, i.e. hatchlings. Such material may be characterized by greater fitness – *vide* Table 1, although due to their smaller size, these could be more at risk from predators. But this would allow for natural selection, a factor always present in the natural habitat.

The accordance of reactions with the dendrograms shown by Rodriguez-Robles and De Jesus-Escobar revealed that captive *Lampropeltis* snakes preserved their natural feeding behaviour. Thanks to their undisputed beauty, Kingsnakes and Milk Snakes have gained enormous popularity among amateur snake keepers. In captive conditions, they are forced to adapt to a rodent based diet. Some specimens adapt to this easily; others are more reluctant. Nevertheless their maintenance in captivity is increasingly easy and the survival rate of hatchlings is gradually increasing. This fact is worthy of attention. The most popular terrarium species may have been subjected to unintentional selection for the ability to adapt to a diet that is atypical for them. Arnold (1981), working on garter snakes *Thamnophis elegans* proved that feeding preferences are genetically polymorphic and of a quantitative nature. Thus selection for diet type could be possible and may also result in inherited changes in feeding customs, not caused by current artificial captive conditions only. This problem is discussed by Conway 2011, focusing on the number of generations in

captivity as a function of potential, undesirable selection.

Snakes play an important role in the trophic chain. In the event of reintroduction of captive material into the natural habitat, the consequences of releasing animals of genetically changed feeding customs could be at the least undesirable, even where the rules of taxonomic and population fidelity have been obeyed. It is worth mentioning that the team working on the reintroduction of the Smooth Snake *Coronella austriaca* (Zieliński and Stanisławski 2001) were offering their captive hatched snakes live small lizards (*Lacerta agilis*), which were preyed on. This reflected in terrarium conditions the rules of natural selection for the snakes' survival. However, the results of this restocking are difficult to evaluate, as the snakes were not tagged for telemetric monitoring as they were in Daltry's (1999) project.

Lack of proper documentation is always the weakest point of any reintroduction project, as was pointed out by Earnhardt (1999). This particularly concerns survival rates in the very first period of release.

The dendrograms and observations presented in this paper lead to conclusion that studied *Lampropeltis* snakes, despite their history of captivity, preserved their natural feeding habits, enabling them to play their natural role in the trophic chain when returned to their natural environment. Likewise the *Pantherophis* snakes, despite their level of domestication, did not differ from one another in the general set of reactions.

CONCLUSIONS

This model experiment shows that captive snakes from multigeneration terrarium colonies can be considered worthy material for reintroduction, on condition that the principles of genetic fidelity are observed.

A planned release should be carried out at a very young age, despite the expected losses of very small and fragile animals, in order to let them undergo a process of natural selection based on fully exposed diversity.

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Streszczenie: Porównawcza analiza zachowań węży z rodziny Colubridae pod kątem przydatności osobników hodowanych w niewoli do reintrodukcji do środowiska naturalnego. W modelowym doświadczeniu przetestowano amerykańskie węże z rodziny Colubridae podzielone według stopnia udomowienia (pokoleniowego stażu w warunkach niewoli). Zwierzęta zostały poddane testowi otwartego pola (OFT), sprawdzającego ich aktywność i odwagę na otwartej przestrzeni oraz konfrontacji z innym wężem zbożowym *Pantherophis guttatus* oraz lancetogłosem królewskim *Lampropeltis getula splendida* i dodatkowo *L.g. californiae*, formą albinotyczną. Test OFT wykazał ujemny wpływ wieku na aktywność terraryjnych zwierząt a konfrontacje z probantami nie ujawniły wpływu udomowienia na reakcje obronne (klasterowa analiza skupień). Nawet u albinotycznych lancetogłosew, uznanych za najbardziej udomowioną formę (zubożały, wsobny genotyp) utrzymywanych od pokoleń na nie-

naturalnej diecie, wystąpiły normalne reakcje drapieżnicze. Stwierdzono zatem przydatność populacji terraryjnej jako ewentualnego banku genów dla strategii ochronnej *ex situ*, przy zaleceniu używania do reintrodukcji materiału możliwie młodego, najwierniej reagującego na presję selekcji naturalnej, wymierzonej w wachlarz reakcji niestłumionych warunkami niewoli.

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