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The character types of the blind guide dogs

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Abstract: *The character types of the blind guide dogs.* The aim of this study was to determine the character types of the blind guide dogs, in correlation with their breed, gender and age. The analysis was based on final results of the training prepared by the Polish Association of the Blind. There were no statistical differences when comparing the final examination results (number of points scored) with regard to breed animals' and their gender. Dependence was found between the dogs character types and the correctness of tasks: dogs in sanguine type are the most suitable for specialized training, but in a group of dogs that successfully completed the exam were also choleric dogs – hyperactive and phlegmatic – slow, which are less predisposed to work with man, but through proper training they can work as a guide the blind.

Key words: dog character types, guide dog the blind, dogs training.

INTRODUCTION

Dogs are used in various fields. In each a different type of behaviors is preferred, and each type of behavior suited to the nature of character, classifying it into the appropriate type of activity (work). Currently, dogs are increasingly committed to working in the service of man. (Palmer, 1983; Kuźniewicz and Kuźniewicz, 2003; Ściesiński, 2004). To perform a variety of tasks, the dog must be properly prepared. The importance of the training process is not only to prepare the dog to his future

work but also learn him the appropriate behavior at a work. (Dennison, 2007; Donaldson, 2007). Kind of behavior, and the character type of dog is the basis to allocate it to the appropriate type of work (Coren, 1997). Dogs trained to the needs of the Polish Association of the Blind are matched in terms of physical structure and must have the appropriate mental abilities (Praca zbiorowa, 2009). Guide dog blind man could be any racial or crossbreed dogs when it enters the appropriate training and can be assigned to the person in need (Praca zbiorowa, 2006). Guide dogs must meet certain criteria, both physical and mental (Praca zbiorowa, 2010). Many dog breeds possess such characteristics, include Labradors (Milewska-Kuncewicz, 2003) and German Shepherds (Nowicka, 1995).

The aim of this study was to determine the character types, which most often occurs in dogs intended for use as a guide the blind, in correlation to their race, gender and age, based on final exam results.

MATERIALS AND METHODS

Materials

The results received by dogs that have completed the final examination success-

fully, and received eligible to work as guide dog the blind were analyzed. The dogs trained in the years 2007–2009 in a training center Mr. J. Przewięda in Piła were involved in the study. The study involved 26 animals: 10 dogs in 2007, and 16 dogs in the years 2008 and 2009, when 8 animals were trained each year. Trained dogs belongs to two breeds: Labradors and German Shepherds. The results of the training included 30 criteria.

Methods

The paper presents an analysis of the obtained final results of the examination of dogs with regard to their race, gender and age. During the exam dogs were rated by 30 criteria: 26 of them were evaluated at a scale of 0–10 points, while the four criteria – in the 0–5 point scale. Dogs can earn a maximum of 280 points. The exam has been passed when dog has got at least 224 points.

Based on the evaluation results of four criteria which are elements of the

exam, while expressing the character of trained dogs, it was developed an own test of the character types of trained dogs, based on psychological pups tests presented in the literature (Brzezicha and Walczyna, 1995; Ściesiński, 2004; Bailey, 2007).

The following criteria were taken into account:

1. Response to auditory stimuli (eg. explosions);
2. Response to other stimuli such as visual and olfactory;
3. Response to orders given to them;
4. Reaction to the recall.

Exam results in the adopted scale are shown in Table 1, and the dogs' types characters corresponding to the number of points obtained in the new-scale are presented in Table 2 (according to the criteria described by Woźniak, 2000).

The estimation statistical differences in the results, using statistical tests: Student's *t* and chi-square, have been done.

TABLE 1. Exam results in the PZN and adopted scale – an estimation of dog's type character

Criteria	Intensity of response	Scale (points)	
		PAB*	Adopted
Response to auditory stimuli (eg. explosions)	No response	10	3
	Weak response	9	2
	Response	8–1	1
Response to other stimuli (visual or olfactory)	No response	10	3
	Weak response	9	2
	Response	8–1	1
Response to orders	Response	10	3
	Weak response	9	2
	No response	8–1	1
Response to the recall	Response	10	3
	Weak response	9	2
	No response	8–1	1

*PAB – Polish Association of the Blind (PZN).

TABLE 2. Dogs' types characters corresponding to the number of points obtained in the adopted scale (according to the criteria described by Woźniak, 2000)

Character types	Points
Sanguine	12–10
Choleric	9–7
Phlegmatic	6–4
Melancholic	3–0

RESULTS AND DISCUSSION

Based on the results, it was observed that the commands: moving off, accelerating, move on the street and free-leash walking were done very well. Moving up the stairs, reactions to the commands and recalls, as well as such commands as sit, beware, stop and retrieving were executed in a good way. Difficult situations on the street and obstacles on the right side, keeping the direction, giving the voice were overcome on the average level. The left side obstacles and barriers on high were most difficult to realize.

The average final exam score for animals who have completed training is 249.3 points (SD = 12.29). The following table show the comparison of final exam results (number of points scored) with regard to animal breed, gender and age (Tab. 3). Student's t-test analysis indicates that the existing differences between groups are statistically insignificant.

Statistically significant difference ($p < 0.05$) was observed by analyzing the level of examination assessment (higher/lower), taking into account the age of animals: an older animals received better results (Tab. 4).

Comparison of animals in terms of their types characters are shown in Table 5. The largest number of dogs (15) was in choleric character, which is hyperactive and unstable, 7 dogs shows the nature of the phlegmatic, slow, and only 4 dogs were in the sanguine type – sustainable. No dog has received as a result less than 4 points, thus not one

TABLE 3. Comparison of final exam results

Criteria		Number of animals	Average result (points)	Standard deviation
Breed	Labrador	17	252.6	9.74
	German Shepherd	9	243.0	14.61
Gender	Males	11	251.5	11.94
	Females	15	247.1	12.69
Age	Up to 23 months	18	248.4	12.10
	Over 23 months	8	251.4	13.24

TABLE 4. The level of examination assessment in relation to average age

Items	Up to 250 points	Over 250 points
Number of animals in group	10	16
Average final score	236.8	257.1
Average age (in months)	19.7 ^a	24.18 ^a

^a the difference statistically significant ($p < 0.05$).

TABLE 5. Comparison of animals in terms to their character types

Criteria		Number of animals	Character types		
			Sanguine	Choleric	Phlegmatic
Breed	Labrador	17	3	11	3
	German Shepherd	9	1	4	4
Gender	Males	11	3	5	3
	Females	15	1	10	4
Age	Up to 23 months	18	2	11	5
	Over 23 months	8	2	4	2

TABLE 6. Comparison the results obtained by certain character types of dogs

Population's average (points)	Character types		
	Sanguine	Choleric	Phlegmatic
249.3 ^{a, B}	262.5 ^a	253.4	233.0 ^B

^a the difference statistically significant ($p < 0.05$).

^B the difference statistically highly significant ($p < 0.01$).

demonstrated the melancholic type. Dogs in the type of phlegmatic and choleric qualified as guide dog, because the training process has built with them the appropriate behavior of allowing the pass the final exam (Coren, 1995; Dennison, 2007; Donaldson, 2007). Performed chi-square test analysis showed that the existing differences between groups are statistically insignificant.

The next table compares the results obtained by certain character types of dogs (Tab. 6). It was found that the highest score obtained dogs with sanguine type, while the lowest – phlegmatic type dogs. These were the values which differ at a statistically significant level to the population's average.

CONCLUSIONS

There were no statistical differences when comparing the final examination

results (number of points scored) with regard to breed animals and their gender. Statistically significant difference was observed by analyzing the level of assessment examination (higher/lower): an older animals obtained better results. Analysis of the participation of certain types characters of animals regard to their race, gender and age indicates that existing differences between groups are statistically insignificant. It was observed that the highest scores obtained dogs in sanguine type, that are most suitable for specialized training. Theirs results were significantly higher than the average for the population, and the difference was statistically significant ($p < 0.05$). Dogs of a choleric – hyperactive characters obtained results similar to the population's average, while dogs with a phlegmatic character – slow, obtained final results much lower than the population's average ($p < 0.01$).

REFERENCES

- BAILEY G., 2008: The perfect puppy. Edit. Hamlyn.
- BRZEZICHA A., WALCZYNA B., 1995: Mój pies i ja. Państwowe Wydawnictwa Rolnicze i Leśne, Warszawa.
- COREN S., 1995: The intelligence of dogs. Edit. Bantam.
- DENNISON P., 2007: Pozytywne szkolenie psów. Wydawnictwo Rebis, Poznań.
- DONALDSON J., 2007: Culture clash. Edit. James & Kenneth.
- KUŹNIEWICZ J., KUŹNIEWICZ G., 2003: Psy w służbie człowieka. Akademia Rolnicza we Wrocławiu, Wrocław.
- MILEWSKA-KUNCEWICZ J., 2003: Golden Retriever. Agencja Wydawnicza MAKO PRESS, Warszawa.
- NOWICKA M., 1995: Owczarek niemiecki. Wydawnictwo Egros, Warszawa.
- PALMER J., 1983: Working dogs. Haynes Publ.
- PRACA ZBIOROWA, 2006: Regulamin przyznawania psów przewodników i psów towarzyszących w Polskim Związku Niewidomych. Zarząd Główny Polskiego Związku Niewidomych, Warszawa.
- PRACA ZBIOROWA, 2009: Protokół z egzaminu wstępnego/końcowego psa przewodnika na 2010 rok. Zarząd Główny Polskiego Związku Niewidomych, Warszawa.
- PRACA ZBIOROWA, 2010: Pies przewodnik osoby niewidomej. Centrum Rehabilitacji Biura Zarządu Głównego Polskiego Związku Niewidomych, Warszawa.
- ŚCIESIŃSKI K., 2004: Hodowla psów. Wydawnictwo SGGW, Warszawa.
- WOŹNIAK J., 2000: Szkolenie psów – porady praktyczne. Wydawnictwo Łowiec Polski, Warszawa.

Streszczenie: *Typy charakterów psów przewodników niewidomych.* Celem badania było określenie rodzaju charakteru psów przewodników niewidomych w korelacji z ich rasą, płcią i wiekiem. Analizę oparto na ostatecznych wynikach szkolenia opracowanego przez Polski Związek Niewidomych. Nie zaobserwowano istotnych różnic przy porównaniu wyników egzaminu końcowego (liczba zdobytych punktów) w odniesieniu do rasy zwierząt i ich płci. Stwierdzono zależność między różnymi typami charakterów psów: psy w typie sangwiniicznym są najbardziej odpowiednie dla szkoleń specjalistycznych, ale w grupie psów, które pomyślnie zaliczyły egzamin były również psy o charakterze cholerycznym – nadpobudliwe, oraz psy w typie flegmatycznym – powolne, które są mniej predysponowane do pracy z człowiekiem, ale poprzez odpowiednie przeszkolenie mogą pracować jako przewodnicy niewidomych.

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Effect of use of herbal feed additive on coccidian invasion level and performance traits in goats

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Abstract: *Effect of use of herbal feed additive on coccidian invasion level and performance traits in goats.* The aim of this study was to investigate if use of phytogetic feed additive based of bioactive herbal compounds affected coccidian invasions and production parameters in goats. This research was carried out on a flock of adult Boer goats and their offspring owned by the WULS-SGGW's Sheep and Goat Research Farm in Żelazna. The experimental and control groups were established regarding 14 and 10 does for fecal analysis, respectively as well as 10 and 7 does for milk performance analysis, respectively. Does were at 2–7 lactation and reared 1–2 kids. The addition of herbal feed additive (ADICOX AP) was applied in feeding of experimental group in doze of 200 g per ton of concentrate. Individual fecal samples were collected directly from the rectum from does (beginning and end of trial time) as well as from kids (end of the experiment). The McMaster counting technique and the flotation method was applied for does and kids respectively. The whole experimental time lasted for approx. 3 months since kidding pick and data regarding milk yield of does and body weights of kids were collected in every 2-weeks period. The results indicated addition of ADICOX AP in preventing of coccidiosis in goats caused several observations regarding experimental group such as: i. advantageous course of lactation curve in

milked goats, ii. higher body weights of kids and higher daily gains during first weeks of rearing, iii. significant lower number of oocytes per gram of faeces in adult goats and iv. decrease of highly infected kids. ADICOX AP demonstrated its high usefulness in prevention of coccidiosis in goats. Moreover, the advantageous trends in production traits, both in adult goats and in kids, gave additional benefit, which indicated the possibility of use ADICOX AP in standard breeding practice. Additionally this study also indicated the necessity of searching for naturally based feeding solutions in animal diets which may be helpful in maintaining parasitic infections and could bring better production yields for breeders.

Key words: goats, phytoncides, feed additive, coccidiosis, production performance.

INTRODUCTION

Coccidiosis is a common parasitic disease of the intestinal tracts of animals, which is spread in many farm animal species around the World (Tenter et al., 2002). It is caused by the coccidian protozoa belonging to the genus *Eimeria*. Coccidiosis is very common in goats and

its typical symptom is diarrhea, especially in kids. The disease development is affected by several factors regarding environment, management and animal immunity, emphasizing the age of animals especially (Balicka-Ramisz, 1999; Smith and Sherman, 2009). While in fact most of adult goats are vectors and asymptomatic, kids suffer very often from coccidiosis, including death in extreme incidents (Kaba et al., 2007). This characteristic indicates the particular economic losses in goat breeding demonstrated by lower daily gains and lower body weights of young goats.

Prevention of coccidiosis is based on the pharmaceutical coccidiostatic treatments, which are used as the feed additives in animal nutrition (Olejnik et al., 2009). However, their time of use is officially limited by the EU until the end of 2012 (1831/2003/EC regulation, Anon., 2003). This facts caused the need of stimulating the development of alternative products preventing coccidiosis incidents and one way is to look for the solutions based on the natural active substances.

ADICOX AP (AdiFeed) is the one of such products containing naturally occurring plant ingredients of important biological effects as phytoncides and phytoalexins and may be helpful and efficient to manage coccidiosis in goats and other farm animals as well (Róžański and Drymel, 2010). Due to its innovative solution effects of ADICOX AP addition on the level of coccidia infection and some production traits in goats as milk yield and growth development should be also recognized, although such study were presented in a limited number (Rochfort et al., 2008; Cornale et al.,

2011; Ghosh et al., 2011; Malecky et al., 2011; Ringdorfer, 2011).

MATERIAL AND METHODS

Animals. The experiment was carried out in 2010 on Boer goats owned by the Warsaw University of Life Sciences (Poland) and housed at the Sheep and Goat Research Farm in Żelazna. Flock of 24 adult Boer goats was split into experimental (n = 14) and control (n = 10) groups. Classification of does to the proper group took place during 14 days after kidding for each goat. The does were at 2–7 lactation and they were suckled by 1 or 2 kids. The feeding of goats from the experimental group with the addition of ADICOX AP (dose: 200 g per ton of concentrate) started during the 14-days of preparatory phase and was maintained at the same level during the right experimental time, which has started since 15th day after kidding and lasted for 3 months. Although the ADICOX AP was designated for the does from the experimental group, their kids might have taken this feed additive unintentionally. Diet regarded following fodders of the own-farm production as hay, straw and concentrates and goats were fed accordingly to norms (Kowalski, 2001). Either goats or kids were kept indoors in barn during the experiment.

Fecal samples and their processing. Level of coccidia infection was evaluated in adult goats as well as in their kids. Fecal samples were collected directly from the rectum to plastic bags and then stored at 4°C. Fecal samples of does were taken twice during the experiment (at the beginning and at the end), whereas the samples from kids were taken once only at the end of experiment.

Microscopic fecal examination of coccidian infections was based on number of oocytes per gram of faeces (OPG) and it was performed using the McMaster counting egg technique in adult goats. Due to the small amount of faeces from kids the simple flotation method in salt saturated solution was applied (Gundlach and Sadzikowski, 1995). The judging scheme for the flotation method in kids' faeces was given in the Table 1.

Milking of does and weighing of kids.

In every two-weeks period, data regarding milking yield of does were obtained accordingly to the method proposed by Konstantinou (1973). At the earliest 7 days after parturition first milking took place. To obtain whole milk from udder, the 5 i.u. of synthetic oxytocine was injected intramuscularly to each milked goat. The machine milking was applied. Moreover body weights of kids were collected during the same time. Data of milking as well as weighting were standardized due to 2-weeks periods regarding

14, 28, 42, 56 and 70 day of lactation and age, respectively.

Statistical calculations. The effects of group and phase of experiment as well as the interaction on the OPG in adult goats were estimated with the LSM method using the SPSS v. 14 software [Anon., 2006]. To establish the effect of group in kids the chi-square test was applied. Also LSM method in milk yield analysis considering effects of group, lactation number, litter size and week of lactation as well as chosen 2-factors interactions (group x litter size, group x week of lactation and litter size x week of lactation) was applied. Statistical model regarding effects of group, birth type and sex as well as chosen 2-factors interactions (group x birth type, group x sex and birth type x sex) were used in LSM analysis of performance traits in kids. Statistical differences between experimental groups were evaluated with F-test (Ruszczyc, 1981). Obtained results were shown in Tables and on Figures.

TABLE 1. Judging scheme for the oocytes number determination using the simple floatation method in salt saturated solution

Symbol	Description criteria
+	absence
++	single (1–5 oocytes)
+++	not numerous (5–30 oocytes)
++++	numerous (30–60 oocytes)
+++++	very numerous (more than 60 oocytes)

TABLE 2. Effect of use ADICOX AP on milk field (l/day) in Boer goats (n = 18)

Trait	N	Group	
		Control (A)	Experimental (B)
		7	11
Milk yield (l/day)	LSM	1.561	3.733
	SE	0.904	0.308
	*	b	a

* statistical significance at: a, b – $p < 0.05$.

RESULTS AND DISCUSSION

Fecal examination. Significantly lower number of oocytes per gram of faeces was observed in the experimental group in contrary to the control group in adult goats (Fig. 1). Differences within the level of coccidia invasion were also observed between kids accordingly to the group (Fig. 2). Kids from the control group demonstrated higher frequency of infected animals, whereas approx. 33% of kids from the experimental group did not presented any oocytes in faeces. The

kids from the experimental group were not intentionally fed with the ADICOX additive, although they had free access to concentrate feeders like their mothers did.

Very few studies were focused on describing the effect of use of medical treatments on the coccidian management in goats (Balicka-Ramisz, 1999). However, it was described by Balicka-Ramisz (1999) that occurrence of oocytes in faeces changes within year and their highest level was presented in March-July and mean value was 1950 OPG

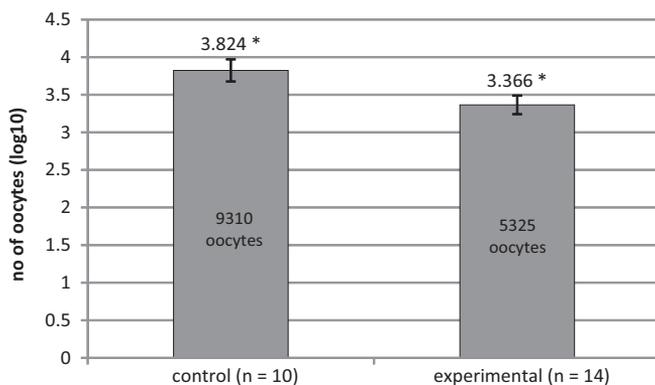


FIGURE 1. Effect of addition of ADICOX AP on the number of oocytes per gram faeces in the adult Boer goats (n = 24, * – p < 0.05)

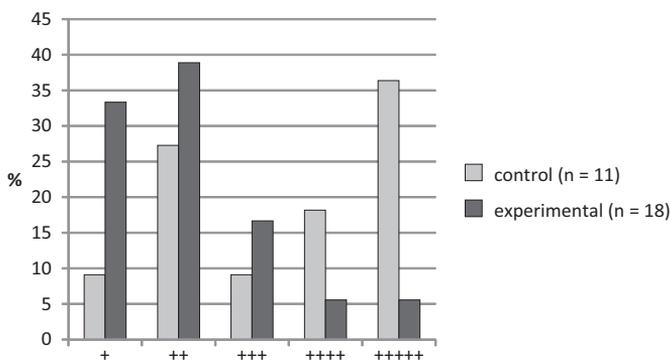


FIGURE 2. Effect of ADICOX AP addition on the coccidian infection level in Boer kids ($X^2(4) = 15.34$; p < 0.01; n = 29)

in adult goats. Own results obtained for kids from control group corresponds indirectly to the high intensity of coccidia invasion in kids at 22 500 OPG during period of spring-summer (March-July) reported by Balicka-Ramisiz (1999), however different technique was used in own studies.

Summing up, obtained results presented lower number of oocytes per gram of faeces in adult goats as well as important decrease of highly infected

kids in experimental group where the phytogetic feed additive was applied.

Production traits – milk yield of does.

The milk traits were examined on 18 goats: 7 and 11 goats from the control and experimental group, respectively. Use of ADICOX resulted in higher milk yield in experimental group, which was important for rearing abilities of does from this group (Tab. 3). Trends in milk yield during first stages of lactation in both groups were also studied on raw

TABLE 3. Effect of group on standardized body weights and daily gains in Boer kids (n = 29)

Traits		Group	
		Control (A)	Experimental (B)
N		12	17
Standardized body weight (kg) at age of:			
At birth	LSM	3.104	4.213
	SE	0.338	0.214
	*	B	A
14 days	LSM	5.507	6.568
	SE	0.581	0.368
28 days	LSM	8.410	11.026
	SE	0.755	0.479
	*	B	A
42 days	LSM	11.854	13.538
	SE	0.819	0.519
56 days	LSM	15.926	15.105
	SE	1.219	0.773
70 days	LSM	16.762	17.825
	SE	1.686	1.069
Standardized daily gains (kg/day) in periods of:			
0–14 days	LSM	0.172	0.168
	SE	0.032	0.020
15–28 days	LSM	0.207	0.318
	SE	0.044	0.028
	*	b	a
29–42 days	LSM	0.246	0.179
	SE	0.052	0.033
43–56 days	LSM	0.291	0.112
	SE	0.076	0.048
	*	b	a
57–70 days	LSM	0.060	0.194
	SE	0.105	0.067

*statistical significance at: A, B – $p < 0.01$; a, b – $p < 0.05$

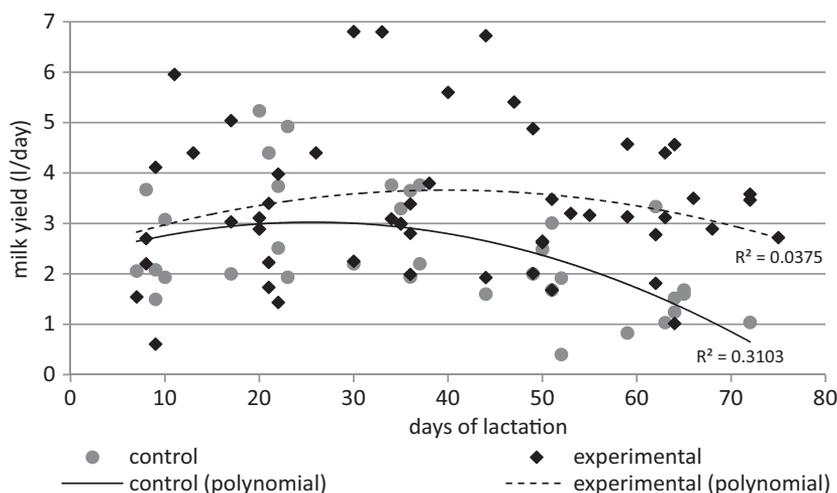


FIGURE 3. Trends in milk yields (l/day) during lactation in the Boer goats (n = 18)

data. That chart (Fig. 3) presented more beneficial lactation curve for does from the experimental group, although these data presents higher dispersion than the control group.

Milk yield plays a very important role in survival and growth of the young, especially in cattle, sheep and goats as well. It was reported that addition of condensed tannin forages (e.g. *L. corniculatus*) in ewes or supplementation of nicotinic acid in dairy cows could increase milk yield (Rochfort et al., 2008). Other studies described absence of effect of supplementation of garlic and juniper berry in dairy cows (Yang et al., 2006). Such studies on goats are poorly documented. However study of Malecky et al. (2009) indicated that use of monoterpene blend oils in diet expressed absence of effect on milk yield in milk goats.

Production traits – body weight and growth performance of kids. Body growth development of kids was examined on the group of 29 kids: 12 and 17

belonging to the control and experimental groups, respectively. The weighing of kids took place at the same time as milking controls of does. Kids from experimental groups were statistically heavier ($p < 0.01$) at birth and at 28th day of age (Tab. 3) than kids from the control group. Generally, kids from the experimental group presented higher body weights during the whole experiment and this trend was demonstrated on the Figure 4. Statistically better results of average daily gains (ADG) were observed in kids from the control group during whole research (Tab. 3), excluding the period of 15–28 day, when the kids from experimental group expressed higher ADG. Analysis of raw data also indicated this trend and it was presented on Figure 5. As it was mentioned previously, the milk yield is strongly correlated with growth development of the young ruminants (Rochfort et al., 2008), so this was also approved by the own results.

Some studies corresponds also to the herbal additives used in fattening of

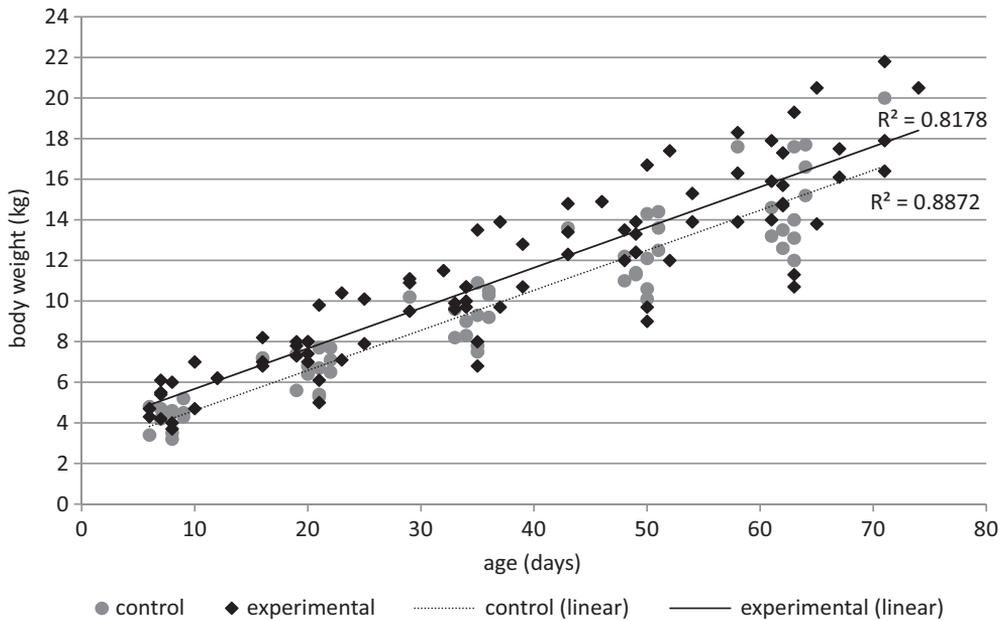


FIGURE 4. Trend in body weight of kids (kg) due to the group and age (n = 29)

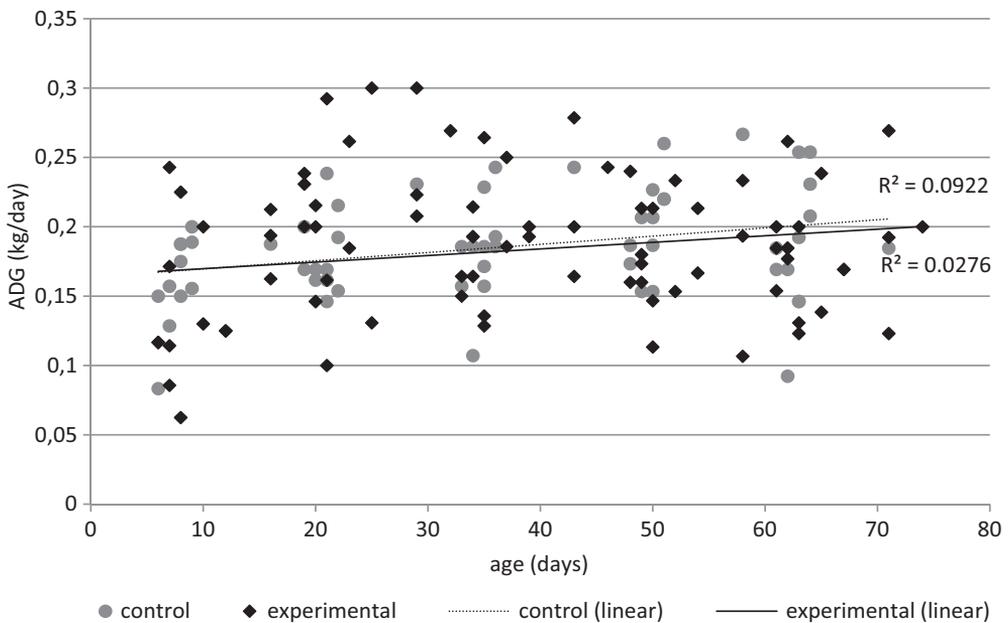


FIGURE 5. Trend in average daily gains – ADG (kg/day) in Boer goat kids due to the group and age (n = 29)

young lambs, calves or finishing bulls. Ringdorfer (2011) reported the absence of effect of use of three types of phyto-genic feed additives on any fattening performance traits as daily gains, dry matter intake or feed conversion in lambs, whereas the statistical ($p < 0.001$) influence of sex was observed. Better results were observed in cattle. Cornale et al. (2011) reported higher ($p < 0.05$) daily gains in finishing bulls after supplementation with plant extracts of ginger (*Zingiber officinale*) and european stoneseed (*Lithospermum officinale*), however these results differed due to breed and farm localization. In the study carried out by Ghosh et al. (2011), use of garlic supplementation in feeding of calves in the pre-ruminant stage caused ($p < 0.01$) higher daily gains, higher feed intake, better quality of faeces and higher costs of feeding.

CONCLUSIONS

Addition of ADICOX AP in preventing of coccidiosis in goats caused several observations due to the experimental group:

1. Significant lower number of oocytes per gram of faeces in adult goats;
2. Decrease of highly infected kids;
3. Advantageous course of lactation curve in milked goats;
4. Higher body weights of kids and higher daily gains during first weeks of rearing.

The ADICOX AP addition demonstrated its high usefulness in prevention of coccidiosis in goats. Moreover, the advantageous trends in production traits, both in adult goats and in kids, gave

additional benefit, which indicated the possibility of use ADICOX AP in standard breeding practice.

Moreover this study also indicated the necessity of researching for naturally based feeding solutions in animal diets which may be helpful in maintaining parasitic infections and bring better production yields for farmers.

REFERENCES

- ANON., 2003: Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 29–43.
- ANON., 2006: Statistical Product and Service Solution base version 14.0 for Windows. SPSS inc. USA. (Manuskrypt).
- BALICKA-RAMISZ A., 1999: Studies on coccidiosis in goats in Poland. Vet. Parasitol. 81, 347–349.
- CORNALE P., TARANTOLA M., LUSSIANA C., TASSONE S., CASTELLINA C, BATTAGLINI L.M., 2011: Effects of ginger (*Zingiber officinale*) and european stoneseed (*Lithospermum officinale*) extracts on performance, meat quality and fatty acid Composition of finishing bulls. Journal of Animal and Veterinary Advances, 10 (9), 1127–1132.
- GHOSH S., MEHLA R.K., SIROHI S.K., TOMAR S.K., 2011. Performance of crossbred calves with dietary supplementation of garlic extract. Journal of Animal Physiology and Animal Nutrition 95, 449–455.
- GUNDEŁACH J.L., SADZIKOWSKI A.B., 1995: Diagnostyka i zwalczanie inwazji pasożytów zwierząt. WAR Lublin.
- KABA J., KLOCKIEWICZ M., OSIŃSKA B., 2007: Kokcydioza u kóz. Życie Weterynaryjne 6, 497–499.
- KONSTANTINOOU A., 1973: Vergleichende Untersuchungen zur Methodik ueber die Milchleistungserfassung bei Fleischschafen sowie ueber die Zusammensetzung der Milch und die Auswirkungen der Milchleistung im Verlauf der Laktation auf die Wollfeinheit von Deutschen Schwarzkoepfigen Fleischschafen und Finnkreuzungen. Institut fuer Tierzucht u.

- Haustiergenetik der Justus Liebig Universitaet Giessen.
- KOWALSKI M., 2001: Normy żywienia kóz mlecznych według INRA. w: Normy żywienia bydła, owiec i kóz. Instytut Zootechniki w Krakowie, wyd. II, Kraków.
- MALECKY M., BROUDISCOU L.P., SCHIDELY P., 2009. Effects of two levels of mono-terpene blend on rumen fermentation, terpene and nutrient flows in the duodenum and milk production in dairy goats. *Animal Feed Science and Technology* 154, 24–35.
- OLEJNIK M., SZPRENGIER-JUSZKIEWICZ T., ŻMUDZKI J., 2009: Kokcydiostatyki w żywności pochodzenia zwierzęcego i paszach – nowe unormowania prawne. *Medycyna Weterynaryjna* 65(12), 807–811.
- RINGDORFER F., 2011: Effect of phytogetic feed additives on performance parameters of fattening lambs. *Book of Abstracts of the 62nd Annual Meeting of the European Federation of Animal Science Stavanger, Norway, 29 August – 2 September 2011. Book of Abstracts No. 17(2011), 45.*
- ROCHFORD S., PARKER A.J., DUNSHEA F.R., 2008: Plant bioactives for ruminant health and productivity. *Phytochemistry*, 69, 299–322.
- RUSZCZYC Z., 1981: *Metodyka badań zootechnicznych*. PWRiL, Warszawa.
- RÓŻAŃSKI H., DRYMEL W., 2010: *AdiCox jako źródło fitoaleksyn i fitoncydów*. *Polskie Drobniarstwo* 12, 17–20.
- SMITH M.C., SHERMAN D.M., 2009: *Goat Medicine*. Lea & Febiger, 312–319.
- TENTER A.M., BARTA J.R., BEVERIDGE I., DUSZYŃSKI D.W., MEHLHORN H., MORRISON D.A., ANDREW THOMPSON R.C., CONRAD P.A., 2002: The conceptual basis for a new classification of the coccidian. *International Journal for Parasitology*, 32, 595–616.
- YANG W.Z., CHAVES A.V., HE M.L., BEN-CHAAR C., MCALISTER T.A., 2006: Effect of monensin and essential oil on feed intake, ruminal fermentation, milk yield and composition of lactating dairy cows. *Canadian Journal of Animal Science*, 86, 598, Abstr.
- paszowych bazujących na bioaktywnych składnikach na poziom zainfekowania kokcydiami oraz na wskaźniki produkcyjne u kóz. Badania prowadzono na stadzie kóz burskich wraz z potomstwem, będącym w posiadaniu Doświadczalnej Fery Owiec i Kóz, RZD Żelazna, SGGW. Stado kóz podzielono na dwie grupy: doświadczalną i kontrolną, w których uwzględniono odpowiednio w analizie kału 14 i 10 matek, a w analizie cech mlecznych – 10 i 7 matek kozich. Kozę znajdowały się w 2–7 laktacji i odchowywały 1–2 kozłat. W żywieniu grupy doświadczalnej zastosowano dodatek roślinnej mieszanki ziołowej (ADOCXP AP) w dawce 200 g/tonę paszy treściwej. Doświadczenie prowadzono przez 3 kolejne miesiące od szczytu wykotów, rejestrując dane co dwa tygodnie: poziom mleczności kóz oraz masy ciała kozłat. W żywieniu kóz użyto pasz pochodzących z gospodarstwa w dawkach wg norm (Kowalski, 2001). Próby kału pobierano indywidualnie bezpośrednio z odbytu, przy czym u kóz dorosłych próbki pobierano dwa razy (na początku oraz na końcu doświadczenia), a u kozłat tylko przy zakończeniu doświadczenia. Stopień zainfekowania kóz dorosłych określono metodą McMastera, podczas gdy u kozłat zastosowano metodę flotacji w nasyconym roztworze NaCl. Przeprowadzone badania pozwoliły zauważyć, że zastosowanie preparatu ADICOX AP w profilaktyce kokcydiozy u kóz spowodowało w grupie doświadczalnej: 1) uzyskanie wyższej produkcji mleka u kóz matek, 2) uzyskanie wyższych mas ciała kozłat oraz wyższych przyrostów masy ciała w trakcie początkowego okresu odchowu, 3) istotne zmniejszenie liczby oocyst w 1 g kału u kóz dorosłych, oraz 4) korzystne oddziaływanie na zmniejszenie stopnia zainfekowania kokcydiami u kozłat. Na podstawie przeprowadzonych badań można stwierdzić wysoką przydatność preparatu ADICOX AP w profilaktyce kokcydiozy u kóz. Ponadto polepszenie cech produkcyjnych stanowi dodatkowy atut przemawiający za wprowadzeniem tego dodatku do standardowego użycia. Niniejsza praca wskazuje także na możliwość poszukiwania takich dodatków paszowych opartych na naturalnych składnikach, które mogłyby zarówno pomóc w opanowaniu infekcji pasożytniczych, jak i podnosić wyniki produkcyjne u zwierząt hodowlanych.

Streszczenie: *Wpływ stosowania ziołowego dodatku paszowego na poziom inwazji kokcydii oraz na wskaźniki użytkowe u kóz.* Celem badawczym było określenie wpływu roślinnych dodatków

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The analysis of chinchilla females reproduction (*Chinchilla laniger* M.), on the example of Polish breeding farm

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Abstract: *The analysis of chinchilla females reproduction (Chinchilla laniger M.), on the example of breeding farm.* Reproduction results of chinchilla females from 1997–2010, were collected on selected Polish breeding farm, and evaluated by means of typical indices. The comparison of monthly births distribution led to conclusion, that pups are born on the farm throughout the year with varying frequency. There was a significant difference between the litter number in winter and summer, although it seems probable that it was not a seasonal tendency but a consequence of maintenance and husbandry. The breeding of chinchillas in optimal environmental conditions likely leads to improved domestication and reproduction.

Key words: chinchilla, reproduction, domestication

INTRODUCTION

Chinchillas are polyestrous herbivores, reaching the sexual maturity in the age of 6 months. Long pregnancy, lasting up to 111 days and small litters cause that breeders aspire to obtain two births per year from female. Good husbandry effects depend, above all, on reproduction results. The size of farm chinchilla litters is currently strongly diversified.

According to the literature, the number of progeny in the litter varies from 1 to 6 (Barabasz, 2001).

Chinchilla's polyestrous reproduction cycles show seasonality symptoms (Sulik et al., 2001). The reproduction performance of chinchillas depends on the farm environment. At the wild, the duration of chinchilla reproductive cycles relies directly on the season, so pups are usually born in the period most abundant in food. In the highland climate of Andes, the typical reproduction time for chinchillas occurs between April and September, which complies to the period from early spring till summer, in European conditions (Gromadzka-Ostrowska, 1998).

There are several reports of chinchillas reproduction seasonality in Poland, however it is still unclear, if over one hundred years of the reproduction of chinchillas in captivity diminished the seasonality of births in particular periods of the year. The aim of this study was to evaluate the results of chinchilla females reproduction results on the selected breeding farm and to describe the annual schedule of births.

MATERIAL AND METHODS

The data was collected for fourteen consecutive years (1997–2010) on the random chinchilla breeding farm, located in central Poland. The farm was established in 1996 on the basis of 4 chinchilla families. In 2002 it gained the status of reproduction farm and since then detailed reproduction records were stored and evaluated.

Animals were housed in two rooms, in polygamy sets of cages (4 females – 1 male) coupled in four levels. Females had individual rooms while the male shared the cage with females, moving through the corridor, having free access to each female. All females had a neck collar, preventing from leaving the cage. Since 2005, animals were housed without bedding, with moved drawers, which appears less labor consuming. A necessary equipment in the cage consists of automatic feeder, the dust bath tub and the teeth wiping stones. Animals were fed with dry pellets with the access to hay. Once a week a mixture of herbs, cereals and rosehips was offered. Drinking water was constantly supplied.

Data were processed with one way ANOVA analysis of variance, performed in SPSS Statistics 17 software (SPSS, Poland). The distribution of means was tested with t-test. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 presents basic indicators of the reproduction of chinchilla females on the farm from since the beginning of animals mating in 1997. It can be noted, that the

growing experience of the breeder resulted in improved reproduction results. The total number of progeny increased every year. In 1997, 90 pups were born from 60 females with 74 weaned (82%). However, in 2000 89 pups were weaned (89%), and in 2006 the percentage of weaned pups reached 95.26% (Tab. 1).

The mortality before weaning ranged from 4.74 to 17.78%. Different authors report mortality rate from 10.4 to 25.1% (Sulik and Barabasz, 1995; Felska et al., 2002; Seremak and Sulik, 2002b). In 1997–1998 weaning index was visibly low (82%). It can be likely explained with the fact, that animals were just purchased and the breeder didn't have the possibility of assessing female's reproductive value. In consecutive years most indicators improved which confirms former notion.

The number of litters and total progeny count showed continuous growth. Consequently, the number of infertile females decreased with reduced mortality of young animals. Seremak and Sulik (2003) reported average annual mortality at 19%, with the highest value reaching 25.1% correlated with low number of litters.

In 2002, 4.08 pups per female were born with 3.53 pups weaned. The following years show a slight decrease in the number of pups born, however the number of weaned pups was constantly > 3 . These are higher values than those reported by Sulik and Barabasz (1995), who reported the fertility of the herd as 2.4 pups born and 2.09 weaned from the female per year. However, there are reports of 3.46 pups born and 3.05 pups weaned from the female per year from females imported from Denmark (Felska and Brzozowski, 2001).

TABLE 1. The reproduction characteristics of chinchilla females (1997–2010)

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Number of females	60	60	60	60	60	60	60	72	72	86	102	102	102	102
Number of born pups	90	167	211	191	230	245	219	271	268	232	300	326	293	342
Number of weaned pups	74	137	181	171	191	212	202	256	246	221	274	307	278	312
% of weaned pups	82.22	82.04	85.78	89.53	83.04	86.53	92.24	94.46	91.79	95.26	91.33	94.17	94.88	91.23
Average litter size	1.76	1.88	1.95	1.84	2.13	2.01	2.01	2.04	1.93	1.90	2	1.86	2.09	2.2
Pups per female	born	1.50	2.78	3.52	3.18	4.08	3.65	3.76	3.72	3.22	2.94	3.19	2.87	3.35
	weaned	1.23	2.28	3.02	2.85	3.53	3.37	3.56	3.42	3.06	2.67	3	2.72	3.06
Number of litters	51	89	108	104	108	122	109	133	139	122	150	175	140	155
Number of litters from female	0.85	1.48	1.8	1.73	1.8	2.03	1.82	1.85	1.93	1.69	1.47	1.72	1.37	1.52

The size of the litter at birth is an essential parameter, influencing the annual reproduction results on the farm. In the present study high values of this index were obtained. During last decade it reached from 1.76 to 2.13, which can be considered satisfying, not differing from the data published in other reports (1.98 – Seremak and Sulik, 2003). Nevertheless there are reports of 1.81 to 2.36 litter size at birth (Socha and Wrona, 2000). It should be noted, that the potential of chinchilla females reproduction is high. Jarosz and Rżewska (1996) report 4 pups born in the litter. In the estrous phase about 4 follicles develop, so in optimal conditions the size of the litter can be 4.

The number of litters from female grew from 0.85 in 1997 to 2.03 in 2002. This value is the highest on this farm and higher than that reported by other authors. Sulik and Barabasz (1995) mentioned 1.5 litter per year, Felska and Brzozowski (2001) – 1.59 for imported chinchillas. Barabasz et al. (2000) however, reported 1.54–1.9 litters per year on examined farms. 2 litters from the female per year is beneficial and in the accordance with the natural annual rhythm of animals. Long pregnancy, low fertility and the numerous litters from females results in poor profitability of breeding of chinchillas. However, three births per year can lead to excessive exhaust of the female organism and cease the reproduction activity.

The second studied aspect was the seasonal pattern of births in chinchilla. Table 2 presents the monthly distribution of births as a % of the total annual number of litters, calculated for years 2002–2010 (the breeding value estimation period on the farm). There were sig-

TABLE 2. The percentage of litters (% of the total number of litters)

	2002	2003	2004	2005	2006	2007	2008	2009	2010
January	4.92	4.59	2.26	6.47	4.10	5.33	3.43	4.29	3.23
February	7.38	8.26	7.52	2.88	2.46	8.67	6.86	5.71	6.45
March	18.03	11.93	6.77	5.76	13.93	12.67	8.57	13.57	8.39
April	7.38	13.76	12.78	9.35	12.30	8.67	10.86	7.86	13.55
May	6.56	8.26	10.53	12.95	8.20	8.67	7.43	10.00	6.45
June	10.66	6.42	9.02	7.19	7.38	12.67	7.43	6.43	7.74
July	12.30	5.50	10.53	6.47	13.93	8.00	9.14	15.00	13.55
August	5.74	6.42	9.77	16.55	6.56	12.00	11.43	11.43	8.39
September	7.38	8.26	9.02	10.07	9.84	10.00	9.71	6.43	7.74
October	6.56	13.76	2.26	7.91	4.92	7.33	8.57	5.71	8.39
November	8.20	7.34	9.77	8.63	9.02	2.67	10.86	7.14	6.45
December	4.92	5.50	9.77	5.76	7.38	3.33	5.71	6.43	9.68

nificant differences in all months of the year and among reproduction years. Pups were born every month of the year, with the increased tendency in March, April, July and August. The lowest values were recorded for January.

Results of the analysis of reproduction indices, presented in Table 3 are similar to those, reported by Seremak (2007). The lowest number of litters were observed in January and December, whereas the most of births occurred in March/April and July/August ($P < 0.01$). Significantly lower performance ($P < 0.05$) was observed in October, November and December. However it should be noted, that there were no overall significant decrease observed in reproduction on the farm in the analyzed period. The differences in the number of pups at birth and weaning were not significant (Tab. 3).

Jarosz and Rżewska (1996), described the increased period of chinchillas mating activity in Poland from November till May, with the particular intensity in January and February. Moreover, animals reared during winter in heated rooms had a distinct seasonal charac-

ter of the reproduction. Nevertheless, females reared in controlled temperature were sexually active through all the year. Gromadzka-Ostrowska (1998) reported chinchilla reproduction time in Poland from November till April with the highest intensity in March. Considering 111 days of pregnancy, the intensive births last from March till August.

However, the high percentage births was also observed in Autumn months but not in a regular pattern (October, 2003; September 2005; November 2008 – Tab. 2). The plausible explanation of the differences in the reproduction can be husbandry and housing conditions on the farm. With the constant light program, atavistic intensification of reproduction due to the elongation of the day tends to disappear.

CONCLUSIONS

We would like to conclude, that the reproduction results on chinchilla farms depend largely on the proper husbandry and housing methods. It seems likely, that there is a distinct mitigation of the

TABLE 3. Comparison of reproduction results

Index	January		February		March		April		May		June		July		August		September		October		November		December	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	5.9 ^A	1.8	8.7	3.3	15.0 ^{Bb}	4.7	14.8 ^{Bb}	3.8	12.1	3.1	11.6	3.5	14.6 ^{Bb}	5.0	13.9 ^{Bb}	5.8	12.1	2.8	10.0 ^B	4.1	10.8 ^a	4.0	9.0 ^a	3.3
2	12.2	4.3	18.1	6.3	29.3	8.4	30.3	8.8	23.3	6.5	23.3	8.9	28.7	8.9	29.7	11.5	24.7	5.4	19.0	8.7	20.0	8.9	18.7	6.4
3	11.7	4.2	16.2	5.2	26.4	7.6	28.3	7.8	22.0	6.1	21.1	8.4	25.7	7.9	27.4	10.4	23.3	4.9	18.4	8.1	18.2	8.8	17.6	6.8

1 – the number of litters

2 – the number of pups born

3 – the number of pups weaned

values are means ± standard deviation

values with different upper case superscript letters are significant at $P < 0.01$

values with different lower case superscript letters are significant at $P < 0.05$

seasonal pattern in chinchillas reproduction activity due to continuous domestication.

REFERENCES

- BARABASZ B., 2001: Szynszyle. Hodowla i użytkowanie. PWRiL, Warszawa.
- BARABASZ B., FORTUŃSKA D., BIENIEK J., 2000: Ocena intensywności użytkowania rozplodowego samic szynszyli. Zesz. Nauk. AR Kraków 369(35), 121–133.
- GROMADZKA-OSTROWSKA J., 1998: Studia nad fizjologią szynszyli ze szczególnym uwzględnieniem rozrodu i odporności. AR Kraków, zeszyt 238. Rozprawa habilitacyjna.
- FELSKA L., BRZOZOWSKI M., 2001: Porównanie wyników rozrodu trzech grup genetycznych szynszyli. Zeszyty Naukowe PTZ 58: 31–38.
- FELSKA L., BRZOZOWSKI M., RZEWUSKA E., 2002: Wyniki rozrodu szynszyli w zależności od poziomu ustawienia klatek i natężenia światła. Zesz. Nauk. PTZ, 64, 97–102.
- JAROSZ S., RZEWUSKA E., 1996: Szynszyle, chów i hodowla. PWRiL, Warszawa.
- SULIK M., SEREMAK B., BIELŃSKA A., MIELEŃCZUK G., 2001: Intensywność użytkowania rozplodowego samic szynszyli na wybranej fermie na Pomorzu Zachodnim. Zesz. Nauk. PTZ, 58, 73–80.
- SULIK M., BARABASZ B., 1995: Porównanie systemów utrzymania rozplodowego na przykładzie wybranych ferm. Zesz. Nauk. AR w Krakowie 297, 159–165.
- SEREMAK B., SULIK M., 2002a: Sezonowa aktywność rozrodcza samic szynszyli na wybranych fermach. Zesz. Nauk. PTZ, 64, 89–96.
- SEREMAK B., SULIK M., 2002b: Charakterystyka wskaźników użytkowania rozrodczego szynszyli na przykładzie wybranej fermy 1997–2000. Acta Scient. Polon., Zootechnica, 1 (1–2), 139–146.
- SEREMAK B., SULIK M., 2003: An attempt to determine causes of lowered values of breeding indices on a chinchilla farm. Scientifur, 26(3), 75–78.
- SEREMAK B., 2007: Wybrane aspekty rozrodu szynszyli (*Chinchilla laniger M.*) w chowie fermowym. AR Szczecin. Rozprawa habilitacyjna.

SOCHA S., WRONA A., 2000: The analysis of the seasonal character of the chinchilla (*Chinchilla velligera* M.) reproduction. *Scientifur*, 24(4), 49–52.

Streszczenie: *Analiza wyników reprodukcyjnych samic szynszyli (*Chinchilla laniger* M.) na przykładzie polskiej fermi reprodukcyjnej. W pracy dokonano analizy ważniejszych wskaźników reprodukcyjnych samic szynszyli zebranych na wybranej fermie reprodukcyjnej w latach 1997–2010. Porównanie rozkładu ocenianych wartości w poszczególnych miesiącach pozwoliło stwierdzić, że młode rodzą się z różnym natężeniem przez cały rok. Liczba miotów uzyskanych w poszczególnych miesiącach roku różniła się istotnie pomiędzy okresem zimowym a letnim, jednak wydaje się że zmiany te nie noszą znamion sezonowości, a są wynikiem zróżnicowania warunków utrzymania i obsługi na fermie. Jest zatem prawdopodobne, że chów szynszyli w optymalnych warunkach środowiskowych spowoduje pogłębienie udomowienia*

i dalsze wyrównanie wskaźników reprodukcyjnych.

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Utilization of the selected microsatellite sequences in optimizing the mating plan in an experimental flock of Ayam Cemani breed

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Abstract: *Utilization of the selected microsatellite sequences in optimizing the mating plan in an experimental flock of Ayam Cemani breed.* The parental flock initially included 2 cocks and 9 hens, that originated from different breeding farms, and then the following F₁ generation included 42 birds (15 hens and 27 cocks). The experimental flock of Ayam Cemani breed was bred at the Poultry Farm of the University of Life Sciences in Wilanów-Obory. Owing to the specific character of the experiment, the following microsatellite sequences were selected based on literature data: MCW0145, MCW0184, MCW0210, LEI0071, and ADL0306, all being linked with the body weight of hens (Atzmon et al., 1998; Atzmon et al., 2008; Weissmann et al., 1998; Tatsuda and Fujinaka, 2001). The earlier investigations demonstrated that the microsatellite sequences investigated in chicken of the Ayam Cemani breed were polymorphic (Gruszczyńska and Łukasiewicz, 2010). Results of molecular analyses enabled determining the genetic distance between all pairs of birds in the flock. It was demonstrated that the chosen microsatellite sequences might be successfully used as genetic markers in the tests concerning chicken origin. The method involving the use of microsatellite sequences in order to determine the genetic distance and then to optimize the mating plan turned out to be successful, for in the experimental flock the hatchability from set eggs increased to a highly significant extent from 50.1% to 74.1%.

Key words: Ayam Cemani breed, microsatellite sequences, genetic distance, mating plan optimization.

INTRODUCTION

Ayam Cemani is a rare black breed of chickens originating from Indonesia. It was first time imported into Europe in 1998 by a Dutch breeder Jan Steverink. The population of Ayam Cemani in Europe is small, they are usually bred as ornamental chickens.

The choice of polymorphic *loci*, suitable for genetic analysis must be preceded by many tests on the ground of which the level of heterozygosity ($H_E > 0.6$ and $PIC > 0.6$), allele frequency, mutation frequency, the possibility of correct genotyping as well as a risk of committing the errors will be, *inter alia*, determined. Microsatellite sequences are short tandem repeats (STRs), consisting of two-, three-, and four-nucleotide motives. They are usually located in the non-coding regions of the genome. They are commonly used in the molecular analysis of animal origin. The polymorphic DNA microsatellites are preferred markers in pedigree control in human as well as in many animal species (Freis et al., 1990; Bowling et al., 1997; Jamieson and Taylor, 1997; Schnabel et al., 2000; Fung et al., 2002; Ganai and

Yadav, 2005; Wenk et al., 2005; Oliveira et al., 2006; Radko, 2008; Riojas-Valdes et al., 2009). The microsatellite sequences are also used in studies addressing the reconstitution of species, as well as in order to determine the genetic relationship between animals and to perform matings that would assure the greatest genetic diversity in a herd and prevent homozygosity increase in the population.

MATERIAL AND METHODS

Animal material and molecular analysis

The experimental flock of Ayam Cemani breed was bred at the Poultry Farm of the University of Life Sciences in Wilanów-Obory. Initially, the flock included 2 cocks and 9 hens originating from different breeding farms (from 1 German breeder: 1♂ and 5♀, and from 1 Dutch breeder: 1♂ and 4♀). The obtained offspring generation included 42 birds (15 hens and 27 cocks). In the first year of the study, results of the hatchability of the imported birds were not satisfactory, which might have been due to high homozygosity between the birds. Hence, an attempt was made to construct a mating plan for the experimental flock of Ayam Cemani breed, based on the specified genetic diversity. Following the plan, 13 pairs (at the age of 1 year) were mated and kept in separate pens throughout the reproductive period. The eggs obtained were incubated in weekly sets of 60 eggs.

The method used to determine the genetic diversity of the investigated population of birds included 5 microsatellite sequences (MCW0145, MCW0184, MCW0210, LEI0071, ADL0306). Owing to the specific character of the experi-

ment, the microsatellite sequences were chosen based on literature data, that in chicken were linked with the body weight (Atzmon et al., 1998; Atzmon et al., 2008; Weissmann et al., 1998; Tatsuda and Fujinaka, 2001). The earlier research demonstrated that microsatellite sequences analyzed in the investigated flock of Ayam Cemani breed were polymorphic (Gruszczyńska and Łukasiewicz, 2010). The values obtained for expected heterozygosity (H_E), observed heterozygosity (H_O), and Polymorphic Information Content (PIC) confirm the usability of the selected microsatellite sequences as markers being a convenient tool in the genetic diversity analysis of a chicken population (Gruszczyńska and Łukasiewicz, 2010).

Statistical analysis of results

Based on the results of the previous study (Gruszczyńska and Łukasiewicz, 2010): the frequency of alleles and genotypes, expected heterozygosity (H_E), observed heterozygosity (H_O), Polymorphic Information Content (PIC), and the exact test of Hardy-Weinberg equilibrium were calculated with the Cervus 3.03. Programme (Kalinowski et al., 2007). Additional determinations were made for the probability of exclusion (PE) and combined probability of exclusion (CPE) (Wenk et al., 2005) and genetic distance between the individuals (Nei, 1972):

1. The a priori probability of exclusion PE was obtained using the formula by Evett and Weir (1998):

$$PE = (H_O)^2 [1 - 2 H_O (1 - H_O)^2]$$

where H_O is the observed frequency of heterozygotes in database of the offspring group ($n = 42$) and the whole population ($n = 53$).

2. The Combined exclusion probability (CPE) was calculated using the formula (Jamieson and Taylor, 1997; Wenk et al., 2005):

$$\text{CPE} = 1 - (1 - \text{PE1})(1 - \text{PE2})(1 - \text{PE3})(1 - \text{PE4})(1 - \text{PE5})$$

3. Nei's standard genetic distance enabling the determination of the genetic diversity between pairs of individuals originating from the common founder population, was computed using the following formula (Nei, 1972):

where f_{Fi} and f_{Bi} denote frequencies of the i -th allele in respectively F and B population.

$$d_{FB} = -\ln \left(\frac{\sum_i f_{Fi} f_{Bi}}{\sqrt{\sum_i f_{Fi}^2 \sum_i f_{Bi}^2}} \right)$$

The results achieved were analyzed with the use of a matrix of distances determined between particular males and females based on the frequency of alleles occurring in the 5 analyzed *loci*.

The values obtained for all combinations of pairs enabled optimizing the mating plan. In this way, pairs were made of birds with possibly the highest value of the genetic distance.

RESULTS AND DISCUSSION

It was found that the number of alleles, varying from 4 to 8, the level of H_E and PIC value (above 0.7) and the probability to identify two individuals with the same genotype 1:2721600 (Tab. 1), as indicated by Crooijmans et al. (1996), Crooijmans et al. (1997), Rosario et al. (2009), Weissmann et al. (1998), Tadano et al. (2007), are sufficiently high to apply the selected microsatellite sequences in checking the origin of the birds derived from the assumed parents. In turn, in the analyzed flock of Ayam Cemani, the probability of finding two birds with the same genotype using these sequences reached 1:6480 (Tab. 1).

Table 2 collates values of the exact test of Hardy-Weinberg equilibrium in terms of the investigated microsatellite sequences in the parental and F_1

TABLE 1. Number of possible genotypes in a domestic chicken and in the investigated flock of Ayam Cemani breed

<i>Locus</i>	Number of alleles in domestic chicken	Number of possible genotypes*	Number of alleles in Ayam Cemani	Number of possible genotypes*
MCW0145	8 ^{a),e)}	36	4	10
MCW0184	6 ^{b)}	21	2	3
MCW0210	4 ^{b),d)}	10	3	6
LEI0071	8 ^{c)}	36	3	6
ADL0306	4 ^{d)}	10	3	6
Probability of finding two individuals with the same genotype		1:2721600		1:6480

^{a)} Crooijmans et al., 1996; ^{b)} Crooijmans et al., 1997; ^{c)} Rosario et al., 2009; ^{d)} Weissmann et al., 1998; ^{e)} Tadano et al., 2007.

* following to the formula: $\{n!/[2(n-2)!]\}+2$, where n – number of alleles.

generation as well as for the entire analyzed population of Ayam Cemani breed. It was demonstrated that in the case of the MCW0210 microsatellite sequence the differences in genotypes distribution were significant, whereas in the case of the ADL0306 sequence – highly significant (Tab. 2).

The computed coefficient of the probability of exclusion (PE) and combined probability of exclusion (CPE), accounting for 0.973 in the parental group and 0.981 in the offspring group (Tab. 3) of the investigated flock, point to the feasibility of applying the selected microsatellite sequences for origin determination in the experimental flock of Ayam Cemani breed.

Based on the frequency of the selected microsatellite sequences, coefficients of observed heterozygosity (H_O), expected heterozygosity (H_E) and Polymorphic Information Content (PIC) were computed in the analyzed population. The calculated values of H_E coefficient were ranging from 0.48 to 0.71 in the parental generation and from 0.39 to 0.64 in the F_1 generation (Tab. 3). Values of the PIC were slightly lower than those of the H_E . The highest number of homozy-

gotes in both generations was observed for the MCW0184 sequence, i.e.: 82% and 81%, respectively. In terms of the ADL0306 sequence, none homozygotes were identified in the parental generation, whereas in the offspring generation they constituted 4.8%. In addition, in the F_1 generation, the loss of 218bp allele was observed in the MCW0145 locus.

Due to the fact that the initial number of birds (parental generation) consisted of 11 birds (2 cocks and 9 hens) and that the origin of the offspring generation was not completely identified, all birds in the flock were genotyped. Owing to the fact that based on the results achieved the origin of the birds was still not inexplicit, use was made of the values of a standard genetic distance computed for each pair of birds based on the frequency of alleles. The mean genetic distance in the parental generation (11 birds) accounted for 0.4877, whereas in the offspring generation (42 birds) – for 0.5432. Values of the mean genetic distance of particular males with a group of females and that of individual females with a group of males were reported in Table 4. The birds were mated so as the genetic distance between

TABLE 2. Results of the exact test of Hardy-Weinberg equilibrium in terms of the analyzed microsatellite sequences in the experimental population of Ayam Cemani breed

Locus	MCW0145	MCW0184	MCW0210	LEI0071	ADL0306
Parental generation	ND	ND	ND	ND	ND
Offspring generation	NS	ND	ND	NS	***
The whole investigated population of Ayam Cemani	NS	ND	*	NS	***

Exact test of Hardy-Weinberg equilibrium:

* significant differences ($p \leq 0.05$)

** highly significant differences ($p \leq 0.01$)

*** extremely significant differences ($p \leq 0.001$)

NS – not significant.

ND – not determined owing to a too few number of groups.

the pairs of mated individuals was as high as possible.

In the first phase (before optimizing the mating plan) of hatching the eggs originating from one-year-old hens of the F₁ generation, despite a satisfactory fertility at a level of 91%, the hatchability of set eggs reached as little as 50.0% (N = 60). The major reason behind that was a high embryonic death rate – at each developmental stage. The conducted bacteriological examinations did not reveal any health disorder of the hens. The likely cause could, thus, be high homozygosity of the mated individuals and misadaptation to a new environment. Once the mating plan had been optimized based on the computed genetic distances, no significant changes were observed in the egg fertility (92%), whereas a highly significant ($p \leq 0.01$) increase was noted in hatchability of set eggs (N = 270) to a level of 74.1%. As reported by Borzemska and Kosowska (1997), losses in hatchings reaching from 7.5% to 20%, depending on the species, breed and utilization of birds, should be considered normal, i.e. within the physiological range. According to Borzemska (2005), in the case of hens the hatchability reaches 80–92%, and even 93.5%. However, an earlier study by this author (Borzemska, 1996) suggests that the hatchability of 78% may point to nutritional errors or wrong flock matching. A high diversity in the body weight of birds observed in the experimental flock of Ayam Cemani (1320 g to 2192 g), may significantly affect the hatchability as well as the quality of chicks. This in turn results in non-simultaneous (desynchronized) hatching of chicks, i.e. early from small eggs and late from large eggs.

This manuscript presented the feasibility of applying microsatellite sequences for identifying birds origin and then for optimizing the mating plan.

CONCLUSIONS

1. The selected microsatellite sequences might be successfully used as genetic markers in the tests concerning chicken origin.

2. The method involving the use of microsatellite sequences in order to determine the genetic distance and then to optimize the mating plan turned out to be successive, for in the experimental flock the hatchability from set eggs increased to a highly significant extent from 50.1% to 74.1%.

REFERENCES

- ATZMON G., BAXTER-JONES C., YONASH N., CHENG H., AVIDAN N., LAVI U., CAHANER A., HILLEL J., 1998: Microsatellite markers associated with quantitative traits in broilers. Proceedings, 10th European Poultry Conference” The Poultry Industry Towards the 21st Century”, Jerusalem Israel, 21–26 June, 1: 191–194.
- ATZMON G., BLUM S., FELDMAN M., CAHANER A., LAVI U., HILLEL J., 2008: QTLs detected in a multigenerational resource chicken population. *The J. Hered.* 99 (5): 528–538.
- BORZEMSKA W.B., 1996: Embriopatologia drobiu. *Drobiarstwo*, 10: 5–9.
- BORZEMSKA W.B., 2005: Choroby drobiu. *Praca zbiorowa pod redakcją M. Mazurkiewiczza*. Wyd. Akad. Rol. we Wrocławiu, 69–124.
- BORZEMSKA W.B., KOSOWSKA G., 1997: Ważniejsze problemy w patologii łęgów u drobiu. *Zesz. Nauk. Przegl. Hod.* 3: 25–31.
- BOWLING A.T., EGGLESTON-STOTT M.L., BYRNS G., CLARK R.S., DILEANIS S., WICTUM E., 1997: Validation of microsatellite markers for routine horse parentage testing. *Anim. Genet.* 28: 247–252.

- CROOIJMANS R.P., DIJKHOF R.J.M., POEL J.J., GROENEN M.A.M., 1997: New microsatellite markers in chicken optimized for automate fluorescent genotyping. *Anim. Genet.* 28: 427–437.
- CROOIJMANS R.P., VAN OERS P.A., STRIJK J.A., VAN DER POEL J.J., GROENEN M.A., 1996: Preliminary linkage map of the chicken (*Gallus domesticus*) genome based on 77 microsatellite markers: 77 new markers mapped. *Poultry Sci.* 75: 746–754.
- EVETT L.W., WEIR B.S., 1998: Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists. Sinauer Associates, Inc., Sunderland, MA.
- FREIS R., EGGEN A., STRANZIENGER G., 1990: The bovine genome contains polymorphic microsatellites. *Genomics* 8: 403–440.
- FUNG W.K., CHUNG Y., WONG D., 2002: Power of exclusion revisited: probability of excluding relatives of the true father from paternity. *Int J. Legal Med* 116: 64–67.
- GANAI N.A., YADAV B.R., 2005: Parentage determination in three breeds of Indian goat using heterologous microsatellite markers. In: H.P.S. Makkar and G.J. Viljoen (eds.) Applications of Gene-Based Technologies for Improving Animal Production and Health in Developing Countries 613–620.
- GRUSZCZYŃSKA J., ŁUKASIEWICZ M., 2010: Microsatellite polymorphism in the study of genetic diversity of an experimental flock of Ayam Cemani breed, *Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci.* 47: 39–44.
- JAMIESON A., TAYLOR S.T.C.S., 1997: Comparisons of three probability formulae for parentage exclusion. *Anim. Genet.* 28: 397–400.
- KALINOWSKI S.T., TAPER M.L., MARSHALL T.C., 2007: Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* 16: 1099–1006.
- NEI M., 1972: Genetic distance between populations. *The American Naturalist.* 106: 283–291.
- OLIVEIRA S., TRINDADE-FILHO A., MENDES C., PAULA K., MAIA F., PAK H., DALTON G., 2006: Power of exclusion of 18 autosomic STR loci in a Brazilian Middle-West region population sample. *International Congress Series* 1288: 433–435.
- RADKO A., 2008: Ocena polimorfizmu markerów mikrosatelitarnych DNA wykorzystywanych w kontroli rodowodów bydła w Polsce. *Wiadomości Zootechniczne, R. XLVI, 4:* 3–8.
- RIOJAS-VALDES V.M., GOMEZ DE LA FUENTE J.C., GARZA-LOZANO J.M., GALLARDO-BLANCO D.C., DE TELLITU_SCHUTZ J.N., WONG-GONZALEZ A., DAVALOS-ARANDA G., SALINAS-MELENDEZ J.A., 2009: Exclusion probabilities of 8 DNA microsatellites in 6 cattle breeds from Northeast Mexico. *J. of Anim. and Vet. Advances* 8(1): 62–66.
- ROSARIO M.F., LEDUR M.C., MOURA A.S.A.T., COUTINHO L.L., GARCIA A.A.F., 2009: Genotypic characterization of microsatellite markers in broiler and layer selected chicken lines and their reciprocal F1s. *Sci. Agric. (Piracicaba, Braz.)* 66: 150–158.
- SCHNABEL R.D., WARD T.J., DERR J.N., 2000: Validation of 15 microsatellites for parentage testing in North American bison, Bison bison and domestic cattle. *Anim. Genet.* 31: 360–366.
- TADANO R., NISHIBORI M., NAGASAKA N., TSUDZUKI M., 2007: Assessing genetic diversity and population structure for commercial chicken lines based on forty microsatellite analyses, *Poultry Sci.* 86: 2301–2308.
- TATSUDA K., FUJINAKA K., 2001: Genetic mapping of the QTL affecting body weight in chickens using a F2 family. *Br. Poultry Sci.* 42(3): 333–337.
- WEISSMANN S., CARMON T., DOUAIRE M., CAHANER A., ZEITLIN G., LAVI U., AVIDAN N., LECLERCQ B., HILLEL J., 1998: Marker gene frequencies within and between divergently selected lines for high and low abdominal fat in chickens. *Proceedings, 10th European Poultry Conference* "The Poultry Industry Towards the 21st Century", Jerusalem Israel, 21–26 June, 1: 280–283.
- WENK R.E., GJERTSON D.W., CHIAFARI F.A., HOUTZ T., 2005: The specific power of parentage exclusion in a child's blood relatives. *Transfusion* 45: 440–444.

Streszczenie: Wykorzystanie wybranych sekwencji mikrosatelitarnych w optymalizacji planu kojarzeń doświadczalnego stada kur rasy ayam cemani. Stado rodzicielskie liczyło

początkowo 2 koguty i 9 kur, które pochodziły z różnych hodowli, a następne uzyskane po nich pokolenie F₁ liczyło 42 ptaki (15 kur i 27 kogutów). Stado doświadczalne pochodziło z fermi drobiu Rolniczego Zakładu Doświadczalnego SGGW Wilanów-Obory. Ze względu na specyfikę prowadzonego doświadczenia na podstawie literatury wybrano sekwencje mikrosatelitarne: MCW0145, MCW0184, MCW0210, LEI0071, ADL0306, które były u kur związane z masą ciała (Atzmon et al., 1998; Atzmon et al., 2008; Weissmann et al., 1998; Tatsuda and Fujinaka, 2001). We wcześniej przeprowadzonych badaniach stwierdzono, że wybrane sekwencje mikrosatelitarne u kur rasy ayam cemani są polimorficzne (Gruszczyńska and Łukasiewicz, 2010). Na podstawie wyników badań molekularnych wyznaczono dystans genetyczny między wszystkimi parami ptaków występujących w stadzie. Stwierdzono, iż wybrane sekwencje mogą być z sukcesem wykorzystywane w identyfikacji pochodzenia u kur. Metoda wykorzystania sekwencji mikrosatelitarnych w celu ustalenia

dystansu genetycznego, a następnie na tej podstawie ustalenia planu kojarzeń okazała się trafna, gdyż w doświadczalnym stadzie wylęgowość z jaj nałożonych wzrosła wysoko istotnie statystycznie z 50,1% do 74,1%.

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A molecular test for the detection of the C295G mutation in the T gene responsible for shortened tail and taillessness in the Pembroke Welsh Corgi

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Abstract: *A molecular test for the detection of the C295G mutation in the T gene responsible for shortened tail and taillessness in the Pembroke Welsh Corgi.* A ban on cutting tail in dogs has been introduced in many countries. In order to exhibit on an official pedigree show a tailless or short-tailed dog, its owner must provide a statement confirming that the short tail is natural characteristic of his dog. The aim of this paper was to design a test to diagnose a C295G dominant mutation in T gene which is responsible for short-tail phenotype in 17 dog breeds. This mutation creates a *BstEII* restriction site. The molecular test designed is based on genomic DNA analysis. A fragment of T gene is amplified in polymerase chain reaction (PCR). Then, the PCR product is treated with restriction enzyme *BstEII*, and the analysis of restriction fragments is carried out in polyacrylamide gel. A genotype of 30 Polish Pembroke Welsh Corgi regarding T gene has been determined. Within the studied group of dogs, 10 heterozygotes and 20 recessive homozygotes have been found. No dominant homozygotes have been present.

Key words: *Canis familiaris*, Pembroke Welsh Corgi, short-tail, taillessness, T-box gene.

INTRODUCTION

The T gene belongs to the family of T-box genes and occurs in many species of animals, both vertebrates and invertebrates. This is a large family of genes

encoding transcription factors expressed during the embryonic development. Most of the T proteins act as transcription activators, only few of them are repressors. The T protein activates genes specific to the mesoderm and is involved in the development of structures derived from the posterior mesoderm, including notochord. The first discovered gene belonging to a T-box gene family is known as Brachyury or T (for tail) gene, which is responsible for the short tail in mice (Kavka and Green, 1997; Papaioannou and Silver, 1998).

In many species the short tail can be conditioned by the presence of mutations in the T gene. T/Brachyury gene at the stage of the embryonic development is involved in the process of differentiation of axial structures, such as notochord and neural tube. Therefore, taillessness and short-tail phenotype induced by the mutation of this gene is associated with the presence of a number of other defects revealing mainly in homozygotes during the embryonic development. Abnormalities in the embryonic development are usually the cause of necrobiosis of these embryos. So in homozygotes the T gene mutation is lethal (DeForest and Basrur,

1979; Adalsteinsson, 1980; Robinson, 1993). Heterozygotes for the T gene either do not demonstrate any genetic defects, or have a shortened or lack of tail. For example, heterozygous mice for the T gene mutation, are short tailed or taillessness, and in addition they have spinal congenital defects. However, the dominant homozygotes decay around the 11th day of pregnancy, and foetuses have no tail and the posterior mesoderm derived structures (Showell et al., 2004). In humans, the T gene mutation is not yet associated with any disease. Contrary, in cats, shortened tail, or tailless is linked to the existence of dominant gene M (Manx), which yet have not been sequenced. The M gene mutation, responsible for the lack of tail in cats also determines other defects. In heterozygotes an urinary and faecal incontinence and abnormal hindlimb action are observed (Robinson, 1993).

The T gene mutation causing taillessness or shortened tail occurs in 17 breeds belonging to two groups of dogs: sheepdogs and hunting dogs (Hytönen et al., 2009). This mutation was also found in the Pembroke Welsh Corgi. In this breed it is inherited in a dominant manner and in heterozygotes it causes bobtail phenotype, while, dominant homozygotes die during embryonic life or shortly after birth (Indrebø et al., 2008).

Knowledge of the genotype for the T gene is essential in the case of purebred, pedigree dogs that will be used for breeding, because an increasing number of countries is prohibiting cutting tails. Molecular test based on the analysis of the genomic DNA allows unambiguous determination of the origin of the short tail.

MATERIAL AND METHODS

Biological material and the isolation of the genomic DNA

The whole blood was collected into K₃EDTA tubes from 30 Pembroke Welsh Corgi born in 1998–2007 and bred in Poland. Out of the 30 dogs from which the blood samples were collected: 20 had a normal tail length, or docked, 6 – short tail, and 4 – no tail. The full pedigree data for each dog were also available, as well as description of parents' tail length. The genomic DNA was isolated from blood using standard phenol-chloroform extraction. The presence and the purity of isolated DNA was checked on a Nanodrop 2000 spectrophotometer (THERMO SCIENTIFIC) and in a 1% agarose gel.

PCR reaction conditions

Polymerase chain reaction (PCR) was performed using the following pairs of primers: 5'-GAAGAGCCTGCAGTACCGAGT-3' in exon 1 and 5'-CACTCTCCGTTTACGTACTTCC-3' in exon 2 of the T gene (Indrebø et al., 2008). The reaction mixture entered: 1 ng DNA, reaction buffer (SIGMA-ALDRICH, USA) 1 × concentrated, 2.5 mM MgCl₂, 200 μM/μl dNTP mix (POLGEN, Poland), 5 pmol primers, 1 U/μl Taq DNA polymerase (SIGMA-ALDRICH, USA). The reaction was carried out under the following conditions: initial denaturation at 95°C for 3 min, 35 cycles of 95°C for 30 s, 61°C for 30 s, 72°C for 45 s, final synthesis at 72°C for 10 min. The PCR product was analysed by the electrophoresis in native 12% polyacrylamide gel. The electrophoresis was carried out in a vertical electrophoresis

apparatus (BIOMETRA, Germany) in 1 × TBE buffer (10 × TBA: 89 mM Tris, 2 mM EDTA pH 8.0, 89 mM boric acid) for 1 h at a voltage of 180 V. After the electrophoresis, the gel was stained with silver, dried and then scanned for further analyses.

Diagnostic test

The purpose of this study was to develop a diagnostic test based on the amplification of a part of the T gene with mutation site, and the differentiation of normal and mutant alleles with differences resulting from restriction enzyme digestion of the PCR product. The substitution of the cytosine for guanine at nucleotide position 189 T gene creates a place recognized by the restriction enzyme *BstEII* from *Bacillus stearothermophilus*. As a result of the PCR reaction, the 702 base pairs fragment is amplified. Through *BstEII* digestion of the PCR product, the 511 bp fragment is obtained, regardless of whether the DNA came from an individual who has the C295G mutation in his genotype or not, and a 191 bp fragment from the wild allele, and 160 and 31 bp from the mutant allele. The additional *BstEII* restriction site for the both types of allele acts as an internal positive control for *BstEII* digestion.

PCR product restriction enzyme digestion

The PCR product was digested using 5 U *BstEII* (BIOLABS, UK) restriction enzyme in the presence of the recommended buffer, and 100 mg/ml bovine serum albumin (BSA) (BIOLABS, UK) for 1 h at 60°C.

Analysis of digested PCR products

Restriction enzyme digestion products were analysed by the vertical electrophoresis in 12% native polyacrylamide gel in 1 × TBE buffer for 90 min at a voltage of 180 V. After the electrophoresis, the gel was stained with silver method, dried and then scanned.

RESULTS AND DISCUSSION

The whole sequence of T-box gene is available in the GenBank (gi:74027283). We amplified 702 bp fragment of T-box gene. This fragment consists of: exon 1 – 173 bp, intron 2 – 406 bp and exon 2 – 123 bp. Sample results of the designed diagnostic test for the T gene mutation in Pembroke Welsh Corgi are shown in Figure 1. Restriction enzyme digestion PCR product indicates the genotype of studied dogs. Recessive homozygous animals have fragments of 511 and 191 bp, and the carriers (heterozygotes) have 511, 191, 160 and 31 bp fragments. Figure 1 does not include the fragment of 31 base pairs, but this fragment is shown on Figure 2.

The presence of the additional restriction site for *BstEII* in all PCR products functions as a positive control ensuring that the appropriate reaction conditions for *BstEII* restriction digestion were provided. Using the diagnostic test developed in this study the genotype of 30 dogs was identified: 20 recessive homozygotes, 10 heterozygotes and no dominant homozygotes were found. Twenty dogs that were recessive homozygous had phenotypically normal tail. Heterozygous dogs for the T gene had either a short tail (6 dogs) or no tail at all (4 dogs). After examining

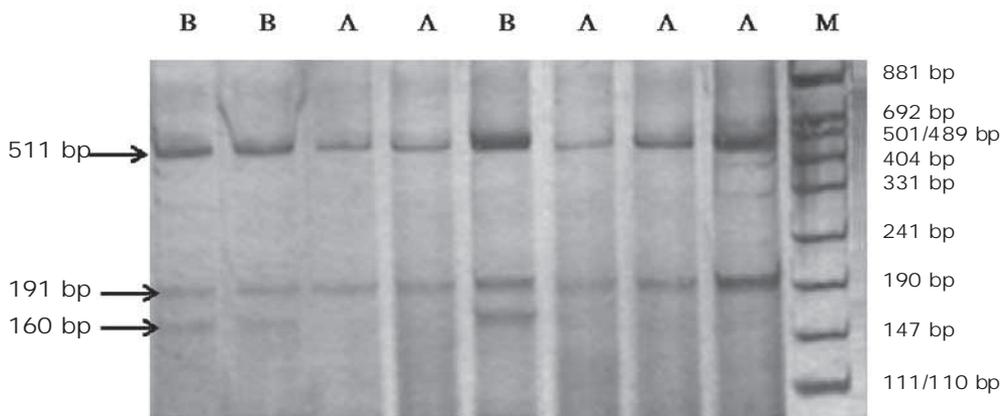


FIGURE 1. Electrophoretic separation of *BstEII* restriction enzyme digestion fragments in native 12 % polyacrylamide gel. M – pUC Mix Marker 8; A – recessive homozygote (511 and 191 bp fragments are visible); B – heterozygote (511, 191 and 160 bp fragments are visible, 31 bp fragment not shown). Arrows marks restriction fragment lengths

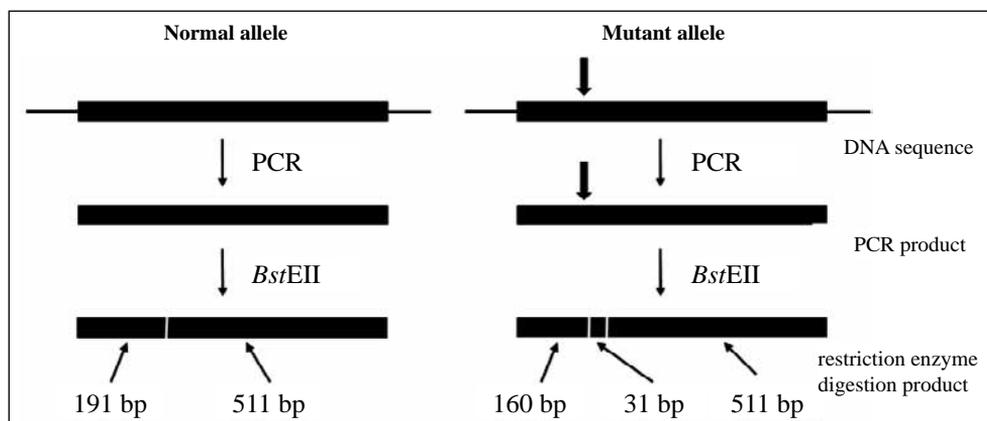


FIGURE 2. Principle of the diagnostic test for detection the C295G mutation of the T gene in Pembroke Welsh Corgi. The length of restriction fragments are given in the last row. Thick arrows showing the site of mutation

the pedigrees of tested dogs, it was found that for all individuals that were heterozygous one of the parents had phenotype of short tail.

British breed standard for the Pembroke Welsh Corgi of April 2004 (http://www.fci.be/uploaded_files/039A2003_en.doc) defines the tail of this breed as a short, and therefore the preferred tail

is a naturally short tail. It was a common practice in many European countries, to cut off tails of dogs that were born with long tails.

Only after the European Convention for the Protection of Pet Animals came into the force, (<http://conventions.coe.int/Treaty/EN/Treaties/Html/125.htm>, Strasbourg, 1987) most countries

introduced a ban on cutting tails. Dogs with their tails docked can not participate in official shows. The owner of a dog with bobtail (short tail or no tail) needs to present a certificate from a veterinarian to confirm the natural origin of a short tail in his dog. In breeds in which a mutation of the T gene was identified, a veterinary observation can be now replaced by a simple genetic test that unambiguously identifies the origin of taillessness (Hytönen et al., 2009).

Canine T-box gene has been cloned by Haworth et al. (Haworth et al., 2001). It is located in the chromosome 1q23. As a result of the analysis of DNA sequences of the T gene C295G mutation was found in exon 1. Substitution of cytosine for guanine (C > G) leads to changes in ATC codon for isoleucine at codon ATG for methionine. After the transcription the mRNA is the codon AUG, which is recognized by the ribosome, as the translation start site (Haworth et al., 2001).

C295G mutation is also known as Ile63Met or C189G mutation (Hytönen et al., 2009). Substitution of cytosine for guanine creates a place recognized by the *BstEII* restriction enzyme from *Bacillus stearothermophilus*.

Haworth et al. compared the canine T gene sequence to the T gene in other species (Haworth et al., 2001). It was found that the isoleucine at 63 amino acid position is conservative in the T protein from *Drosophila* to tunicates to man. The location of this isoleucine in the highly conserved region of T domain may indicate that the substitution of isoleucine for methionine at this position changes the ability of mutant T protein to DNA binding (Papapetrou et al., 1997).

In this paper, primers published by Indrebø et al. were used for the polymerase chain reaction (Indrebø et al., 2008). As a result of the PCR reaction, DNA segment with a length of 702 bp was obtained, which consists of a fragment of exon 1, intron 2 and a part of exon 2.

Haworth et al. (2001) applied two starters – both in exon 1 and an amplified fragment length of 317 bp to the PCR reaction exon 1 of the T gene with a mutation site (Haworth et al., 2001). Also Hytönen et al. (Hytönen et al., 2009) applied the same pair of primers that Haworth et al. (Haworth et al., 2001), and primers designed by themselves within exon 1 of the T gene.

C295G mutation in the designed DNA test was identified by the analysis of the length of restriction fragments generated in *BstEII* digestion of the PCR product. After the electrophoresis of restriction fragments in polyakrylamide gel, the gel was stained with silver method. Polyacrylamide gels are characterized by a higher resolution than agarose gels. By contrast, the method of silver staining of DNA fragments is more sensitive to ethidium bromide staining. Indrebø et al. analysed the sequenced PCR product (Indrebø et al. 2008). By contrast, Haworth et al. and Hytönen et al. subjected the PCR product to both *BstEII* enzyme digestion and sequencing (Haworth et al., 2001; Hytönen et al., 2009).

Among the dogs studied by Haworth et al. in order to analyse T gene mutations, there was no dominant homozygote even in the litter from mating two short tailed dogs (Haworth et al., 2001). All the dogs with a shortened tail were carriers of the C295G mutation. This may mean that the T gene mutation is lethal

in homozygous animals (Haworth et al., 2001). Also mice heterozygous for the T gene mutation have short tail or no tail and it is associated with skeletal defects. Moreover, mice homozygous for the T gene mutation exhibit severe malformations, and fetuses survive to the 10.5 day of the gestation (Meisler, 1997). While in the fish *Danio rerio* carrying the mutation in the *ntl* gene, homologous to the mouse T gene, there were no visible phenotypic changes that would be associated with shortening of the tail. The *ntl* gene phenotypic effect in homozygous fish is similar to the phenotypic effect of gene Brachyury in homozygous mice (Schulte-Merker et al., 1992, Schulte-Merker et al., 1994).

Indrebø et al. examined 19 naturally short tailed Pembroke Welsh Corgi heterozygous for the T gene mutation in order to determine their suitability for congenital spine (Indrebø et al., 2008). All dogs were subjected to a radiological examination. The results showed no spinal congenital defects connected with the T gene mutation, which means that the only phenotypic effect of the C295G mutation observed in the carriers of the Pembroke Welsh Corgi is short tail or tailless.

Indrebø et al. reported also two Pembroke Welsh Corgi puppies, which were dominant homozygous for the T gene mutations (Indrebø et al., 2008). One of them survived only a few seconds after birth, and the second was euthanized. Both puppies were radiographically and genetically tested and submitted to an autopsy. In addition to the lack of tail and anorectal atresia, they showed a series of spinal defects, for example: shortened cervical, thoracic and lumbar vertebral

bodies, and no sacral or caudal vertebrae, scoliosis of thoracic and lumbar spine and kyphosis of the thoracic spine (Indrebø et al., 2008).

There are no other reports of homozygous puppies born or stillborn fetuses, which suggests that in most cases, the homozygotes for the C295G mutation is lethal even in an early fetal development (Haworth et al., 2001; Indrebø et al., 2008). Until now there was only one report of homozygous puppies born alive. Thus, it appears that there is a possibility for these puppies to be born and be able to survive for a short period of time. The reasons for such phenotypic differences in dogs with the same genotype regarding T gene, have not been explained yet. Natural variability in the length of the tail in dogs of Pembroke Welsh Corgi with short tails indicates that there may be differences in the sequences of other genes directly interacting with the T gene (Indrebø et al., 2008). It was demonstrated that the genes belonging to a family of genes with a T-box and their cofactors may have complex interactions (Packham and Brook, 2003).

After crossing a short tailed with a long tailed dog it can be expected that about 50% of litter are short tailed (heterozygous). In the same time, when crossing two short tailed dogs there is a possibility of getting homozygous dominant puppies for the C295G mutation. However, the probability of obtaining homozygotes in the progeny is relatively low. Indeed, in the past, such crossing was conducted in many countries, but so far there has been no information about puppies with congenital defects indicating the presence of the T gene mutations in homozygous genotype. Nevertheless, mating of dogs

with genotypes that could cause an increased presence of hereditary defects should be prohibited by law (Indrebø et al., 2008).

Hytönen et al. examined 23 breeds of dogs and demonstrated the presence of the C295G mutation in all short tailed dogs belonging to 17 breeds (Hytönen et al., 2009). In the remaining 6 breeds, neither mutation Ile63Met, nor any other T gene mutations were detected. 9 breeds of dogs, which phenotypes do not exhibit natural taillessness were also studied. The results show that the T gene mutation is not present in all breeds of dogs, in which short tailed phenotype is observed. Thus, there must be some other genetic factor that determines the length of the tail in dogs (Hytönen et al., 2009). The possible second mutation affecting this trait might explain while some of T gene heterozygous dogs had short tail while others had no tail at all. All tested dogs with short tails were heterozygous for the C295G mutation, and all dogs with long tails were not carriers of this mutation. This indicates full penetration of the T gene and its lethality in the homozygous configuration. The T gene lethality was further confirmed by statistical analysis, based on comparison of the litter size of two mating groups (long tailed × long tailed and short tailed × short tailed) of Swedish Vallhund. When two parents were short tailed a 29 percentage decrease in the litter size was observed. This litter size reduction is likely due to the lethality of the embryos in the uterus, which is consistent with the expected 25-percent decrease in the litter size when crossing two heterozygous par-

ents. As expected 1/4 of all puppies should inherit a mutation from both parents and became lethal homozygous (Hytönen et al., 2009).

Seventeen breeds of dogs, in which the presence of the Ile63Met mutation was detected, belong to two groups of dogs (sheepdog and hunting dogs). This may suggest an ancestral origin of the T gene mutation. To determine the pattern of inheritance and the reasons for taillessness in other breeds of dogs further research are needed (Hytönen et al., 2009).

In the present study the genotype of 30 dogs for the T gene C295G mutation in Pembroke Welsh Corgi was determined. Ten dogs proved to be the carriers of the mutation and the other 20 showed no presence of the T gene mutation. As in the previous studies conducted by other authors (Haworth et al., 2001; Indrebø et al., 2008; Hytönen et al., 2009), all heterozygous dogs were short tailed (6 dogs) or taillessness (4). In turn, all dogs without the C295G mutation were born with a long tail. There were no dominant homozygotes for this mutation, because they died during embryonic or perinatal period, as shown by the work of other authors (Haworth et al., 2001; Indrebø et al., 2008; Hytönen et al., 2009).

Using the designed diagnostic test allows to determine the T gene mutation carriers that are responsible for taillessness or short tailed phenotype in Pembroke Welsh Corgi. This is important because tail docking is prohibited in many countries. If a dog is to take part in pedigree shows a certificate to ascertain a natural short tail will be required; such certificate can be based now on objective molecular analysis.

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REFERENCES

- ADALSTEINSSON S., 1980: Establishment of Equilibrium for the Dominant Lethal Gene for Manx Taillessness in Cats. *Theoretical and Applied Genetics* 58 (2): 49–53.
- DEFOREST M.E., BASRUR P.K., 1979 – Malformations and the Manx Syndrome in Cats. *The Canadian Veterinary Journal* 20, 304–314.
- HAWORTH K., PUTT W., CATTANACH B., BREEN M., BINNS M., LINGAAS F., EDWARDS Y.H., 2001: Canine homolog of the T-box transcription factor T; failure of the protein to bind to its DNA target leads to a short-tail phenotype. *Mammalian Genome* 12: 212–218.
- HYTÖNEN M.K., GRALL A., HÉDAN B., DRÉANO S., SEGUIN S.J., DELATTRE D., THOMAS A., GALIBERT F., PAULIN L., LOHI H., SAINIO K., ANDRÉ C., 2009: Ancestral T-box Mutation Is Present in Many, but Not All, Short-Tailed Dog Breeds. *Journal of Heredity* 100 (2): 236–240.
- INDREBØ A., LANGELAND M., JUUL H.M., SKOGMO H.K., RENGMARK A.H., LINGAAS F., 2008: A study of inherited short tail and taillessness In Pembroke Welsh corgi. *Journal of Small Animal Practice* 49 (5): 220–224.
- KAVKA A.I., GREEN J.B.A., 1997: Tales of tails: Brachyury and the T-box genes. *Biochimica et Biophysica Acta* 1333 (2): F73–F84.
- MEISLER M.H., 1997: Mutation watch: Mouse brachyury (T), the T-box gene family, and human disease. *Mammalian Genome* 8 (11): 799–800.
- PACKHAM E.A., BROOK J.D., 2003: T-box gene in human disorders. *Human Molecular Genetics* 12 (1): R37–R44.
- PAPAIOANNAOU V.E., SILVER L.M., 1998: The T-box gene family. *BioEssays* 20 (1): 9–19.
- PAPAPETROU C., EDWARDS Y.H., SOWDEN J.C., 1997: The T transcription factor functions as a dimer and exhibits a common human polymorphism Gly-177-Asp in the conserved DNA-binding domain. *The Federation of European Biochemical Societies Letters* 409 (2): 201–206.
- ROBINSON R., 1993: Expressivity of the Manx Gene in Cats. *Journal of Heredity* 84(3): 170–172.
- SCHULTE-MERKER S., EEDEN VAN F.J.M., HALPERN M.E., KIMMEL C.B., NÜSSEIN-VOLHARD C., 1994: No tail (ntl) is the zebrafish homologue of the mouse T (Brachyury) gene. *Development* 120: 1009–1015.
- SCHULTE-MERKER S., HOR K., HERRMANN B.G., NÜSSEIN-VOLHARD C., 1992: The protein product of the zebrafish homologue of the mouse T gene is expressed in nuclei of the germ ring and the notochord of the early embryo. *Development* 116: 1021–1032.
- SHOWELL C., BINDER O., CONLON F.L., 2004: T-box Genes in Early Embryogenesis *Developmental Dynamics* 229 (1): 201–218.
- European Convention for the Protection of Pet Animals Strasbourg, 13 XI 1987, <http://conventions.coe.int/Treaty/EN/Treaties/Html/125.htm>
- FCI – Standard Welsh Corgi (Pembroke) No39/28.11.2003/GB, http://www.fci.be/uploaded_files/039A2003_en.doc

Streszczenie: *Molekularny test wykrywania mutacji C295G w genie T odpowiedzialnego za krótkoogoniastość i bezogoniastość u pembroke welsh corgi.* Gen T należy do dużej rodziny genów z motywem T, która koduje czynniki transkrypcyjne, ulegające ekspresji podczas rozwoju zarodkowego. Białko T aktywuje geny specyficzne dla mezodermy i jest zaangażowane w proces rozwoju struktur wywodzących się z tylnej części mezodermy, m.in. struny grzbietowej. Mutację genu T warunkującą krótkoogoniastość i bezogoniastość zidentyfikowano u 17 ras psów (Hytönen i wsp., 2008). U psów rasy pembroke welsh corgi jest ona dziedziczona w sposób autosomalny dominujący i u heterozygot warunkuje fenotyp o skróconym lub szczątkowym ogonie. Homozygoty dominujące giną w okresie płodowym lub zaraz po urodzeniu (Indrebø i wsp., 2007). Obecnie w wielu krajach prawnie zabroniono cięcia ogonów u psów. Aby pies bez ogona lub też z ogonem szczątkowym mógł uczestniczyć w oficjalnych pokazach lub

wystawie psów rasowych, jego właściciel musi mieć zaświadczenie, potwierdzające „naturalność” krótkiego ogona u psa. Celem niniejszych badań było zaproponowanie testu diagnostycznego, identyfikującego mutację C295G w genie T oraz identyfikacja w polskiej populacji psów rasy pembroke welsh corgi heterozygot mutacji genu T warunkującego krótkoogoniastość i bezogoniastość. W tym celu wykorzystano enzym restrykcyjny *BstEII*, który tnie sekwencję w 191 pozycji nukleotydowej w eksonie 1 genu T. W wyniku mutacji powstaje dodatkowe miejsce restrykcyjne w pozycji 160. Spośród 30 przebadanych psów zidentyfikowano 20 homozygot recesywnych i 10 heterozygot. Psy heterozygotyczne pod względem genu T miały fenotypowo

ogon krótki (6 psów) lub szczątkowy (4 psy). Zmienność długości ogona u krótkoogoniastych psów rasy pembroke welsh corgi wskazuje na istnienie być może różnic w sekwencjach innych genów, bezpośrednio oddziałujących z genem T.

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Ecological and hydrological aspects of small river catchment management in Nature 2000 site

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Abstract: *Ecological and hydrological aspects of small river catchment management in Nature 2000 site.* ‘Water Framework Directive’ stresses the maintaining of good ecological status of water ecosystems as well as holistic approach to water resources management. The aim of the study was to determine the possibilities of joint management measures of ecological and hydrological management of two small lowland river catchments: Zagożdżonka and Zwoleńka rivers, located in central Poland. Both are natural rivers, with lots of meanders and abounding biodiversity of extensive agriculture and forest areas. The field investigations were carried out to describe the habitat preferences of amphibians in two analyzed river catchments and to record the hydrological values of the catchments. During considered period the hydrological condition were above average (more wet) in a sense of rainfall and runoff. During the whole observation period nine amphibian species were recorded: *Rana esculenta* complex, *R. temporaria*, *R. arvalis*, *Bufo bufo*, *Hyla arborea*, *Pelobates fuscus*, *Triturus vulgaris*, *Bombina bombina* and *Triturus cristatus*. The most important species occurring in investigated catchments, listed in II Annex to Habitat Directive that require protection inside Natura 2000 sites are *Triturus cristatus* and *Bombina bombina*.

Key words: amphibians, catchment management, Zwoleńka River, Zagożdżonka River.

INTRODUCTION

Water management became an important task for EU Member States since December 2000 when “Framework for Community Action in the Field of Water Policy” was established (European Commission, 2000). The main objective raised by this document (called also ‘Water Framework Directive’ – WFD) is for all surface waters, artificial and heavily modified waters and groundwaters to achieve good water status by 2015. It also aims to promote sustainable water use (sufficient provision of drinking water and water for other economic requirements) and focus on mitigation of the effects of floods and droughts. It stresses the maintaining of good ecological status of water ecosystems, which means that only a slight departure from the biological values that may be expected in conditions of minimal anthropogenic impact will be allowed. Achievement of ecological objectives should consider an integrated approach to three basic components: water quality, water quantity, and physical structure which interact with each other (Logan, 2001).

This strong focus on the ecological aspects of freshwater is something new in Poland for water management authorities of different stages as well as scientists dealing with water managements. Holistic approach to water management and protection of the aquatic environment suggests organization based on catchment areas. Although wider units may be of great value for this activities (Moog et al., 2004), it is confirmed by other authors that regional level of planning and implementing measures also plays an important role in achieving the directive's regulations (Braukmann and Pinter, 1997; Verdonshot, 2000; Dorge and Windolf, 2005).

The first step for planning management measures for such unit is the analysis of actual situation (condition) of the water body. The next steps should consider: definition of main threats, quantitative assessment of deficits, determination of potential measures together with analysis and selection of conservation and/or restoration scenarios. After the implementation of measures the proper monitoring of results should be conducted (Kolisch et al., 2000).

The aim of this study is to present the combination of ecological and hydrological analysis of two small lowland river catchments to determine the joint management measures.

STUDY AREA AND METHODS

The research was conducted within two catchments: Zagożdżonka and Zwoleńka rivers. They are located in central Poland (South-Mazovian Lowland) about 100 km south of Warsaw and both are the left bank inflows of the Vistula river. The

length of the Zagożdżonka river is 39.9 km and the size of the whole catchment is 568.5 km². The size of the subcatchment till Płachty Stare gauge is 82.4 km². The absolute relief of the watershed to Płachty Stare gauging point is 37 m (maximum and minimum elevation 185 and 148 m a.s.l. respectively). The mean slopes of the main channels are in the range of 2.0–3.5%. The existing valleys are narrow and shallow. The dominant soils are loamy soils and about 10% are peats. The 50–70% of watershed is under cultivation. In streams valleys the pasture are dominant. The main crops are rye, wheat-rye and oat together with potato. The forest cover about 40% of catchment to Płachty Stare gauging point. The subcatchment till Płachty Stare gauge is controlled in a sense of rainfall and runoff since 1963 (Hejduk et al., 2010). The average rainfall (years 1963–2008) for this area is estimated for 606 mm and runoff for 107 mm (Banasik, 2009). The measured rainfall in hydrological years 2009 and 2010 shows values above the average (661 mm and 808 mm respectively) what can describe those years as wets. The runoff for hydrological year 2009 was 66.6 mm and for 2010 was 119.8.

The Zwoleńka is a natural river, with lots of meanders and abounding biodiversity. Its length is 34.1 km and the catchment size is 230.2 km². The average flow in 2002 was estimated for 1.09 m³/s for a whole catchment. Zwoleńka is a neighbor catchment of Zagożdżonka (Fig. 1) so the rainfall in both catchments can be estimate as comparable. Most of the research was done in the middle part of the Zwoleńka river close to Siekierka gauge (186.8 km²), however there is no

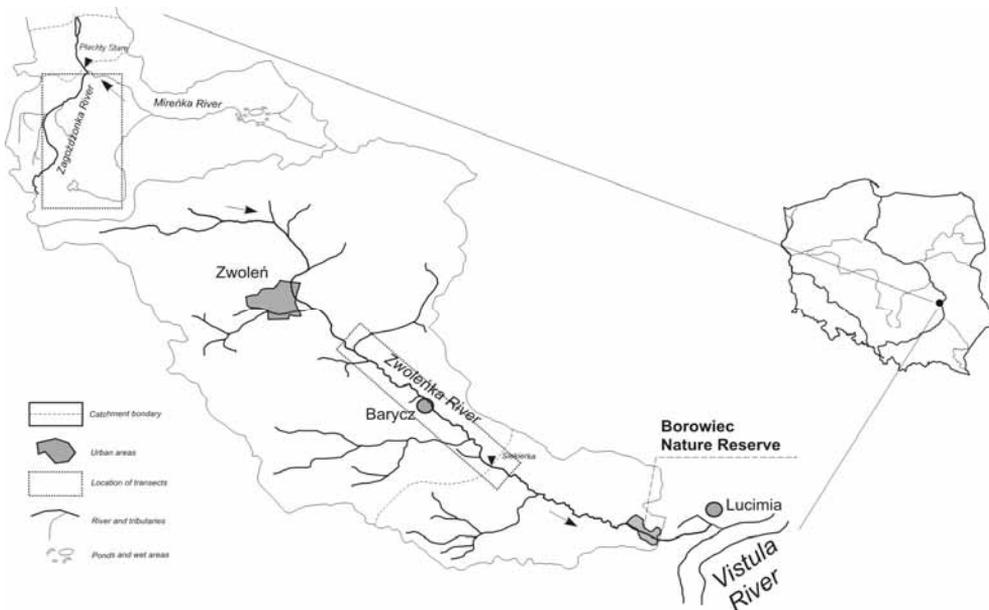


FIGURE 1. Schematic location of Zagożdżonka and Zwoleńka catchments and transects

long time hydrological observation for this gauge, so the runoff was calculated based on empirical equation estimated on the basis of semi-simultaneous flow measurements and peak hydrograph records. The runoff for year hydrological 2009 was 75.8 mm and for 2010 was 114.5 mm.

The dominant soil are loamy soils and sand. In the valley there is also peat. The main crops are rye and mixtures of corn and oat. The Zwoleńka valley is Natura 2000 site (PLH140006). In this area there are two nature reserves Borowiec and Ługi Helenowskie. There are about 80 species of birds and 19 species of mammals. According to Habitats Directive the most valuable species of vertebrates occurring in this site are: european weatherfish *Misgurnus fossilis*, european pond turtle *Emys orbicularis*, fire bellied toad *Bombina orientalis*, marsh harrier *Circus aeruginosus*, corncrake *Crex*

crex, black tern *Chlidonias niger*, kingfisher *Alcedo atthis*, european beaver *Castor fiber*. There are also 3 species of invertebrates from II Annex of Habitats Directive: narrow-mouthed whorl snail *Vertigo angustior*, green snaketail *Ophiogomphus cecilia*, yellow-spotted whiteface *Leucorrhinia pectoralis*. All those species are strictly connected with wetlands or slowly floating river valleys. The main purpose of establishing Natura 2000 was the preservation of lowland hay meadows (code 6510) covering 20% of site surface, natural eutrophic lakes (code 3150) covering 10% of the surface and Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (code 91E0) covering 8% of the site.

During two spring seasons 2009 and 2010 the field investigations were carried out to describe the habitat preferences of amphibians in two analyzed river catchments. This group of animals due to its

two-staged life cycle, needs two kinds of habitats to fulfill their life requirements: aquatic and terrestrial, so threats to either habitat can affect their populations. They seem to be a very fragile indicator of environmental processes also because as an adult form they are predators. The small river catchment with its habitat variety can be a good example of how such dual threats influence amphibians. The species composition has been recorded for different examples of water bodies (river, wetlands, peatbogs) and terrestrial surroundings (forests, meadows, fields, etc.). 16 transect for Zagożdżonka river and 5 sites for Zwoleńka river were designated as representative for different habitats. Each transect was 100m long and covered terrestrial habitat next to water. The amphibians occurrence was recorded by the use of direct observations of adults, larvae and eggs, netting and vocal recordings. Each transect or site was visited at least three times (one time at dark). The field observations were carried out from the beginning of April to the end of June 2009 (Zagożdżonka) and the same part of year 2010 for Zwoleńka river, to cover the higher breeding activity of amphibians.

RESULTS

During the whole observation period nine amphibian species were recorded: *Rana esculenta* complex, *R. temporaria*, *R. arvalis*, *Bufo bufo*, *Hyla arborea*, *Pelobates fuscus*, *Triturus vulgaris*, *Bombina bombina* and *Triturus cristatus*. The detailed results of this investigation are presented in paper Klimaszewski et al. 2009. For each species the habitats needs were assessed (Tab. 1). As an addition to this

comparison we can divide amphibians according to dispersal power: *Triturus* species have extremely low dispersal power (few hundred meters), whilst all frogs and toads can travel much longer distances (more than 1 km).

Two years research period is too short for estimation of river regime and for prediction of species in relation to hydrological condition. During considered period the hydrological condition were above average (more wet) in a sense of rainfall and runoff. It is difficult to make a long term relation based only on this data specially where there is no information about species observation during dry periods. One of the most important period from species point of view is a breeding activity time – from beginning of April to the end of June. In case of Zagożdżonka river, based on long term research, is possible to estimate the monthly distribution of rainfall and runoff (Fig. 2).

Periods from April to June are the months with relatively high average runoff during April and low average rainfall in the same months. The high flows during springtime are often caused by snowmelt or rain on snow situations. On the other hand, the height rainfall and runoff can occur during other months. This kind of situation were measured in May and September 2010 (Fig. 2).

As the river valley areas are the most preferable habitats during breeding and post-breeding time (Tab. 1), the determination of flood range can have the important influence on land management and species protection. The small ponds are important due to two-stage life cycle of amphibians, so the research

TABLE 1. Habitat preferences of amphibian species recorded in Zagożdżonka and Zwolenka river catchments (“-” – of no importance for species, “+” – possible to use, “++” – suitable, “+++” – most preferable)

	<i>Rana esculenta complex</i>	<i>Rana temporaria</i>	<i>Rana arvalis</i>	<i>Bufo bufo</i>	<i>Hyla arborea</i>	<i>Triturus vulgaris</i>	<i>Triturus cristatus</i>	<i>Bombina bombina</i>	<i>Pelobates fuscus</i>
Breeding habitats									
River (with slowly running water)	-	+	+	+	-	-	-	-	-
River valley (meadows with Seasonally filled water bodies)	+	+++	+++	+++	+	++	+	-	+
Small ponds with dense water vegetation)	++	++	++	++	+++	+++	+++	++	++
Big shallow water bodies (in peatbogs)	+++	++	++	++	+++	++	+	++	+
Post-breeding habitats									
Water bodies and surroundings	+++	+	+	+	+	++	+++	+++	+
River valleys (meadows)	+	+++	+++	+++	++	+++	++	+	++
Arable fields	-	+	+	++	-	-	-	-	+
Wastelands with shrub and bushes	-	++	++	+++	+	++	+++	-	+++
Forests	-	+++	+++	+++	++	++	++	-	+

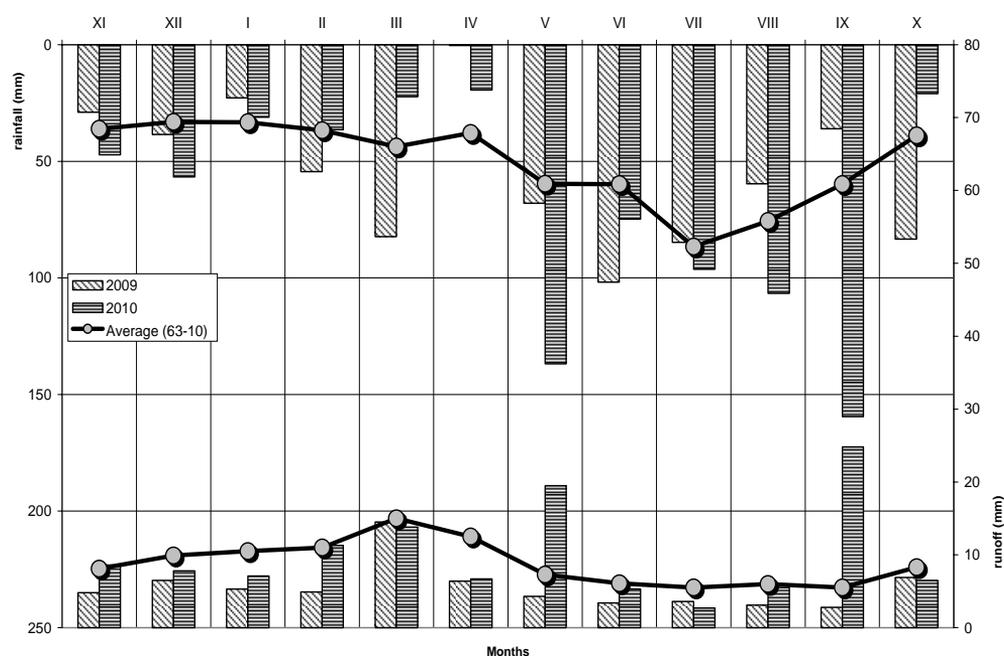


FIGURE 2. Monthly distribution of rainfall and runoff during investigation period and long term averages for Plachty Stare gauge

for determination of condition of drying such ponds can be an important issue for protected species management to fulfill habitat directive requirements.

CONCLUSIONS

1. Management of the catchment should consider preservation of all habitats used by amphibians. First – the condition of water bodies cannot be changed, second – the water flow in the rivers should allow existence of seasonal, small water bodies.

2. The most important species occurring in investigated catchments, listed in II Annex to Habitat Directive that require protection inside Natura 2000 sites are *Triturus cristatus* and *Bombina bombina*.

3. As the investigations has been carried during wet years it is important to conduct them during dry years to avoid the influence of specific condition on estimation of species occurrence.

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REFERENCES

ACHLEITNER S., TOFFOL S., ENGELHARD C., RAUCH W., 2005: The European Water Framework Directive: Water Quality Classification and Implications to Engineering

- Planning. Environmental Management. Vol: 35, Issue: 4. Springer-Verlag.
- BANASIK K., 2009: Czasy opóźnień odpływu wezbraniowego i fali rumowiska z małych zlewni rolniczych. Projekt badawczy Specjalny MNISW nr. COST 21/2005. Maszynopis.
- BRAUKMANN U., PINTER I., 1997: Concept for an integrated ecological evaluation of running waters. Acta Hydrochim. Hydrobiol., 25: 113–127.
- DORGE J., WINDOLF J., 2005: Implementation of the water framework directive – can we use models as a tool in integrated river basin management Intl. J. River Basin Management Vol. 1, No 2, pp. 165–171.
- EUROPEAN COMMISSION, 2000: DIRECTIVE 2000/60/EC. Establishing a framework for community action in the field of water policy. European Commission PE-CONS 3639/1/100 Rev. 1, Luxembourg.
- GOETHALS P.L.M., DE PAUW N., 2001: Development of a concept for integrated ecological river assessment in Flanders. (Belgium). J. Limnol. 60: 7–16.
- HEJDUK L., BANASIK K., HEJDUK A., 2010: Monitoring ilości i jakości wody w małej zlewni. Hydrologia w inżynierii i gospodarce wodnej. T. 1 (pod red. Beniamina Więżika, Monografie) Polska Akademia Nauk. Komitet Inżynierii Środowiska, nr 68.
- KLIMASZEWSKI K., JAROSIŃSKA P., ROMAŃSKI B., 2009: The habitat preferences of amphibians in the catchment area of the Zagózdźonka River. Ann. Warsaw Agricult. Univ. – SGGW, Anim. Sci. No 63.
- KOLISCH G., LONDONG J., RENNER J., 2000: Integrated and sustainable river basin management by German river associations. Water, 10: 38–41.
- LOGAN P., 2001: Ecological quality assessment of rivers and integrated catchment management in England and Wales. In: Ravera O. (ed.), Scientific and legal aspects of biological monitoring in Freshwater. J. Limnol. 60 (Suppl. 1): 25–32.
- MOOG O., SCHMIDT-KLOIBER A., OFENBÖCK T., GERRITSEN J., 2004: Does the ecoregion approach support the typological demands of the EU 'Water Framework Directive'? Hydrobiologia, Volume 516, Number 1, pp. 21–33(13). Springer.

VERDONSCHOT P.F.M., 2000: Integrated ecological assessment methods as a basis for sustainable catchment management. *Hydrobiologia*, Vol. 422–423, No 0, pp. 389–412

Streszczenie: *Ekologiczne i hydrologiczne aspekty gospodarowania w małej zlewni na obszarze Natura 2000.* W pracy przedstawiono wyniki badań dla dwóch zlewni (Zagożdżonki i Zwolenki) o dominującym rolniczym zagospodarowaniu, w granicach których wyznaczone zostały obszary Natura 2000. Przeprowadzono badania występowania różnych gatunków płazów w latach 2009 i 2010. Ta grupa zwierząt, ze względu na dwustopniowe cykle życia, potrzebuje dwóch rodzajów siedlisk – wodnego i lądowego, więc zagrożenia dla tych siedlisk mogą mieć wpływ na ich populacji. Dodatkowo, na podstawie badań hydrologicznych, ustalono okresy, których warunki hydrologiczne mogą mieć wpływ na cykl życiowy płazów. Praca jest wynikiem połączenia badań ekologicznych i hydrologicznych dla uzyskania

wspólnych działań gospodarowania zlewniami w aspekcie ekologicznym.

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Human – animal relations: the case of two primate species kept at Warsaw Zoo

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Abstract: *Human – animal relations: the case of two primate species kept at Warsaw Zoo.* In this work authors tried to compare in visitors of Warsaw Zoo attraction of two primate species: the tufted capuchin monkey (*Cebus paella*) and the hamadryas baboon (*Papio hamadryas*). The other aim was to estimate the effect of visitors on animal activity. Number, sex and age of visitors was determined. Special scales to measure the whole monkey group activity were also elaborated. The exhibit of hamadryas baboon attracted more visitors than capuchin monkey and greater number of behaviour acts directed to animals was also recorded there. Pointing at animals and laughter turned out to be the most frequent behaviour types of visitors. The sex of visitors had more pronounced effect on their behaviour than an age. The effect of visitor number on the activity of whole group of monkeys was significant in both primates. This study should be supplemented by the detailed observation of primate behaviour.

Key words: Zoo, visitors, behaviour, hamadryas baboon, tufted capuchin monkey.

INTRODUCTION

The relationship between the visitors presence and behaviour and animals behaviour at zoological garden is well-known fact (Hosey et al., 2009). Animals evoke various reactions in humans and stimulate them to express opinions on animals and on an exposition. But op-

posite is also true. Presence and behavior of visitors can influence animal activity. These facts were established partly as an results of observations of captive primates. For example previous study of author (Kaleta et al., 2004) carried out at Warsaw Zoo showed that chimpanzee (*Pan troglodytes*) agonistic and begging behaviour were to some degree evoked by visitor verbal and non-verbal signals. The non-verbal signals embraced pointing at animals, throwing food items and objects, waving hands, clap and attempt to come close to the cage. Verbal signals were either addressed to animals or remarks concerning opinions on enclosure, characteristics of chimpanzees etc. On the other hand, chimpanzee used various begging signals to obtain food items from visitors.

The main aim of this study was to compare the effect of visitors on the behaviour of two primate species quite popular in zoological gardens: hamadryas baboon (*Papio hamadryas*) and tufted capuchin monkey (*Cebus apella*). The other aim was to show hypothetical relationship between visitor behaviour and characteristic such as an age and sex.

The mode of life of monkey species studied is different. Hamadryas baboon

is an animal of open often semi – arid plains, which usually live in one-male and rather numerous group. Capuchin monkey is adapted to life in tropical forest. The group of this species comprises several individuals of both sexes. The feeding of both species involves wide array of plant and animal food (Napier and Napier, 1985).

At Warsaw Zoo both species were kept under standard conditions. Hamadryas baboons occupied old-fashioned “modernist” exhibit made of artificial rock with the moat as a barrier between an animal and visitor. In the case of tufted capuchins they lived in double inner and outdoor enclosure. Outdoor enclosure has been recently renewed by using glass panels which partially replaced the wire. As regards furnishing baboon exhibit was rather barren with small number “trees” whereas in capuchin enclosure there were many enrichment items usually used in monkeys at zoos (boughts, ropes etc.).

MATERIAL AND METHODS

In sum, the group of hamadryas baboons comprised of 44 individuals: 25 males, 15 females and 4 young of indeterminate sex. There was some fluctuation in number of baboons during the observation period. In the case of hamadryas baboon exhibited at Warsaw Zoo knowledge concerning age of animals was limited. The oldest female was 34 years old, and an age of many other individual (females and males) was around 10 years old. The age of the oldest male was indeterminate, probably equal to the oldest female.

The group of tufted capuchins comprised of 15 individuals: 2 males,

11 females and 2 young monkeys of indeterminate sex. The oldest female was 26 years old, the youngest – one year old. From two males first was 16 years old, the second – very young (was born previous year). The number of capuchin monkeys also fluctuated during the observation period.

The observations were carried out between the May and September of 2010. Observer tried to select the days and hours with potentially the highest level of visitors presence at the Warsaw Zoo (e.g. weekends, sunny days). In total there were 66 hours of ad libitum observations divided equally into the observation of baboon and capuchin exhibits. Prior to recorded observation there was one hour pilot observation. The schedule of observations was shown in Table 1.

During the study period observer has occupied determined place near the exhibit which enabling him the best possible view of animals and approaching visitors. The observation simultaneously focused on the visitors and animals. Number of visitors near the exposition was counted every 10 minutes. Verbal and non-verbal reactions of visitors were also recorded. The classification of visitors gestures is based on the previous study on chimpanzee (Kaleta et al., 2004). In the case of the visitor behavior his/her sex and age was determined.

In the case of animal behaviour it was need to elaborate the system of recording the whole group activity. It is shown in Table 2. Taking into account differences between species behavior (ascertained in the pilot observation) two independent ordinal scales were used. In the case of baboon the animals could not hide in shelter during daily activity. Therefore,

TABLE 1. The observation schedule

Day	Hours of capuchin observation	Hours of baboon observation	Day	Hours of capuchin observation	Hours of baboon observation
02.05.2010	12 ⁰⁵ –13 ¹⁵	13 ³⁰ –14 ³⁰	01.08.2010	12 ⁴⁰ –13 ⁴⁰	14 ⁰⁰ –15 ⁰⁰
23.05.2010	12 ²⁰ –13 ²⁰	13. ⁴⁵ –14 ⁴⁵	07.08.2010	12 ³⁰ –13 ³⁰	14 ³⁰ –15 ³⁰
29.05.2010	11 ³⁵ –12 ³⁵	13 ¹⁰ –14 ¹⁰	14.08.2010	14 ³⁰ –15 ³⁰	12 ¹⁰ –14 ¹⁰
30.05.2010	11 ²⁵ –12 ²⁵	12 ⁴⁵ –13 ⁴⁵	15.08.2010	14 ³⁵ –16 ³⁵	13 ¹⁵ –14 ¹⁵
06.06.2010	12 ³⁰ –13 ³⁰	13 ⁴⁵ –14 ⁴⁵	21.08.2010	12 ⁰⁵ –14 ⁰⁵	14 ²⁰ –15 ²⁰
12.06.2010	11 ¹⁵ –12 ¹⁵	12 ²⁰ –13 ²⁰	22.08.2010	12 ⁰⁰ –13 ³⁰	14 ⁰⁰ –15 ³⁰
13.06.2010	13 ³⁰ –14 ³⁰	12 ¹⁵ –13 ¹⁵	28.08.2010	12 ⁴⁰ –14 ¹⁰	14 ²⁰ –15 ²⁰
26.06.2010	11 ²⁰ –12 ²⁰	12 ³⁵ –13 ²⁵	29.08.2010	12 ⁰⁰ –13 ³⁰	13 ⁵⁰ –15 ²⁰
27.06.2010	15 ³⁰ –16 ³⁰	14 ⁰⁵ –15 ⁰⁵	04.09.2010	11 ³⁵ –13 ⁰⁵	13 ²⁵ –14 ⁵⁵
03.07.2010	17 ⁰⁰ –18 ⁰⁰	15 ⁴⁰ –16 ⁴⁰	05.09.2010	12 ³⁵ –13 ³⁵	14 ⁰⁰ –16 ⁰⁰
10.07.2010	13 ³⁵ –14 ³⁵	15 ⁰⁰ –16 ⁰⁰	11.09.2010	11 ⁴⁰ –12 ⁴⁰	10 ³⁵ –11 ³⁵
11.07.2010	14 ³⁰ –15 ³⁰	15 ³⁵ –16 ³⁵	12.09.2010	11 ⁰⁰ –13 ⁰⁰	13 ³⁰ –15 ⁰⁰
17.07.2010	13 ³⁰ –14 ³⁰	14 ³⁵ –15 ³⁵	25.09.2010	12 ²⁵ –13 ²⁵	13 ⁴⁰ –15 ⁴⁰
18.07.2010	12 ³⁵ –13 ³⁵	11 ²⁰ –12 ²⁰			
31.07.2010	10 ²⁰ –11 ²⁰	12 ⁴⁰ –13 ⁴⁰			

TABLE 2. Scales used in primate reaction classification

Hamadryas baboon		Tufted capuchin	
Activity/Behaviour	Points	Activity/Behaviour	Points
		No animal in outdoor enclosure	0
Resting and basking of the whole group	1	Presence of up to 3 individuals, low level of activity	1
Low level of activity: mutual grooming, looking around, looking for food items in moat, washing food	2	Same number, high level of activity	2
Walking, climbing, wading and waiting for food	3	Presence of 5 or 6 individuals, high level of activity	3
Playing, running, copulations, receiving food items from visitors	4	At least half of a group visible, high level of activity physical and/or vocal	4
High level of arousal, conflicts over female, agonistic displays, vocalization	5		

the score “0” was excluded in this case. On the other hand, the behaviour and activity level was more varied in baboon than in the capuchin. Hence, the “5” score was granted in this case.

Concerning the analysis of results of observation two questions seems to be the most important:

(1) Have the age and sex of visitors effect on the visitors behaviour (particularly gestures)?

(2) Have the visitors effect on the animals activity and behaviour?

The following statistical methods were used to test above-mentioned questions: chi-square test in the case of (1)

and Spearman correlation in the case of (2) question.

RESULTS AND DISCUSSION

After little training it was possible to determine the number of visitors looking at primate exhibits quite efficiently. The total number of visitors recorded over the study period was over 2000 persons. However, the exact number of persons is hard to determine due to the fact that the same persons could be counted twice or more for example when they stayed longer near the exhibit or returned to it. Nevertheless, markedly greater number of visitors of hamadryas baboon exhibit than the capuchin exhibit was recorded. Mean number of visitors of baboon exhibit in 10 minutes period was approximately twice as in the case of capuchin. The groups of visitors were balanced as regards an age and sex (Tab. 3).

TABLE 3. The number, sex and age of visitors

Total number of visitors during the whole observation period	
TCE*	7 475
HBE**	14 083
The mean number of visitors in 10 min period (33 observations)	
TCE	22.6
HBE	42.7
% of adults	
TCE	66.7
HBE	62.0
% of male adults	
TCE	47.0
HBE	48.0
% of boys	
TCE	48.0
HBE	49.0

* TCE – Tuft capuchin exhibit

** HBE – Hamadryas baboon exhibit

The total number of gestures and other types of behaviour (GOB) performed by visitors is shown in Table 4. Their predominance in the case of hamadryas baboon exhibit was clear consequence of difference in number of visitors shown above. However, it is interesting that behaviour of visitors was more frequent in adults than children and this difference was more visible near the baboon exhibit. Moreover, women were more prone to non-verbal reactions than men.

TABLE 4. Total number of behaviour performed by visitors of various sex and age

Total number of gestures and other behaviours (GOB) recorded	7 330
% of GOB recorded in the whole group	
TCE	39.0
HBE	61.0
% of GOB recorded in adults	
TCE	26.0
HBE	41.0
% of GOB recorded in children	
TCE	12.0
HBE	20.0
% of GOB recorded in men	
TCE	12.0
HBE	19.0
% of GOB recorded in boys	
TCE	6.0
HBE	10.0

The analysis of behaviours performed by visitors (Tab. 5) showed that the nearly all previously assumed behaviour categories were present in the studied group. Pointing at animals gesture was observed far more frequently than others. Important were also laugh (in children) and placing child on the rail separating exhibit (apparently to enable them to have a better view). The last behaviour

TABLE 5. Frequency of visitor behaviour types (%)

	TCE				HBE			
	Adults		Children		Adults		Children	
Gestures and other behaviours	♀	♂	♀	♂	♀	♂	♀	♂
Pointing at animals	70.7	63.0	84.3	80.0	70.6	59.2	75.1	67.1
Waving hands	1.0	0.7	2.3	0.7	0.2	0.1	0.6	1.0
Clap	0.4	0.8	0.5	0.7	0.4	0.5	0.6	0.7
Attempt to approach cage	3.0	2.4	3.2	5.3	0.1	0.1	0.0	0.5
Throwing food items	1.5	1.9	0.7	1.6	3.2	4.5	1.4	2.5
Throwing other objects	0.1	0.1	0.5	0.0	0.2	0.3	0.1	0.4
Imitating monkeys	0.6	0.5	1.4	1.1	0.2	0.4	0.6	2.2
Other non-verbal signals directed to animals	0.6	4.8	1.4	1.8	0.8	1.1	0.4	1.4
Laughter	4.6	4.2	5.9	8.7	15.4	17.9	21.0	24.3
Placing child on the rail separating exhibit from visitors	11.5	21.4	x	x	8.9	16.0	x	x

was performed more often by men than women. Oddly enough there was relatively low number of throwing food and throwing other items, types of visitor behaviour directed to primates commonly described in various zoos (Hosey et al., 2009). Comparing behaviour of visitors as a whole near two exhibits on the basis of frequencies shown in Table 5 it may be concluded that the clear-cut difference was not observed. For example, there was predominance of “pointing” in capuchin exhibit in all age and sex groups of visitors, whereas in the case of baboon exhibit frequency of “laughter” was higher.

In the previous work concerning human animal relation at Warsaw Zoo on chimpanzee (*Pan troglodytes*) “pointing” was also the most frequent visitors behaviour followed by “imitating”, “clap” and “waving”. Attempts to approach cage were rare and the placing children on barrier was not observed

Using more detailed analysis the relationship between the sex and age

of visitors and particular behaviour was determined. In the case of age (both sexes were taken into account) only its effect on “laugh” ($p = 0.42$) and “throwing other objects” ($p = 0.531$) was estimated as highly significant, and “attempt to approach cage” as significant ($p = 0.03$).

More important factor turned out to be the sex of visitors. Its effect on behaviour in majority of cases was estimated as highly significant (Tab. 6)

TABLE 6. The effect of sex on the visitors behaviour

Behaviour of visitor	P-value
Waving	0.151
Clap	0.263
Attempt to approach cage	0.364
Throwing other objects	0.65
Imitating	0.0593
Other non-verbal signals	0.664

The last point was to evaluate the relationship between number of visitors and activity and behaviour of animals.

Correlation coefficients yielded were 0.38 and 0.45 for tufted capuchin monkeys and the hamadryad baboon accordingly. Both correlations were significant ($p < 0.05$). To comment these findings shortly, more visitors meant in the case of capuchin also more specimens in an outdoor enclosure. The hamadryad baboon showed in this time high level of motor activity, more conflicts and agonistic displays. Both, capuchins and baboons when “met” visitors jumped and ran excitedly (especially baboons) as if they clearly expected the food items provided by visitors. However, according to method authors did not prepare detailed analysis of primate behaviour (particularly signs of abnormal behaviour) only used it to score the activity.

There are some works concerning effect of zoo visitor on primates behaviour. In fact, “most of the studies on zoo visitor effect have been done on primates” (Hosey et al., 2009). However, these studies focused rather on qualitative analysis of animal behaviour and showed bad effect of visitors presence on animals (Hosey, 2000). In the present study authors emphasized only positive correlation between number of visitors and level of primates activity and difference between two primates in this respect.

It should be emphasized that neither hamadryad baboon, nor capuchin monkey did show begging reaction directed to visitors. This behaviour is commonly observed in apes and bears.

CONCLUSIONS

1. Observations carried out at Warsaw Zoo showed that hamadryad baboon

attracted more visitors than tufted capuchin monkeys in the same period. The reason is probably far larger group of baboons with more complex social life. The hamadryad baboons were also forced to stay “on display” and had no access to indoor enclosure as capuchins did.

2. Visitors did not only prefer hamadryad baboon but showed also greater number of behaviour acts directed to observed animals.

3. “Pointing at animal” and “laugh” turned out to be the most important types of visitor behaviour.

4. Measured in the context of behaviour differentiation the effect of visitor sex on behaviour was greater than an age.

5. In both primates, there was significant effect of visitors presence on animal activity. Only the effect on the whole group of monkeys was studied. The effect of visitors on particular behaviour types (especially abnormal behaviour) is left to be done.

REFERENCES

- HOSEY G., 2000: Zoo animals and their human audiences: what is the visitor effect? *Animal Welfare* 9, 343–357.
- HOSEY G., MELFI W., PANKHURST S., 2009: *Zoo Animals. Behaviour, Management and Welfare*. Oxford University Press, Oxford, New York.
- KALETA T., TUMIALIS D., MAŁODOBRA M., 2004: The introductory study concerning human-animal relations in Warsaw Zoological Garden – the case of chimpanzee (*Pan troglodytes*). *Annals of Warsaw Agricultural University-Animal Science* 42, 3–7.
- NAPIER J., NAPIER P., 1985: *The natural history of the primates*. The MIT Press, Cambridge (Mass).

Streszczenie: *Relecje ludzie – zwierzęta: przypadek dwóch naczelnych gatunków.* W pracy autorzy starali się porównać popularność u zwiedzających dwóch gatunków naczelnych w ZOO Warszawa: kapucynki czubatej (*Cebus paella*) i pawiana płaszczowego (*Papio hamadryas*). Innym celem było oszacowanie wpływu liczby zwiedzających na aktywność zwierząt. Określano liczbę, płeć i wiek zwiedzających. Opracowano także skalę do mierzenia aktywności całej grupy małp. Wybieg pawianów płaszczowych przyciągał więcej widzów, niż ekspozycja kapucynek i odnotowano tam również większą liczbę zachowań zwiedzających, kierowanych do zwierząt. Wskazywanie ręką na zwierzęta i śmiech okazały się być najczęściej występującymi zachowaniami zwiedza-

jących. Płeć zwiedzających miała większy wpływ na ich zachowanie się niż wiek. Wpływ liczby zwiedzających na aktywność całej grupy małp był istotny u obu gatunków naczelnych. Badanie to powinno być uzupełnione szczegółowymi obserwacjami zachowania się zwierząt.

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Fatty acid composition of milk from Brown Swiss and Holstein-Friesian black and white cows kept in a certified organic farm

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Abstract: *Fatty acid composition of milk from Brown Swiss and Holstein-Friesian black and white cows kept in a certified organic farm.* The objective of the present study was to evaluate the fatty acid composition of cows milk, kept in a certified organic farm, independent of breed and season of nutrition. In the selected dairy farm, cows of two different breeds, i.e. Brown Swiss (BS) and Holstein-Friesian black and white (HF) between 45th–100th day of lactation, were managed under the same environment conditions. There were highly significant effects of the season on the SFA content in milk, where in SFA content were higher in the winter season compared with the summer season. The studies showed a significant influence of the season on the level of: BA, TVA, LA, CLA9, CLA10, EPA, DPA, DHA. Milk from BS cows was characterized by higher concentration of SFA, BA, AA, EPA and DPA during summer season of feeding. However, milk from HF cows was characterized higher concentration of MUFA, PUFA, OA, LA, TVA and CLA and lower of SFA. In the winter season milk from BS cows was characterized by a higher concentration of SFA, MUFA, BA, TVA and CLA compared with milk of HF breed. Finally, milk from HF breed in organic system during summer and winter season was characterized much higher nutritional value.

Key words: milk quality, organic milk, CLA, fatty acid.

INTRODUCTION

Fatty acids (FA) found in cows milk can be divided into: SFA (saturated fatty acid) and UFA (unsaturated fatty acid). Functional properties of FA are associated with: chain length, saturation and position of the first double bond (n-3, n-6, n-9). Pro health properties have following fatty acids: BA (C4:0), OA (18:1 *cis*-9), LA (C18:2), LNA (C18:3), CLA (C18:2 *cis*-9, *trans*-11), TVA (C18:1 *trans* 11), AA (C20:4), EPA (C20:5) and DHA (C 22:6). There are multiple potential isomers of CLA, but the *cis*-9, *trans*-11 (CLA9) and *trans*-10, *cis*-12 (CLA10) isomers are thought to be active as potential antioxidant, anticarcinogenic, immune-modulating agents and also may normalized glucose level in blood (McGuire and McGuire, 2000). The uniqueness of these effects was recognized in the National Academy of Science report *Carcinogens and Anticarcinogens in the Human Diet*, which stated that CLA is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals (NRC, 1996).

Many factors affect the FA composition of bovine milk, including: breed (White et al., 2001), season of feeding, stage of lactation, age of cows (Nałęcz-Tarwacka, 2006), access to fresh grass (Nałęcz-Tarwacka and Grodzki, 2005), system of production (Metera et al., 2010) and supplementation (Puppel, 2011). However, the effect of dairy nutrition on milk fat composition is much stronger than the effects of other agronomic factors. For example, increasing fresh forage intake (Nałęcz-Tarwacka and Grodzki, 2005), or the use of fish oil and linseeds supplements (Puppel, 2011) have been shown to reduce the content of SFA and increase the content of OA, LA, LNA, TVA, EPA, DHA and CLA9. In contrast, feeding conserved forage reduces the concentrations of CLA and ALA, and increases SFA concentrations in milk fat (Elgersma et al., 2003). Research has also suggested that fatty acid composition of milk by cows under organic management differ from those produced by cows under conventional management (Ellis et al., 2006; Collomb et al., 2008; Metera et al., 2010).

There were no references in the literature about fatty acid composition of milk from Brown Swiss and Holstein-Friesian cows kept in a certified organic farm in Poland. This is a new solution and makes it possible to analyze changes occurring in content selected fatty acid in cows milk caused by the organic system.

MATERIALS AND METHODS

The study was conducted in a certified organic farm. In the farm, dairy cows of two different breeds: Brown Swiss (BS) and Holstein-Friesian (HF) were

kept. Fifteen BS and fifteen HF lactating primiparous, between 45th–100th day of lactation, were chosen to the experiment. The cows of the both breeds in the farm were kept under the same environmental conditions in a free stall dairy barn.

In the surveyed farm in the feeding of cows used the following feed. In the summer season: hay meadow; yellow lupine, pea; oats and field pea; forage grass with red clover and spelled. But in the winter season: corn meal; oats with field pea meal, rye meal, grass silage with red clover and grass hay with red clover (Tab. 1).

TABLE 1. Components of the treatments in experiment

Composition	Treatment	
	Summer season	Winter season
Ingredient (kg/d)		
Hay meadow	8.68	–
Yellow lupine and pea	0.50	–
Oats and field pea	2.00	–
Forage grass with red clover	50.00	–
spelled 1	1.00	–
spelled 2	1.00	–
Corn meal	–	1.00
Oats with field pea meal	–	4.00
Rye meal	–	2.00
Grass silage with red clover	–	23.50
Grass hay with red clover	–	6.50
JPM	18.37	17.21
BTJE	1381.90	1370.70
BTJN	1950.70	1734.60

Sampling

Representative milk samples were collected from each cow during evening milking by means of a milk meter. Milk

were placed in sterile bottles, preserved Mlekostat CC. After milking samples were immediately submitted to the Cattle Breeding Division (Milk Testing Laboratory of Warsaw University of Life Sciences) for compositional analysis.

Fatty acid analyses

For examination fatty acid composition, extraction of fat was performed according to Röse-Gottlieb procedure (AOAC, 1990) at a room temperature. Fatty acid methylation was performed according to the transesterification method by Kramer et al. (1997). The identification with fatty acid standards and quantitative determination of individual fatty acids in crude fat was conducted in Hewlett Packard 6890 GC with HP Chem software, a flame-ionization detector CP-Sil 88 (100 m length, 0.25 mm in diameter and 0.25 μm thick). The helium carrier gas was maintained at a linear velocity of 25 cm/s.

The separation was performed at pre-programmed temperature: 130°C for 1 min; 130–170°C at 6.5°C/min; 170–215°C at 2.75°C/min; 215°C for 12 min, 215–230°C at 20°C/min and 230°C for 3 min. Other parameters were: split sample injector (40:1); injector temperature 220°C; and detector temperature, 240°C.

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:

$$Y_{ijk} = \mu + A_i + B_j + (A_i \cdot B_j) + e_{ijk}$$

Where: Y_{ijk} = dependent variable, μ – general mean, A_i – breed effect ($i = 1 - 2$), B_j – season of the feeding ($j = 1 - 2$), $(A_i \cdot B_j)$ – fixed interaction effect between breed and season of the feeding.

RESULTS AND DISCUSSION

Table 2 shows the changes in the contents of fatty acids independent of breed and season of nutrition. Studies demonstrated a statistically higher content of each group of fatty acids in the summer season i.e.: SFA, PUFA, n-3, n-6 and n-3/n-6. Only significant interaction season*breed for n-3/n-6 was recorded. Milk from dairy cows grazing on pasture was characterized by a favorable concentration of all groups of fatty acids compared with the winter season.

There were significant effects (Tab. 2; $P \leq 0.001$) of the season on the SFA content in milk, wherein SFA concentrations were higher in the winter season compared with the summer season. Study has shown, that grazing of both grass and red clover swards were associated with a significantly lower SFA content in milk compared with no grazing. In the case of SFA, favorable (lower) level obtained in the milk of HF cows, and it was in the range from 58.48 to 69.12 g/100 g of fat. Ellis et al. (2006) has been shown that increased SFA level is recorded, when white clover silage, red-clover silage, or

TABLE 2. Fatty acid of cows milk depending the season of feeding, and race in organic farm (g/100 g of fat)

Parameters	Significant			Season							
				Summer feeding				Winter feeding			
	season	breed	season* breed	BS		HF		BS		HF	
				LSM	SE	LSM	SE	LSM	SE	LSM	SE
SFA	***	NS	NS	61.60	3.941	58.48	4.300	69.14	2.049	69.12	2.914
BA	***	NS	NS	2.239	0.353	2.097	0.416	1.885	0.127	1.842	0.205
BCFA	***	NS	NS	4.77	0.616	5.05	0.617	5.45	0.497	5.38	0.510
MUFA	NS	NS	NS	21.31	4.858	22.38	5.286	21.87	2.073	21.72	2.720
TVA	***	NS	NS	3.324	1.797	4.281	2.030	1.208	0.256	1.043	0.365
OA	NS	NS	NS	14.394	2.768	14.424	2.342	16.652	2.329	16.719	2.283
PUFA	***	NS	NS	4.89	0.788	5.45	1.364	3.48	0.351	3.53	0.399
LA	***	NS	NS	1.952	0.241	2.090	0.442	1.445	0.138	1.498	0.156
CLA9 (<i>cis-9,</i> <i>trans-11</i>)	***	NS	NS	1.594	0.760	1.998	0.859	0.698	0.108	0.629	0.154
CLA10 (<i>trans-10,</i> <i>cis-12</i>)	***	***	***	0.144	0.037	0.276	0.055	0.105	0.016	0.095	0.021
AA	*	NS	NS	0.114	0.031	0.106	0.042	0.084	0.025	0.098	0.020
EPA	***	NS	NS	0.095	0.017	0.092	0.032	0.107	0.012	0.119	0.016
DPA	***	NS	NS	0.051	0.011	0.050	0.029	0.161	0.031	0.173	0.038
DHA	***	NS	NS	0.019	0.005	0.019	0.006	0.032	0.006	0.033	0.007
n-3:n-6	***	NS	*	1.97	0.333	2.28	0.512	1.34	0.148	1.32	0.131

LSM – least square mean; SE – standard error of the mean;
NS – no significant, * $P \leq 0,05$; ** $P \leq 0,01$; *** $P \leq 0,001$

grass silage were fed compared with no silage feeding. Lower SFA content in milk is advantageous from the point of view of the consumer's health, but it does not apply to butyric acid (BA) level which has health-promoting properties (Parodi, 1999; Chilliard et al., 2001). The BA level was higher in milk of BS cows both in the summer and winter season of feeding and ranged from 2.23 to 1.88 g/100 g of fat respectively. Kelsey et al. (2003) also reported higher level of BA in milk of BS compared to HF breed.

The studies showed a significant influence of the season on the level of: BA,

TVA, LA, CLA9, CLA10, EPA, DPA, DHA. Study has been demonstrated a significant effect of breed only in the case of CLA10. The statistical interaction season * breed has been demonstrated also for CLA10.

There were no significant effects of the season and breed on the monounsaturated fatty acid (MUFA) content in cows milk. The concentration of MUFA was higher in the winter season compared with the summer season in milk of BS cows, but in the case of HF cows higher concentration of MUFA in the summer season was recorded. There were highly

significant effects (Tab. 2; $P \leq 0.001$) of the season on the *trans*-vaccenic acid (TVA) concentration in milk of both breed of cows. Higher concentration of TVA was recorded in the summer feeding season. Summarize grazing had a significant effect on the level of this acids in the cows milk. Ellis et al. (2006) shown also, that grazing grass or white clover is associated with increased vaccenic acid concentration in cows milk. Research has also shown higher concentration of TVA in milk of HF cows in the summer season, but in the winter season higher concentration of this acid was recorded in BS cows milk.

There were highly significant effects of the season on the concentration of polyunsaturated fatty acids (PUFA) in the cows milk (Tab. 2; $P \leq 0.001$). The concentration of PUFA was higher in the summer season compared with the winter season in both breed of cows. The concentration of PUFA was higher in the milk of HF cows, both in the summer and winter season. The milk from BS cows was characterized by higher concentration of AA, EPA and DPA during summer season. However, milk from HF cows was characterized by a higher concentration of LA during this season.

There were highly significant effects of the season on the concentration of CLA in cows milk in both breed. A seasonal effect was seen with increased CLA concentration during summer season. Grazing forage with red-clover was associated with higher concentrations of CLA in milk. The results of many works indicate that the content of daily ratio, especially grazing on pasture, has the greatest

impact on the level of CLA9 (Ellis et al., 2006; Nałęcz-Tarwacka, 2006). The CLA content was higher in the milk of HF cows in the summer season, compared with milk of BS cows (1.99 g/100 g of fat vs. 1.594 g/100 g of fat). But in the winter season concentration of rumenic acid were at similar level and ranged from 0.62 g/100 g of fat (for HF) to 0.69 g/100 g of fat (for BS). Kelsey et al. (2003) show the level 4.4 mg/g of fatty acid for HF and 4.1 mg/g of fatty acid for BS breed. Lawless et al. (1999) reported that breed had only a small effect on the CLA content in milk.

BS milk has got a lover ratio of n-3/n-6 in the summer season ($P \leq 0.001$) compared to HF cows milk. The ratio of n-3/n-6 was higher in the milk of HF cows – 2.28, in the summer season. But in the winter trends were different, a higher ratio of n-3/n-6 has been demonstrated in HF breed – 1.34.

CONCLUSIONS

The milk from BS cows was characterized by higher concentration of SFA, BA, AA, EPA and DPA during summer season of nutrition. But milk from HF cows was characterized by a higher concentration of MUFA, PUFA, OA, LA, TVA and CLA and lover of SFA (which is advantageous from the point of view of the consumer's health). In the winter season milk from BS cows was characterized by a higher concentration of SFA, MUFA, BA, TVA and CLA compared with milk of HF breed. Finally, milk from HF breed in organic system during summer and winter season was characterized much higher nutritional value.

Acknowledgement

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REFERENCES

- COLLOMB M., BISIG W., BÜTIKOFER U., SIEBER R., BREGY M., ETTER L., 2008: Fatty acid composition of mountain milk from Switzerland: Comparison of organic and integrated farming systems. *Int. Dairy J.* 18: 976–982.
- CHILLIARD Y., FERLAY A., DOREAU M., 2001: Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest. Prod. Sci.* 70: 31–48.
- ELLIS K.A., INNOCENT G., GROVE-WHITE D., CRIPPS P., MCLEAN W.G., HOWARD C.V., MIHM M., 2006: Comparing the fatty acid composition of organic and conventional milk. *J. Dairy Sci.* 89: 1938–1950.
- ELGERSMA A., ELLEN G., VAN DER HORST H., MUUSE B.G., BOER H., TAMMINGA S., 2003: Comparison of the fatty acid composition of fresh and ensiled perennial ryegrass (*Lolium perenne* L.), affected by cultivar and regrowth interval. *Anim. Feed Sci. Technol.* 108: 191–205.
- KELSEY J.A., CORL B.A., COLLIER R.J., BAUMAN D.E., 2003: The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *J. Dairy Sci.* 86: 2588–2597.
- KRAMER J.K.G., PARODI P.W., JENSEN R.G., MOSSOBA M.M., YURAWECZ M.P., ADLOF R.O., 1998: Rumenic acid: a proposed common name for the major conjugated linoleic acid isomer found in natural products. *Lipids* 33: 835.
- LAWLESS F., STANTON C., L'ESCORP P., DEVERY R., DILLON P., MURPHY J.J., 1999: Influence of breed on bovine milk cis-9, trans-11 conjugated linoleic acid content. *Livest. Prod. Sci.* 62: 43–49.
- McGUIRE M.A., Mc GUIRE M.K., 1999: Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects on human health. *Proc. Am. Soc. Anim. Sci.* <http://www.asas.org/jas/symposia/proceedings/0938.pdf>.
- METERA E., SAKOWSKI T., SŁONIEWSKI K., REMBIAŁKOWSKA E., KUCZYŃSKA B., 2010: Wpływ systemu produkcji na zawartość substancji bioaktywnych w mleku krów – przegląd piśmiennictwa. *Przegl. Hod.* 2: 1–4.
- NAŁĘCZ-TARWACKA T., 2006: Wpływ wybranych czynników na zawartość funkcjonalnych składników tłuszczu mleka krów. Rozprawa habilitacyjna – Rozprawy Naukowe i Monografie, Wydawnictwo SGGW.
- NAŁĘCZ-TARWACKA T., GRODZKI H., 2005: Influence of early spring feeding on fatty acid levels of cow's milk. *Pol. J. Food Nutr. Sci.* 1: 67–70.
- NRC, 1996: Carcinogens and Anticarcinogens in the Human Diet. National Academy Press, Washington DC.
- PARODI P.W., 1999: Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *J. Dairy Sci.* 82, 6: 1339–1349.
- PUPPEL K., 2011: The influence of fish oil and linseed supplementation on the fat and the protein fraction content of cow's milk. Doctoral Thesis, Warsaw University of Life Sciences.
- WHITE S.L., BERTRAND J.A., WADE M.R., WASHBURN S.P., GREEN J.T., JENKINS T.C., 2001: Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. *J. Dairy Sci.* 84: 2295–2301.

Streszczenie: Zawartość kwasów tłuszczowych mleka krów rasy Brown Swiss i Holsztyńsko-Fryzjskiej odmiany czarno-białej utrzymywanych w certyfikowanym gospodarstwie ekologicznym. Celem prezentowanej pracy było określenie wpływu wybranych czynników, takich jak: sezon żywienia czy rasa, na kształtowanie się zmian w zawartości poszczególnych kwasów tłuszczowych. Badania przeprowadzono w certyfikowanym gospodar-

stwie ekologicznym, w którym utrzymywane są krowy dwóch ras: Brown Swiss (BS) i Holszynie-Fryzyjskiej odmiany czarno-białej (HF). Grupę badawczą stanowiło 30 krów (15 BS i 15 HF). Mleko do analiz pobierane było 2-krotnie od każdej krowy: w sezonie letnim (gdzie podstawę żywienia stanowiło pastwisko) oraz w sezonie zimowym. Badania wykazały istotny wpływ sezonu żywienia na poziom następujących kwasów tłuszczowych: BA, TVA, LA, CLA9, CLA10, EPA, DPA, DHA. W sezonie letnim, mleko pochodzące od krów rasy BS charakteryzowało się wyższą zawartością: SFA, BA, AA, EPA i DPA. Natomiast mleko pozyskane od krów rasy HF wyróżniało się wyższą koncentracją: MUFA, PUFA, OA, LA, TVA i CLA oraz niższym poziomem SFA. W sezonie zimowym w mleku krów rasy BS wykazano wyższą koncentrację SFA, MUFA, BA, TVA i CLA w porównaniu do mleka krów rasy HF. Podsumowując, mleko pochodzące od krów rasy HF zarówno w sezonie letnim, jak i zimowym charakteryzowało się znacznie wyższą wartością odżywczą.

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Technological usefulness of milk from Brown Swiss and Holstein-Friesian black and white cows kept in a certified organic farm

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Abstract: *Technological usefulness of milk from Brown Swiss and Holstein-Friesian black and white cows kept in a certified organic farm.* The objective of the present study was to evaluate the chemical composition and technological usefulness of cows milk kept in a certified organic farm. In the selected dairy farm, cows of two different breeds, i.e. (BS) and Holstein-Friesian (HF) between 45th–100th day of lactation, were managed under the same environment conditions. Results showed that in the summer and winter season milk yield, percentages of protein and fat, and also somatic cell score (SCC) were higher in milk of BS breed. However, protein to fat ratio, percentages of lactose and concentration of urea were higher in milk of HF breed. Only protein to fat ratio was higher in milk of BS breed during winter season. Milk from BS cows was characterized by a better usefulness of technological parameters, mainly lower coagulability time against the rennet and a higher of casein and beta-lactoglobulin content, which indicates that it is a better raw material for processors.

Key words: milk quality, organic milk, technological properties.

INTRODUCTION

Organic farming is a way of farming also known as biological, organic or

alternative. Depends on such farm management to the methods of both crop and livestock were friendly to the environment. In our country, as opposed to organic plant production, the animal equivalent of only enters the path of development. Organic milk accounts for less than 0.22% of all organic products chosen by consumers. Currently in Poland there are about 7700 certified farms where it is held about 14 000 dairy cows from which milk is harvested for human consumption (Kuczyńska and Puppel, 2010). The production of organic foods do not use of agricultural, veterinary and food chemicals, which results in the so-called high primary biological quality of manufactured products. Therefore, first ask yourself how organic pasture is differs from the conventional pasture? Reply to this question is very simple: a kind of intensity of fertilization. Organic pasture based on the maximum use of natural habitat productive capacity, while supporting only farm fertilizers. Although it occurs, however much lower yield per hectare, nutritional value resulting from

a large variety of plant species growing cancel out the differences. Nutritional qualities of organic pasture due to large amounts of plant species found in the sward, which directly translates to both the quality and nutritional value of feed.

Recent studies have pointed out significant differences in the chemical composition and an improved nutritional quality of milk and dairy products obtained by organic with respect to conventional farming systems (Borell and Sorensen, 2004; Olivo et al., 2005; Bloksma et al. 2008; Metera et al., 2010).

Quality and quantity of milk heavily depends upon two main group of factors: genetic and environmental. Considering environmental ones nutrition is the most important determinant of milk chemical composition, followed by production system, health status, as well as effect of herd and labour (Nałęcz-Tarwacka, 2006; Puppel, 2011). Within genetic factors breed of the cow seems to have substantial influence on milk compounds (Barłowska, 2007).

Technological quality of milk depends on its chemical composition, especially on casein and beta-lactoglobulin content and coagulability time against the rennet (Barłowska et al., 2006).

There were no reference in the literature about chemical composition and technological usefulness of milk from Brown Swiss and Holstein-Friesian cows kept in a certified organic farm in Poland. This is a new solution and makes it possible to analyze changes occurring in chemical composition and technological usefulness caused by the organic system.

MATERIALS AND METHODS

The study was conducted in a certified organic farm – Juchowo Sp. z o. o. In the farm, dairy cows of two different breeds: Brown Swiss (BS) and Holstein-Friesian (HF) were kept. Fifteen BS and fifteen HF lactating primiparous, between 45th –100th day of lactation, were chosen to the experiment. The cows of the both breeds in the farm were kept under the same environmental conditions in a free stall dairy barn.

In the surveyed farm in the feeding of cows used the following feed: in the summer season: hay meadow; yellow lupine, pea; oats and field pea; forage grass with red clover and spelled. But in the winter season: corn meal; oats with field pea meal, rye meal, grass silage with red clover and grass hay with red clover (Tab. 1).

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Rye meal	–	2.00
Grass silage with red clover	–	23.50
Grass hay with red clover	–	6.50
<hr/>		
JPM	18.37	17.21
BTJE	1381.90	1370.70
BTJN	1950.70	1734.60

Sampling

Representative milk samples were collected from each cow during evening milking by means of a milk meter. Milk were placed in sterile bottles, preserved Mlekostat CC. After milking samples were immediately submitted to the Cattle Breeding Division (Milk Testing Laboratory of Warsaw University of Life Sciences) for compositional analysis.

Milk analyses

Basic parameters of the milk were 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).

β -lactoglobulin (B-LG) was examined on HPLC – Agilent 1100 series. In the investigations on the development of the method for separation and identification of whey proteins' components, the following parameters were checked using literature data (Kuczyńska et al., 2011).

Coagulability time against the rennet was examined by Shern method (Jurczak, 2003).

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:

$$Y_{ijk} = \mu + A_i + B_j + (A_i \cdot B_j) + e_{ijk}$$

Where: Y_{ijk} = dependent variable, μ – general mean, A_i – breed effect ($i = 1 - 2$), B_j – season of the feeding ($j = 1 - 2$), $(A_i \cdot B_j)$ – fixed interaction effect between breed and season of the feeding.

RESULTS AND DISCUSSION

The milk performance of cows according to the study of breed and the season of nutrition dose is presented in the Table 2. Milk yield from BS cows was higher by 2.25 kg/d from HF cows, in the summer season. In winter, a slightly higher milk yield was recorded in HF breed, by 0.72 kg/day from the BS. Study demonstrated statistically significant differences in relation to the daily milk yield between seasons of the nutrition. Barłowska (2007) showed milk yield for the BS breed from 24.15 to 28.40 and from 31.95 to 37.75 for HF breed. While Kelsey et al. (2003) 38.0 kg/d and 31.5 kg/d respectively. Neja et al. (2010) give the value of annual production for the national population of HF – 7041 kg, and for BS – 6350 kg. Milk yield is consequently less in organic production system (Borell and Sorensen, 2004), which is also reflected in this studies.

A few years ago in the manufacturing industry the largest role in milk paid to the fat content. Currently intensively in production of dairy products and the

TABLE 2. Performance and the chemical composition of milk from cows depending the season of feeding and breed in organic farm

Parameters	Significant			Season							
				Summer feeding				Winter feeding			
	season	breed	season*	BS		HF		BS		HF	
				LSM	SE	LSM	SE	LSM	SE	LSM	SE
Milk yield (kg/d)	*	NS	NS	26.09	5.21	23.84	7.54	21.03	3.70	21.75	5.97
Protein (%)	NS	**	NS	3.48	0.286	3.37	0.28	3.49	0.38	3.11	0.19
Fat (%)	NS	NS	NS	3.93	0.894	3.65	1.07	3.75	0.59	3.59	0.533
Protein: fat ratio	NS	NS	NS	0.93	0.241	0.98	0.241	0.97	0.282	0.88	0.143
Lactose (%)	NS	NS	NS	4.71	0.185	4.74	0.206	4.78	0.133	4.80	0.120
Urea (mg/l)	*	NS	NS	220	65.59	185	48.27	226	43.65	233	48.23
SCC ¹ (tys/cm ³)	NS	NS	NS	162	297.04	126	235.74	189	235.54	135	159.92

¹ SCC – somatic cell count;

LSM – least square mean; SE – standard error of the mean;

NS – no significant, *P ≤ 0.05; **P ≤ 0.01.

demand for high-quality assortment of taste and appropriate, make a basic ingredient used in milk has become a protein, and ratio this component to fat. In the case of total protein levels study showed a statistically highly significant differences between the investigated breeds of cows. BS cows produced milk with a protein content without significant differences between dietary periods. Neja et al. (2010) showed comparable levels of protein (3.48%) in milk of this race, both in the organic and conventional farms. In the case of HF cows, protein content was at a lower level. Fleszar (2009) gives the average protein content on the level of 3.32% for HF, while Barłowska (2007) 3.45% for the spring – summer of feeding season and 3.56% in the autumn – winter. Olivo et al. (2005) also reported lower protein, casein and fat content in the organic cows milk with respect to the

conventional one. The reason for lower content of protein in organic system, can be lack of sugar-rich juicy feed, which stimulates production of butyric acid used for protein synthesis (Zagorska and Cipovica, 2008).

The most preferred protein-fat ratio (that is closer to 1.0) was demonstrated in the case of HF cows in the summer season (0.98). Barłowska et al. (2006) cyt. by Reklewski (1997) reported a lower protein-fat ratio, from 0.64 to 0.85.

In our study, there was no statistically significant differences in the impact of factors studied on the fat content of cows milk. The content of milk fat averaged from 3.84% (BS) to 3.62% (HF). While, Kelsey et al. (2003) give 3.86% for BS, and 4.41% for HF. Higher fat content in milk of both breeds showed the Soyeurt and Gengler (2008) 4.16% and 3.70% respectively. This is in line with the gen-

eral observation that fat is the most sensitive component of cows milk to dietary changes.

The content of lactose in the milk of experimental cows were at a similar level and ranged from 4.71% in the BS in the summer to 4.80 during the winter in HF. Barłowska (2007) showed a level of 4.87% in HF, but in other studies, this value fluctuates around 4.78% (Matwiejczuk and Król, 2009).

Lower protein, casein and fat amounts, were observed in examined organic milk, as previously reported by Olivo et al. (2005).

The studies also showed, that BS milk was characterized by a higher levels of SCC compared with milk of HF.

The factors influencing on the technological usefulness of milk are presented in Table 3. Technological quality of milk depends on its chemical composition, especially on casein content. Studies have shown a significant effect of breed on the content of casein in milk as well as casein in the total protein content and at the coagulation time.

Coagulability induced by rennet is a valuable indicator for the assessment of milk usefulness at the cheese making

TABLE 3. Technological properties of cows milk depending the season of feeding, and race in organic farm

Parameters	Significant			Season							
				Summer feeding				Winter feeding			
	season	breed	season* breed	BS		HF		BS		HF	
				LSM	SE	LSM	SE	LSM	SE	LSM	SE
Coagulation time (min)	NS	***	NS	5.46	0.436	7.12	0.666	5.63	0.411	7.16	0.525
Casein (%)	NS	***	*	2.60	0.189	2.49	0.189	2.68	0.291	2.34	0.164
Beta-lactoglobulin (g/l)	**	**	NS	5.385	0.653	4.603	0.691	4.621	0.582	4.41	0.583
Casein in total protein (%)	***	***	NS	74.81	2.42	73.96	2.42	76.59	1.109	74.99	1.402
TS (%) ¹	NS	NS	NS	13	1.570	12.67	1.570	13.10	0.586	12.63	0.584
Citric acid (%)	***	NS	NS	0.13	0.031	0.13	0.031	0.14	0.023	0.15	0.018
FPD (°C) ²	NS	NS	NS	-0.534	0.0521	-0.531	0.0521	-0.535	0.089	-0.534	0.059
Density (g/cm ³)	**	NS	*	1.029	0.45	1.030	0.45	1.029	0.41	1.028	1.161
Total acidity (°SH)	NS	NS	*	17	2.028	19	2.028	18	2.11	17	1.457
FFA ³	***	NS	NS	0.31	0.160	0.33	0.160	0.80	0.101	0.74	0.110

LSM – least square mean; SE – standard error of the mean;

NS – no significant; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

¹ TS – Total Solids;

² FPD – Freezing Point Depression;

³ FFA – Free Fatty Acid.

process. Milk BS cows was characterized by a higher protein content (Tab. 2) and its coagulation time was significantly shorter compared to the milk of HF cows. The studies showed the level of 5.46 min. for BS and 7.12 for HF in the summer season, and 5.63 min. for BS and 7.16 for HF in the winter season. This is confirmed by the study of De Marchi et al. (2007). Budslawski (1973) claims that milk whose coagulation time is 4–10 min is recognized appropriate and suitable for cheese making.

Quantity, quality and types of proteins in milk influence the yield and technological and health-beneficial properties of milk. The value of milk proteins is more than twice as valuable as milk fat. The amount of proteins produced by cows strongly depends upon many factors including: cows' diet, health, stage of lactation, breed and part of the year (Puppel, 2011). Milk is synthesized in mammary secretory epithelial cells and contains 2 major protein groups: casein's (insoluble) and whey proteins (soluble). The major milk proteins are α S1-CN, α S2-CN, β -CN, κ -CN, α -LA, and β -LG.

In our study, there were statistically significant differences in the impact of factors studied on the beta-lactoglobulin (B-LG) content. The highest level was recorded in BS breed – 5.385 g/l during summer season, the lowest level was recorded in HF breed during winter season.

Casein's, are important for cheese yield, milk coagulation time, and curd firmness (Wedholm et al., 2006). The studies showed the level from 2.60 to 2.68% for BS and from 2.49 to 2.34% for HF. Also in the case of casein, study showed higher levels of this component in the milk of BS cows. Flow of cheese

from milk with a low content of casein is less suitable, because it creates a structure of a clot. The optimal ratio of fat to casein in milk, allowing to get the best cheddar cheese is 1:0.7.

Urea levels in milk should be on a level from 150 to 300 mg/l (Lach, 2003). The concentration of this component in milk is closely related to both the amount and type of feed included in the ration. The high level of urea in milk during pasture feeding season can be tolerated and even economically justified. In the analyzed milk showed a significant relationship between winter feeding season and urea content in milk of both breeds. In the studies of Litwińczuk et al. (2006) differences between the two seasons in the content of urea in HF breed was at the level 20 mg/l, while in our study it was the level 48 mg/l.

The study also showed a significant effect of feeding season on the FFA content in milk of cows.

Density of milk was at the level 1.029 for BS cows and 1.029–1.030 g/cm³ for HF cows. Proteins, carbohydrates and minerals increase, but fat reduces the density of milk. The density of raw milk varies under the influence of the lactation period, the breed of cows and their health.

Freezing point is a temperature which milk passes from the liquid state to solid. It depends on the concentration of lactose and soluble salt content. Also depend upon the acidity of milk. The freezing point in the milk of BS cows was at the higher level and ranged from –0.534 to –0.535°C.

The quality of raw milk is very important, the milk may have at least 5 types of undesirable traits, what caught be a problems in its processing into cheese. These include the follow-

ing: the high content of bacteria, affects the formation of defects in texture and appearance of the cheese. High somatic cells count reduces clotting time of milk. Additionally, antibiotics, sulfonamides may cause inhibition effects on microorganisms introduced with the acid. Therefore, that in organic system chemicals are eliminated in production, the possibility of their penetration into milk is equal to zero. What is a proof, that the milk of cows from organic farms is a better product for processing.

CONCLUSIONS

In conclusion the milk from BS cows was characterized by a better usefulness of technological parameters, mainly lower coagulability time against the rennet and a higher of casein and beta-lactoglobulin content, which indicates that it is a better raw material for production of fermented milk products or cheese.

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REFERENCES

BARŁOWSKA J., 2007: Wartość odżywcza i przydatność technologiczna mleka krów 7 ras użytkowanych w Polsce. Rozprawy naukowe Akademii Rolniczej w Lublinie.
BARŁOWSKA J., LITWIŃCZUK Z., KRÓL J., TOPYLA B., 2006: Technological usefulness

of milk of cows of six breeds maintained in Poland relative to a lactation phase. *Pol. J. Food Nutr. Sci.* 15: 56, 17–21.
BLOKSMA J., ADRIAANSEN-TENNEKES R., HUBER M., VAN DE VIJVER L.P.L, BAARS T., DE WIT J., 2008: Comparison of organic and conventional raw milk quality in the Netherlands. *Biolog. Agr. Hort.* 26: 69–83.
BORELL E., SØRENSEN J.T., 2004: Organic livestock production in Europe: aims, rules and trends with special emphasis on animal health and welfare. *Liv. Prod. Sci.* 90: 3–9.
BUDSŁAWSKI J., 1973: *Consumable milk.* WPLiS, Warszawa.
DE MARCHI M., DAL ZOTT R., CASSANDRO M., BITTANTE G., 2007: Milk coagulation ability of five dairy cattle breeds. *J. Dairy Sci.* 90: 3986–3992.
FLESZAR J., 2009: Ilościowa i jakościowa wydajność produkcji mleka w gospodarstwie ekologicznym. *J. Res. Applicat. Agricul. Engin.* 54: 75–78.
JURCZAK M.E., 2003: *Mleko produkcja, badanie, przerób.* Wydawnictwo SGGW, Warszawa.
KELSEY J.A., CORL B.A., COLLIER R.J., BAUMAN D.E., 2003: The effect of breed, parity, and stage of lactation conjugated linoleic acid (CLA) in milk fat from dairy cows. *Am. Dairy Sci. Assos.* 86: 2588–2597.
KUCZYŃSKA B., GOŁĘBIEWSKI M., PUPPEL K., BRZOZOWSKI P., 2011: Comparison of fat and protein fractions of milk constituents of Montbeliarde and Polish Holstein – Friesian cows originated from the selected farm in Poland. *Acta Vet. Brno* (in print).
KUCZYŃSKA B., PUPPEL K., 2010: Mleko ekologiczne – niezastąpione źródło bioaktywnych składników. *Przegl. Mleczarski* 9, 4–9.
LACH Z., 2003: Narzędzia do oceny prawidłowego zarządzania stadem krów mlecznych. *Przegl. Hod.* 6: 8–11.
LITWIŃCZUK A., BARŁOWSKA J.K., RÓL L., SAWICKA W., 2006: Porównanie składu chemicznego i zawartości mocznika w mleku krów czarno-białych i simentalskich z okresy żywienia letniego i zimowego. *Annales UMCS Lublin* 10: 67–72.
MATWIEJCZUK A., KRÓL L., 2009: Profil kwasów tłuszczowych w mlku krów różnych ras w okresie wiosenno-letnim. *Przegl. Hod.* 7: 3–6.

- METERA E., SAKOWSKI T., SŁONIEWSKI K., REMBIAŁKOWSKA E., KUCZYŃSKA B., 2010: Wpływ systemu produkcji na zawartość substancji bioaktywnych w mleku krów – przegląd piśmiennictwa. *Przegl. Hod.* 2: 1–4.
- NAŁĘCZ-TARWACKA T., 2006: Wpływ wybranych czynników na zawartość funkcjonalnych składników tłuszczu mleka krów. Rozprawa Habilitacyjna – Rozprawy Naukowe i Monografie, Wydawnictwo SGGW.
- NEJA W., SAWA A., BOGUCKI M., 2010: Zmiany wydajności i składu mleka krów krajowej populacji aktywnej. *Przegl. Hod.* 5: 4–7.
- OLIVO C.J., BECK L.I., MOSSATE GABBI A., SANTINI CHARÃO P., SOBCZAK M.F., GOMESUBERTY L.F., DÜRR J.W., ARAÚJO FILHO R., 2005: Composition and somatic cell count of milk in conventional and agro-ecological farms: a comparative study in Depressão Central, Rio Grande do Sul state, Brazil. *Liv. Res. Rural Dev.* 17, 6: <http://www.cipav.org.co/lrrd/lrrd17/6/oliv17072.htm>.
- PUPPEL K., 2011: The influence of fish oil and linseed supplementation on the fat and the protein fraction content of cow's milk. Doctoral Thesis, Warsaw University of Life Sciences – SGGW.
- SOYEURT H., GENGLER N., 2008: Genetic variability of fatty acids in bovine milk. *Biotech. Agron. Soc. Envir.* 12: 203–210.
- WEDHOLM A., HALLEN E., LARSEN L.B., LINDMARK-MÅNSSON H., KARLSSON A.H., ALLMERE T., 2006: Comparison of milk protein composition in a Swedish and a Danish dairy herd using reversed-phase HPLC. *Acta Agric. Scand. Sec. t. A, Animal Sci.* 56: 8–15.
- ZAGORSKA J., CIPROVICA I., 2008: The chemical composition of organic and conventional milk in Latvia. *Foodbalt* 10–14.
- cji, sezon żywienia czy rasa – na kształtowanie się zmian w zawartości podstawowego składu chemicznego mleka, jak również jego przydatności technologicznej. Badania przeprowadzono w certyfikowanym gospodarstwie ekologicznym, w którym utrzymywane są krowy dwóch ras: Brown Swiss (BS) i Holszyńsko-Fryzyjskiej odmiany czarno-białej. Grupę badawczą stanowiło 30 krów (15 BS i 15 HF). Mleko do analiz pobierane było 2-krotnie od każdej krowy: w sezonie letnim (gdzie podstawę żywienia stanowiło pastwisko) oraz w sezonie zimowym. Badania wykazały, że w trakcie sezonu letniego, krowy rasy BS charakteryzowały się wyższą wydajnością, ale też mleko od nich pobrane wyróżniało się wyższym poziomem białka oraz tłuszczu. Natomiast wyższy poziom laktozy, mocznika, a także korzystniejszy stosunek – białko: tłuszcz – wykazano w mleku krów HF. Mleko krów rasy BS charakteryzowało się lepszymi parametrami przydatności technologicznej: krótszym czasem koagulacji oraz wyższym poziomem kazeiny i beta-laktoglobuliny.

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Streszczenie: *Przydatność technologiczna mleka krów rasy Brown Swiss i Holszyńsko-Fryzyjskiej odmiany czarno-białej utrzymywanych w certyfikowanym gospodarstwie ekologicznym.* Celem prezentowanej pracy było określenie wpływu wybranych czynników, takich jak: system produk-

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Effect of breed and season on the boar's semen characteristics

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Abstract: *Effect of breed and season on the boar's semen characteristics.* The investigation was carried out on 6627 boars ejaculates collected from the following breeds: Polish Large White, Polish Landrace, Pietrain, Duroc × Pietrain. Ejaculates were obtained in 2003–2008 in Breeding Centre for Animal Reproduction in Łowicz. The aim of this study was to assess the impact of boars breed and season on some characteristics of ejaculates: volume, sperm concentration, % of live sperm in the ejaculate. Among the four studied breeds, the largest volume of ejaculate was found in Pietrain, when the smallest one in Duroc × Pietrain. The concentration of spermatozoa in ejaculates was the highest in Duroc × Pietrain and the lowest in Pietrain breed. The lowest % of live spermatozoa in the ejaculate was observed for PL. The parameters of ejaculates was also influenced by the month of collection. The largest volume of ejaculate was found at autumn-winter season. In these months the concentration of spermatozoa was the lowest. The highest concentration of sperm and a high % of live spermatozoa were noticed in ejaculates collected during the late winter and spring time. It was proved that the season and breed has an effect on the volume, sperm concentration and the ratio of live sperm in ejaculates obtained from AI boars.

Key words: boar, semen, breed, season.

INTRODUCTION

Insemination of sows with boar's semen coming from Station of Sow Insemination improve herd of pigs in terms of important traits from an economic point of

view, in particular, must characteristics and production health and traits. This can be achieved only when for insemination were used ejaculates of boars with high performance features, with the highest breeding value, and better production predisposition.

Pigs are considered a species devoid of seasonality of reproduction. Selection led to the fact that sows are polyestral, and sexual activity of boars is observed throughout the year. However, as shown by numerous studies, sperm production by males depends on many factors, both genetic (Kondracki, 2003) and environmental (Kunavongkrit and Prateep, 1995), what can significantly affects the quality and quantity of obtained sperm.

The aim of this study was to analyze the influence of breed and season of semen collection on the volume, sperm concentration and percentage of spermatozoa with proper mobility in boars ejaculates used in artificial insemination.

MATERIAL AND METHODS

The study was conducted on 6627 ejaculates collected from 95 boars, including 22 boars of Polish Large White breed, 48 Polish Landrace, 9 Pietrain and 16 Duroc

× Pietrain hybrids, used in 2003–2008 at the Station of Sow Insemination in Zielkowice. Semen was collected by manual method. Obtained ejaculates was evaluated according to quantitative and qualitative methodology used in the Polish Stations of Sow Insemination in accordance to the National Animal Breeding Centre. The following parameters were evaluated:

- volume of ejaculate (ml);
- concentration of spermatozoa in the ejaculate (thous./mm³);
- percentage of live sperm in the ejaculate (showing progressive motion).

The data were divided into 12 subgroups, including ejaculates collected in different months of the year (Tab. 1).

The values of the characteristics of ejaculates were statistically verified according to breed group.

Performed multivariate analysis of variance with least squares method using SPSS 14.0. according to the following statistical model:

$$Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$$

Where: Y_{ijk} – value of trait, μ – the average of the trait, a_i – effect of boars breed, b_j – effect of month of the year, ab_{ij} – breed × month interaction, E_{ijk} – error.

The significance of differences was estimated with Fischer test.

RESULTS

The data contained in Table 2 show the diversity of boars sperm parameters of different breeds.

The largest volume characterised ejaculates collected from Pietrain boars, whose average volume was 270.36 ml and differed significantly ($P \leq 0.01$) from ejaculates breed Duroc × Pietrain (mean 231.75 ml) and Polish Large White (mean 255.25 ml). In presented study, the smallest volume of semen showed a Duroc × Pietrain breed (average 231.75 ml), and thus highly significantly differed from the average volume of the ejaculates of the other breeds.

The concentration of spermatozoa of all tested breeds was an average of 446.86 thous./mm³ (Tab. 2). Pietrain boars had the lowest concentration of spermatozoa (mean 406.53 thous./mm³) in semen, and this value differed significantly ($P \leq 0.01$) from the average concentration of spermatozoa in ejaculates of other studied breeds. Boars Duroc × Pietrain (average 475.13 thous./mm³) characterized the highest concentration of sperm in the ejaculate and significantly differed ($P \leq 0.01$) from the values obtained in Polish Landrace (435.26 thous./mm³) and Pietrain (406.53 thous./mm³) breeds.

The average level of sperm motility was 78.76% (Tab. 2).

TABLE 1. Number of ejaculates collected from boars of various breeds in different months of the year

Breed	1	2	3	4	5	6	7	8	9	10	11	12	Σ
Duroc × × Pietrain	148	138	160	162	179	168	206	177	161	160	140	140	1939
PL	217	234	257	264	260	244	230	203	210	223	231	272	2845
Pietrain	46	44	54	48	47	50	57	54	45	47	43	46	581
PLW	110	100	125	127	133	112	106	90	82	92	90	95	1262
Σ	521	516	596	601	619	574	599	524	498	522	504	553	6627

TABLE 2. Ejaculate characteristics depending on the breed

	Trait					
	volume		concentration		% of sperm moving	
	LSM	SE	LSM	SE	LSM	SE
Breed total	256.18	1.23	446.86	1.62	78.76	0.09
Duroc × Pietrain	231.75AB	1.74	475.13A	2.31	79.34A	0.12
PL	267.38AC	1.52	435.26AB	2.01	77.58ABC	0.11
Pietrain	270.36B	3.38	406.53AC	4.46	78.95B	0.24
PLW	255.25BC	2.70	470.51BC	3.56	79.15C	0.19

A, B, C are different at $P \leq 0.01$;

LSM – least square mean;

SE – error.

The smallest percentage of sperm moving with an advancing motion showed a Polish Landrace breed (average 77.58%) and significantly differed ($P \leq 0.01$) from the other breeds (Tab. 2). The greatest value of this trait was observed in the Duroc × Pietrain breed (79.34%).

In Table 3 the parameters of ejaculates in the particular months was summarized. The volume of all studied ejaculates averaged 256.18 ml. The largest volume characterised by ejaculates collected in November (mean 295.23 ml) and the lowest in April (average 225.35 ml). Month of semen collection significantly influenced the volume of ejaculate received ($P \leq 0.05$).

The results at presented research show that the highest concentration of spermatozoa were characterised by ejaculates collected in winter and spring season: in February (average 475.59 thous./mm³) – winter, in March (average 468.95 thous./mm³), April (average 477.36 thous./mm³) – spring (Tab. 3). Mean values of this trait obtained in February and April differ significantly ($P \leq 0.05$) from concentration of spermatozoa in semen recorded in the remaining months

of the year. The lowest average concentration (420.48 thous./mm³) were observed in October (autumn). In the summer and autumn season, the smallest concentrations of sperm in the ejaculate were noticed and were statistically significantly different from concentrations in spring and winter.

In presented studies one can observe the relationship between the volume and concentration of spermatozoa in semen. The highest volume was found in ejaculates collected in the autumn months, and thus the concentration of sperm in these months was the lowest. The largest concentration of spermatozoa in ejaculates was in the spring months and the same volume of ejaculates at that time were the smallest (Tab. 3).

The percentage of live sperm in the ejaculate showing the correct movement, on average was steady at 78.76% (Tab. 3). The highest percentage of live sperm was found from January to May (winter and spring season), and in August and September (late summer). The lowest values of this feature coincided with the period from October to December (autumn season), and in June and July (summer). November (autumn

TABLE 3. Semen parameters according to the month of collection

Month	Trait					
	Volume (ml)		Concentration (thous/mm ³)		% of sperm moving	
	LSM	SE	LSM	SE	LSM	SE
	256.18	1.23	446.86	1.62	78.76	0.09
January	243.73abc	3.96	459.70ab	5.24	78.93A	0.28
February	236.24ade	4.04	475.59c	5.34	79.05Bb	0.29
Marz	229.45dfg	3.69	468.95ac	4.87	78.94C	0.26
April	225.35fh	3.78	477.36c	4.99	79.01Dd	0.27
May	226.75egh	3.76	453.78bd	4.98	79.02Ee	0.27
June	247.27bi	3.80	439.17e	5.01	78.70f	0.27
July	251.68ci	3.67	425.17fg	4.85	78.74g	0.26
August	262.34	3.85	437.45ef	5.09	78.93H	0.27
September	278.55j	4.09	441.40de	5.41	79.05Ii	0.29
October	289.55jk	3.67	420.48g	5.24	78.68j	0.28
November	295.23k	4.12	427.61eg	5.45	77.81ABCDEfgHIj	0.29
December	288.08jk	4.00	435.61eg	5.29	78.22bdei	0.28

Volume: a, b, c, d, e, f, g, h, i, j, k, do not differ at $P \leq 0.05$;

Concentration: a, b, c, d, e, f, g do not differ at $P \leq 0.05$;

% of live sperm cells: A, B are different at $P \leq 0.01$, a, b differ at $P \leq 0.05$.

season) was characterised by the lowest value of that trait (average 77.81%), and statistically significant ($P \leq 0.01$) differed from the average values of this trait obtained in the months from January to May (winter and spring season) and August and September (summer).

In Figure 1 was presented the dynamics of change of ejaculates volume of different breeds depending on the season. Minimal volume of ejaculate was observed in boars of Duroc \times Pietrain breed (mean 214.19 ml) in February (winter season), and (an average of 214.68 ml) in May (spring season). However, the greatest value of this trait was found in the breed Pietrain (mean 326.56 ml) in October (autumn season).

Figure 2 shows the dynamics of the spermatozoa concentration of different breeds, depending on the month. The highest concentration had ejaculates,

which showed the lowest volume (Duroc \times Pietrain breed). Pietrain breed had the lowest concentration of spermatozoa per ejaculate, and the highest volume of semen. The biggest changes in the different seasons of the year showed the Pietrain breed. This breed received the highest concentrations of sperm in April (an average of 457.69 thous./mm³), and thus the volume of ejaculate in this month was the lowest. The lowest concentration in October (an average of 374 thous./mm³) and November (average 373.1 thous./mm³), and also the largest volume in these months (Fig. 2).

In Figure 3 variations in the proportion of live sperm in ejaculates depending on the breed of the boar in each month of the year was shown. The largest percentage of live sperm was found for Duroc \times Pietrain hybrids and purebred PLW. Duroc \times Pietrain in January, February

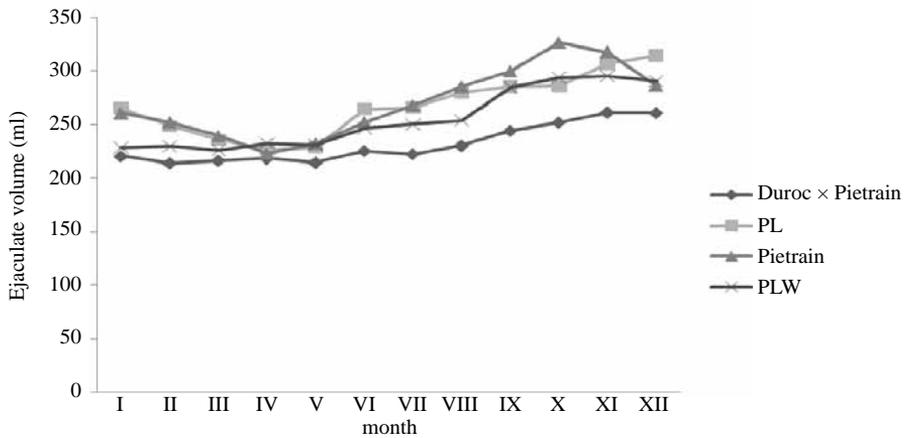


FIGURE 1. Changes in volume of ejaculate depending on the season and breed

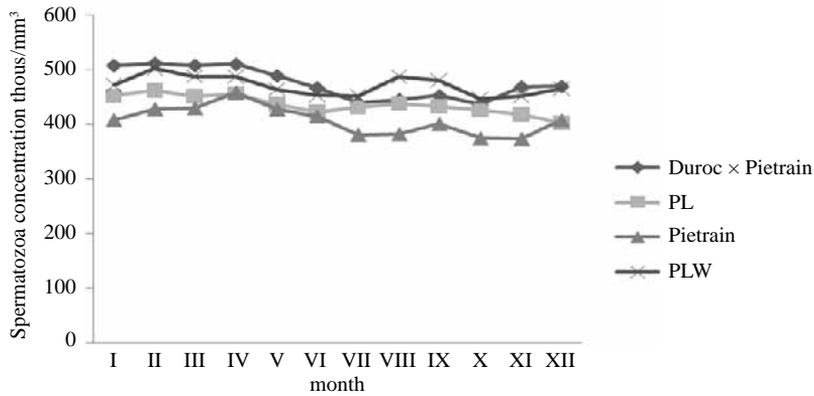


FIGURE 2. Changes in concentration of spermatozoa, depending on the month of sperm collection and breed

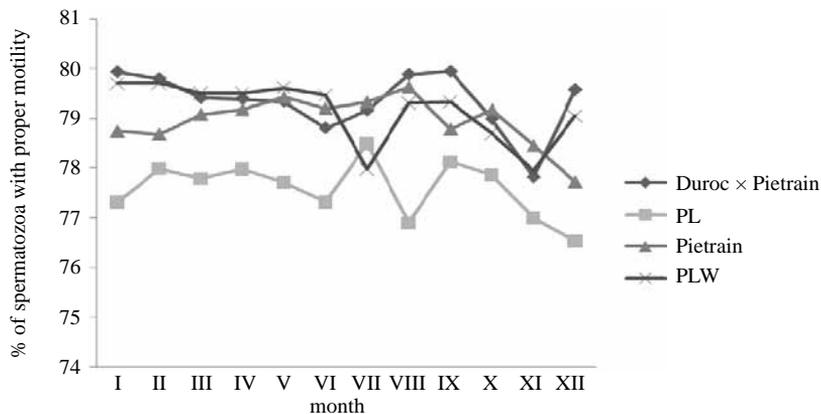


FIGURE 3. Changes in sperm motility in boars ejaculates depending on the month

and August, September showed greater motility of spermatozoa, while from September to November was characterised by a significant decrease in sperm motility, as well as breeds PLW, PL, Pietrain. The biggest changes in sperm motility were seen in July (an increase for Polish Landrace and the decline for PLW).

DISCUSSION

Breed and sperm parameters

Obtained results suggest that the breed of boar has a substantial effect on semen volume, sperm concentration and % of live spermatozoa in the ejaculate.

The average ejaculate volume of an adult boar is estimated as around 200–250 ml. The volume of ejaculate of the boar range from 100 to 1000 ml. Effect of breed on the volume of the boar ejaculate also showed in their study of Gączarzewicz et al. (2000), Udała et al (2005), Kondracki (2003), Dubiel (1987).

The analysis of literature data shows that hybrids characterized the better semen parameters than the purebred boars. In Gączarzewicz et al. (2000) observed that the boars of Polish Large White breed, Pietrain, Hampshire, Duroc ejaculates obtained lower volume than hybrids. Ones thus, these studies suggest that purebred boars had worse the quantitative parameters of semen than hybrids, which is not consistent with presented results. However, the results by Wysokińska et al. (2008) confirm the results obtained by authors. These authors also showed that the volume of ejaculates of purebreds (Duroc breed is an exception) exceeds the amount of

volume of semen obtained from Duroc × Pietrain hybrids.

The concentration of spermatozoa (sperm density) is estimated based on the number of sperm per its volume (1 mm³). Normally, the volume of ejaculate is closely related to its concentration. There is a correlation between the volume and concentration of spermatozoa in the ejaculate. The volume is inversely proportional to the concentration of spermatozoa. The larger volume of ejaculate means the lower concentration of sperm and vice versa. The average number of sperm in 1 ml of ejaculate varies from 150×10^6 to 600×10^6 (Strzeżek, 1999).

As indicated by literature data and own research the level of concentration of spermatozoa depends on the boar's breed. In studies of Kondracki (2003), the hybrids Duroc × Pietrain showed an intermediate concentration 559.97 thous./mm³ then the purebreds (Duroc and Pietrain), but it was closer to the Duroc breed. The concentration of spermatozoa of the Pietrain breed was an average of 445.12 thous./mm³ and differed significantly ($P \leq 0.01$) by over 140 thous./mm³ the concentration of the breed Duroc boars 588.17 thous./mm³. Paternal heterosis resulted as an example: Duroc × Pietrain generally characterised the better semen quality parameters, a stronger libido, and larger testes than Pietrain breed. These studies confirm the results achieved in our study, where in Pietrain and Duroc hybrids was the highest concentration of spermatozoa in contrast to the much lower values of this trait in the other purebreds. In studies Milewska (2007) the highest concentration of sperm cells can be observed in boars of the Pietrain breed (388 thous./mm³), but statistically

significant differences between the races was not observed. Among the hybrids the highest concentration showed Duroc × Pietrain boars average of 356 thous./mm³, which also confirms the results obtained in our study. Research by Szostak (2003) confirms significant differences in sperm concentration between ejaculates of different breeds, and indicate a correlation between the volume and concentration of spermatozoa in the ejaculate. The highest concentration had ejaculates, which showed the smallest volume. In our research, this relationship was also observed in boars of the Pietrain breed, which were characterised by a large volume of semen, but a small concentration of spermatozoa, while in the hybrids ejaculates were of low volume, but with a high concentration of spermatozoa.

Also Milewska et al. (2003) in the study showed differences in sperm concentration in tested ejaculates of boars. The highest concentration in ejaculate showed boars of PLW breed (365 thous./mm³), Pietrain (358 thous./mm³), and hybrid 990 × Pietrain (348 thous./mm³). This results highly significant ($P \leq 0.01$) differed from the concentration of Duroc boars ejaculates (190 thous./mm³) and significantly ($P \leq 0.05$) from Hampshire boars (287 thous./mm³) and Hampshire × Pietrain hybrids (267 thous./mm³). The authors of these studies confirm the significant influence of boar breed on spermatozoa concentration in the ejaculate (Milewska et al., 2003).

Assessment of sperm motility (% of live sperm in the ejaculate) is one of the most important sperm parameters. The percentage of motile sperm, ejaculate volume, sperm concentration, and – on the basis of these three parameters, labo-

ratory staff assess whether the semen meets the minimum criteria for suitability for artificial insemination. (Brucka-Jastrzębska et al., 2008; Gasiński, 1999).

Brucka-Jastrzębska et al. (2008) in the studies comparing the sperm parameters of different breeds showed the better semen parameters of hybrids in relation to purebreds ones. The largest percentage of live sperm per ejaculate were in hybrids Pietrain × Duroc, Duroc × Pietrain. Also, the results of Kondracki et al. (2003) in hybrids Duroc × Pietrain, and purebreds Duroc and Pietrain, the sperm motility characteristics, show that the hybrids are better than purebreds. This was caused by heterosis effect on the sperm motility. Duroc breed was characterised by a sperm motility of higher than about 0.53% of the Pietrain breed. Sperm motility of hybrids was higher about 3.31% from Duroc and about 3.84 from Pietrain, which was statistically significant ($P \leq 0.01$). In conclusion ejaculates of different breeds may differ significantly by semen parameters, including percentage of motile spermatozoa, which also confirmed in our research, where the greatest percentage of motile sperm were characterized by ejaculates collected from hybrids Duroc × Pietrain.

Season of the year and sperm parameters

Seasonality exploitation of boars influences the number and quality of collected semen. In our study, the most quantitatively ejaculates were collected in autumn and winter. Similar results ie, significantly higher volumes of ejaculates of boars in the fall and winter season have shown in studies by Pokrywka and Ruda (2004), Wysokińska et al. (2005),

Kondracki et al. (1997). Influence of season on semen volume obtained is explained by the authors according to the length of light, ambient temperature, seasonal variations in male sexual activity. Shorter days and lower temperature in autumn-winter season, had a beneficial effect on the quantity of sperm produced (the process of spermatogenesis). During the summer the authors have received ejaculates from boars with less favorable characteristics, since in this period, boars have a weaker libido and reduced sperm production. In our study, the smallest volume was obtained during the spring (III–VI), which can be explained by reduced male sexual activity during this period.

The time of year had also significantly effect on the concentration of spermatozoa (Szostak, 2003; Bronicka and Dembiński, 1999; Kondracki et al., 1997; Milewska, 2003; Sławeta and Strzeżek, 1984; Wysokińska et al., 2005). The analysis made by Kondracki et al. (1997) shows that the highest concentration of sperm were characterised by ejaculates collected from August to November (autumn season) above 470 thous./mm³, and the lowest concentration in a month: January, February, March (winter season) below 420 thous./mm³. Research of Sławeta and Strzeżek (1984) also showed the highest concentration of sperm in the autumn season (an average of 472.67 thous./mm³), and the lowest in winter (average of 340.51 thous./mm³). According to Bronicka and Dembiński (1999), ejaculates collected in the autumn season has the highest concentration of sperm as a result of restoring the functionality of testis after the summer. Low libido in the summer is manifested as a result of sea-

sonal changes in the behavior of sexually active boar. Autumn season positively affects the process of spermatogenesis, as a result of lower ambient temperature. Also Szostak (2003) in his study received the largest concentration in the autumn season (430.61 thous./mm³) and spring (415.82 thous./mm³) and the lowest in winter (403.27 thous./mm³). Presented results differ from those obtained in above mentioned studies. The highest concentrations of sperm were obtained in the winter-spring season. At the same time presented research confirms the negative correlation between the volume and concentration of spermatozoa. The highest volume had ejaculates of the autumn months and therefore the concentration of sperm in these months was the lowest. The largest concentration of spermatozoa in ejaculates was in the spring months and the same volume of ejaculates at the time were the smallest.

Live sperm motility in the ejaculate is another parameter dependent on environmental factors. The highest percentage of live sperm was found in the winter and spring season and late summer. The lowest values of this feature for the season coincided with the autumn and summer. Similar results were obtained by Kondracki et al. (1997), from August to November (the end of summer, the season of autumn) the percentage of sperm with progressive movement was much smaller. The highest percentage of live sperm was from January to April (winter, early spring). In the summer time, there is a reduction of sperm motility, due to greater ambient temperature, which in turn leads to disturbances in the process of spermatogenesis. Influence of season on the % of live spermatozoa

in the ejaculate were confirmed also in studies by other authors Pokrywka and Ruda (2004) but Wysokińska et al. (2005) have shown no significant impact seasons on sperm motility.

CONCLUSION

Based on the results concluded that season and breed has an effect on volume, sperm concentration and sperm live in ejaculates obtained from boars semen used for insemination.

REFERENCES

- BRONICKA A., DEMBIŃSKI Z., 1999: Aktualne kryteria oceny oraz uwarunkowania jakości nasienia knura. *Medycyna Wet.* 55 (7), 436–439.
- BRUCKA-JASTRZĘBSKA E., BIAŁEK M., BRZEZIŃSKA M., KAŃCZUGA D., DREWNOŃSKI W., LISIECKI L., 2008: Parametry ejakulatu w zależności od rasy świń. *Medycyna Wet.* 64 (10), 1248–1251.
- DUBIEL A., 1987: Wpływ wieku oraz leków układu adrenergicznego na odruchy płciowe i właściwości nasienia knurów wybranych ras. *Zesz. Probl. Post. Nauk Roln.* 340, 121–140.
- GASIŃSKI M., 1999: Zasady postępowania z nasieniem knura. *Trzoda chlewna* 4: 24–26.
- GĄCZARZEWICZ D., UDAŁA J., LASOTA B., BŁASZCZYK B., 2000: Kształtowanie się wybranych wskaźników oceny jakościowej i biochemicznej nasienia knurów eksploatowanych w zakładzie unasienniania zwierząt. *Zesz. Nauk. Chów Hod. Trzody Chlewniej* 48 (1), 93–101.
- KONDRACKI S., 2003: Wykorzystanie inseminacji w chowie świń. *Hodowca Trzody Chlewniej* 2, 32–35.
- KONDRACKI S., ANTOLIK A., ZWIERZ B., 1997: Cechy nasienia knura w zależności od pory roku. *Rocz. Nauk. Zoot. T.* 24, z. 3, 67–76.
- KONDRACKI S., WYSOKIŃSKA A., KOWALCZYK Z., 2003: Wpływ krzyżowania ras duroc i pietrain na cechy ejakulatów knurów mieszańców dwurasowych. *Zeszyty Naukowe Przeglądu Hodowlanego* 68 z. 2, 105–112.
- KUNAVONGKRITA., PRATEEP P., 1995: Influence of ambient temperature on reproductive efficiency in pigs: (1) boar semen quality. *Pig J.* 35, 43–47.
- MILEWSKA W., 2007: Ocena przyżyciowa knurów rasy hampshire i pietrain oraz mieszańców dwurasowych, a efekt użytkowania rozplodowego w stacjach unasienniania loch. *Medycyna Wet.* 63 (6), 708–711.
- MILEWSKA W., ELJASIAK J., TYMIŃSKI K., 2003: Długość użytkowania przyczyny brakovania oraz jakość nasienia knurów inseminacyjnych. *Zeszyty Naukowe Przeglądu Hodowlanego* 68 z. 2, 123–130.
- POKRYWKA K., RUDA M., 2004: Wpływ sezonu eksploatacji na wybrane wskaźniki ilościowe i jakościowe nasienia knurów rozpoczynających użytkowanie w różnym wieku. *Zeszyty Naukowe Przeglądu Hodowlanego* 72 z. 2, 85–92.
- SŁAWETA R., STRZEŻEK J., 1984: Pora roku a właściwości biologiczne konserwowanego nasienia knura. *Medycyna Wet.* 40(10), 619–622.
- STRZEŻEK J., 1999: *Andrologia*. Wyd. Platan, Kraków, 201–215.
- SZOSTAK B., 2003: Wpływ genotypu, wieku knura i sezonu eksploatacji na wybrane cechy ejakulatów. *Zeszyty Naukowe Przeglądu Hodowlanego* 68 z. 2, 147–152.
- UDAŁA J., GĄCZARZEWICZ D., LASOTA B., BŁASZCZYK B., SEREMAK B., STANKIEWICZ T., 2005: Charakterystyka zmian budowy morfologicznej plemników knurów użytkowanych w inseminacji. *Folia Univ. Agric. Stetin. Zootechnika* 243 (47), 161–172.
- WYSOKIŃSKA A., KONDRACKI S., BANASZEWSKA D., 2005: Wpływ pory roku na cechy fizyczne ejakulatu knurów mieszańców duroc x pietrain i hampshire x pietrain oraz rasowych knurów ras duroc, hampshire i pietrain. *Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego*, t. 1, nr 3, 533–541.

Streszczenie: *Wpływ sezonu oraz rasy knura na wybrane cechy nasienia.* Badania przeprowadzono na 6627 ejakulatach pobranych od knurów następujących ras: wielka biała polska, polska biała zwiśłoucha, pietrain, duroc x pietrain. Dane o ejakulatach pobieranych w latach 2003–2008 pochodziły z Mazowieckiego Centrum Hodowli i Rozrodu

Zwierząt w Łowiczu. Celem badań była ocena wpływu rasy knura i pory roku na niektóre cechy nasienia: objętość, koncentrację plemników oraz procent plemników żywych w ejakulacie. Spośród czterech badanych ras największą objętość ejakulatu stwierdzono u rasy pietrain, najmniejszą zaś u duroc × pietrain. Koncentracja plemników w nasieniu była najwyższa u duroc × pietrain i najniższa u pietrain. Najniższy procent żywych plemników w ejakulacie zaobserwowano u pbz. Miesiąc, w którym pobierano nasienie od knurów, również wpłynął na parametry ejakulatów. Największe objętości ejakulatów uzyskiwano w sezonie jesienno-zimowym. W tych miesiącach również koncentracja plemników była najmniejsza. Najwyższą koncentrację plemników w nasieniu i wysoki pro-

cent plemników żywych zanotowano podczas późnej zimy i wiosny. Podsumowując, można stwierdzić, że sezon pobrania nasienia i rasa knura mają wpływ na objętość, koncentrację plemników i odsetek plemników żywych w ejakulatach uzyskiwanych od samców używanych do inseminacji.

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The assessment of fattening value of White-head Synthetic Line lambs kept in Ciosny farm in Łódź province

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Abstract: *The assessment of fattening value of White-head Synthetic Line lambs kept in Ciosny farm in Łódź province.* The observation was carried out on 277 lambs born and reared on private farm Ciosny during 6 successive years. The influence of sex, birth type, and ewe's lambing number on birth weight, body weight at 28th, 56th and 70th day of life was evaluated. The ram lambs obtained higher daily gains in comparison with ewe lambs. The lack of differences on daily gains between singles and twins in 28th day of life suggested good milk performance of their mothers. The highly statistically lightest birth weight was delivered by first time lambing ewe. Obtained results confirmed good fattening performance of ram lambs as well as ewe lambs of White-head Synthetic Line.

Key words: sheep, synthetic line, fattening.

INTRODUCTION

In the realities of market economy of our country, the profits from sheep production are obtained mainly from the selling slaughter's lambs. The lack of domestic market for lambs' meat and mutton and in the same time opportunities to export, led to regard slaughter lambs as the main sheep product. The quality of meat is an increasing importance to consumers in Europe and has gained preference over

quantity. To produce young lean lambs' meat farmers needs the synthetic lines, which are an early maturing, have good meat performance and can be used for commercial crossbreeding with ewes of local breeds. The White-head Synthetic Line is meat line created in Poland. The aim of this study was estimation the fattening value of these synthetic line kept in private farm situated in central Poland.

MATERIAL AND METHODS

The observations were carried out in private farm situated in Łódź province. Based on breeding records, the fattening value of 277 White-head Synthetic Line lambs born and reared during 6 successive years was evaluated. The mothers were between 2nd and 5th year of age and nursed single or twin litters. The number of animals in each investigated group shows Table 1.

The ewes were fed according to standards with farm produced fodder like: grass hay, corn grain, rapeseed meal, red carrot. Throughout the rearing the lambs were with mothers and milk was their basic food. From 2nd week of age, they were given additional concentrate (oats, barley, soybean meal) and grass hay ad libitum.

TABLE 1. Number of animals in each investigated group

Year	n
2000	17
2001	23
2002	43
2003	55
2004	61
2005	78
Lambing number	
1	60
2	65
3	46
4	55
5	29
6	22
Sex	
Ram lambs	137
Ewe lambs	140
Birth type	
Singles	164
Twins	113

The influence of sex, birth type, and ewe's lambing number on birth weight, body weight on 28th, 56th and 70th day of life was evaluated.

The obtained values have been processed statistically using SPSS 14.0 (2003).

RESULT AND DISCUSSION

The birth body weight difference between ram and ewe lambs amounted mean value of 400 g and was statistically significant. In each observed periods ram lambs obtained significantly higher (over 1 kg) body weight in comparison with ewe lambs (Tab. 2).

The mean body weigh of ewe lambs at 70th day of life was 25.3 kg while ram lambs achieved 26.7 kg. The highest daily gains up to 28th day of both sexes (overpass 340 g) were in accordance with expectations. The daily gains of rams compared to ewes were higher in all investigated periods which was confirmed statistically (Tab. 3). Probably it was caused by greater food intake of rams when the forage was a base of feeding. Usually the daily gains as well as finishing body weight of ram lambs are higher then obtained in the same age by ewes, which was also confirmed the other authors (Borys and Osikowski, 2001; Bromley et al., 2001).

The type of birth did not influence significantly body weight and daily gains of lambs up to 28th day of life, which suggests good milk performance of theirs mothers.

TABLE 2. Influence of sex and type birth on body weight of lambs (kg)

Traits		Birth body weight	Body weight on 28 day	Body weight on 56 day	Body weight on 70 day
Ram lambs	LSM	7.3	17.3	23.0	26.7
	SE	0.1	0.3	0.2	0.2
Ewe lambs	LSM	6.9	16.2	21.7	25.3
	SE	0.1	0.3	0.2	0.2
Significance		**	**	**	**
Single	LSM	7.2	16.7	21.9	25.5
	SE	0.1	0.2	0.2	0.2
Twins	LSM	7.0	16.9	22.9	26.6
	SE	0.1	0.4	0.3	0.3
Significance		ns	ns	**	**

** $P \leq 0,01$; * $P \leq 0,05$; ns – not significant effect.

TABLE 3. Influence of sex and type birth on daily gains of lambs (kg)

Trails		Daily gains 1–28 days	Daily gains 1–56 days	Daily gains 1–70 days	Daily gains 28–56 days	Daily gains 28–70 days	Daily gains 56–70 days
Ram lambs	LSM	0.371	0.286	0.281	0.203	0.223	0.263
	SE	0.012	0.004	0.004	0.007	0.005	0.006
Ewe lambs	LSM	0.344	0.269	0.266	0.197	0.216	0.255
	SE	0.012	0.004	0.004	0.007	0.005	0.006
Significance		*	**	**	ns	ns	ns
Singles	LSM	0.355	0.269	0.266	0.186	0.209	0.254
	SE	0.110	0.003	0.003	0.006	0.004	0.005
Twins	LSM	0.360	0.286	0.281	0.214	0.231	0.264
	SE	0.170	0.005	0.006	0.010	0.007	0.009
Significance		ns	**	*	*	**	ns

**P ≤ 0,01; *P ≤ 0,05; ns – not significant effect.

When lambs were older the forage intake increased and the compensatory growth of twins took place. The differences in growth rate between singles and twins have been confirmed statistically in periods between 1st–56th and 1st–70th day as well as 28th–56th and 28th–70th day. The twin’s body weight at 56th and 70th day of life was higher then singles (Tabs 2, 3). The other authors affirmed higher growth rate of singles (Stanisz et al., 1999; Yilmaz et al., 2007). The difference of discussed results in presented study may be caused

probably by similar birth body weight of singles and twins (Tab. 2). It was also confirmed by not statistically significant correlation between birth type and body weight and daily gains (Tab. 5).

The influence of ewe’s lambing number on birth and rearing body weight of their lambs was examined. Results showed, that progeny of the mothers after third parturition were significantly heavier at 28th (p ≤ 0.01), 56th (p ≤ 0.05) and 70th (p ≤ 0.05) day of life than ewe’s which were lambed first time (Tab. 4). The dif-

TABLE 4. Influence of ewe’s lambing number on body weight of lambs (kg)

Ewe’s lambing number		Birth body weight	Body weight on 28 day	Body weight on 56 day	Body weight on 70 day
1	LSM	7.1	15.7 C	21.6 c	25.0 c
	SE	0.2	0.5	0.4	0.5
2	LSM	7.0	16.8	22.2	25.7
	SE	0.1	0.3	0.2	0.3
3	LSM	6.7	18.1 A	22.8 a	26.7 a
	SE	0.2	0.4	0.3	0.3
4	LSM	7.2	16.8	22.4	26.0
	SE	0.1	0.3	0.2	0.3
5	LSM	7.3	16.8	22.8	26.5
	SE	0.2	0.5	0.3	0.4
6	LSM	7.4	16.6	22.7	26.3
	SE	0.2	0.6	0.4	0.5

A, B, C – P ≤ 0,01; a, b, c – P ≤ 0,05; ns – not significant effect.

TABLE 5. Interaction sex and birth type x body weight of lambs as well as sex and birth type x daily gains of lambs (kg)

Trials	sex	Birth type	LSM	Se	Daily gains	sex	Birth type	LSM	Se
Birth body weight	rams	single	7.1	0.114	1–28 days	rams	single	0,373	0.014
		twins	7.6	0.164			twins	0,370	0.020
	ewe	single	7.1	0.109		ewe	single	0,337	0.013
		twins	6.8	0.168			twins	0,351	0.020
Body weight on 28 day of life	rams	single	17.2	0.294	1–56 days	rams	single	0,277	0.004
		twins	17.5	0.424			twins	0,295	0.006
	ewe	single	16.2	0.283		ewe	single	0,261	0.004
		twins	16.3	0.435			twins	0,277	0.006
Body weight on 56 day of life	rams	single	22.3	0.204	1–70 days	rams	single	0,273	0.004
		twins	23.8	0.295			twins	0,290	0.006
	ewe	single	21.5	0.197		ewe	single	0,259	0.004
		twins	22.1	0.302			twins	0,273	0.006
Body weight on 70 day of life	rams	single	25.9	0.257	28–70 days	rams	single	0,208	0.005
		twins	27.5	0.371			twins	0,238	0.008
	ewe	single	25.0	0.248		ewe	single	0,209	0.005
		twins	25.7	0.380			twins	0,223	0.008

**P ≤ 0,01; *P ≤ 0,05.

TABLE 6. Ewe's lambing number influence on daily gains of lambs (kg)

Lambing number		Daily gains 1–28 days	Daily gains 1–56 days	Daily gains 1–70 days	Daily gains 28–56 days	Daily gains 28–70 days	Daily gains 56–70 days
1	LSM	0.318 C	0.263 C	0.259 C	0.209	0.221	0.245
	Se	0.250	0.008	0.008	0.015	0.010	0.013
2	LSM	0.363	0.275	0.271	0.191	0.212	0.253
	Se	0.160	0.005	0.005	0.010	0.006	0.008
3	LSM	0.422 A	0.293 A	0.290 A	0.168 e	0.205	0.280
	Se	0.190	0.006	0.006	0.011	0.007	0.010
4	LSM	0.354	0.275	0.271	0.199	0.218	0.256
	Se	0.160	0.005	0.005	0.009	0.006	0.008
5	LSM	0.349	0.281	0.277	0.216 c	0.231	0.262
	Se	0.220	0.007	0.007	0.013	0.009	0.001
6	LSM	0.340	0.277	0.273	0.217	0.230	0.256
	Se	0.260	0.008	0.008	0.016	0.010	0.013

A, B, C, D – P ≤ 0,01; a, b, c, d – P ≤ 0,05; ns – not significant effect.

ferences were confirmed statistically in daily gains between 1–28th, 1–56th and 1–70th day of life (Tab. 6). The growth rate of lambs depends on many factors. One of them may be the ewe maturity.

The first time lambing ewes have not got a fully mature udder. They are usually nursing lower litters than older ewes and it can be reason of lower milk yield. The milk secretion of ewes nursing multiple

litters may rise from 20 to 40% (Bromley et al., 2001). The findings of Gruszecki et al., 2002 and Ayestaran et al., 2004 confirmed the effect of lambing number on milk amount, showing increased yield from the first to the third lactations. Up to 28th day of lamb's life the milk is their basic food so the daily gains in that fattening period mostly depends of the milk productivity of their mothers, that's why the biggest differences in body weight of lambs was noted at that period. In older lambs the differences of body weight were lower however statistically confirmed.

The differences between body weight and daily gains of lambs in analyzed years reached 20%. The effect of year of investigation is known to be a significant source of variation, which has been attributed to factors such as fodder availability, changes in feeding method or differences in weather conditions during crucial production stages (Gruszecki et al., 2002; Niedziółka and Pieniak-Lędzion, 2005; Kuźnicka and Rant, 2008).

CONCLUSIONS

The ram lambs obtained higher daily gains in comparison with ewe lambs. The lack of differences on daily gains between singles and twins in 28th day of life suggested good milk performance of their mothers. The highly statistically lightest birth weight was delivered by first time lambing ewe. Obtained results confirmed good fattening performance of ram lambs as well as ewe lambs of White-head Synthetic Line.

REFERENCES

- AYESTARAN O., ALFONSO L., PEREZ DE ALBENIZ J.I., 2004: Milk production by Assaf sheep. *Albeitar*, 78, 26–28.
- BENSON M.E., HENRY M.J., CARDELLINO R.A., 1999: Comparison of weigh–suckle–weigh and machine milking for measuring ewe milk production. *Journal of Animal Science*, 77, 9, 2330–2335.
- BORYS B., OSIKOWSKI M., 2001: Wpływ metody żywienia jagniąt mieszańców merynosa z rasami plennymi i mięsna na wartość tuczną i rzezną przy tuczu do wyższych standardów wagowych (30–40 kg). *Roczniki Naukowe Zootechniki*, 28, 1, 119–135.
- BROMLEY C.M., VAN VLECK L.D., SNOWDER G.D., 2001: Genetic correlations for litter weight weaned with growth, prolificacy, and wool traits in Columbia, Polypy, Rambouillet, and Targhee sheep. *Journal of Animal Science*, 79, 2, 339–345.
- GRUSZECKI T., SZYMANOWSKA A., LIPECKA C., MARKIEWICZ J., 2002: Produktynność owiec utrzymywanych w warunkach chowu ekstensywnego. II. Obserwacje nad mlecznością maciorek. *Annales Universitatis Mariae Curie-Skłodowska, Lublin – Polonia*, XX, 27, 193–197.
- GUT A., KOZAL E., ŚLUSARZ P., 1995: Znaczenie syntetycznych linii w programach hodowli owiec w kraju, *Wydawnictwo Akademii Rolniczej, Poznań*, 9–17.
- KUŹNICKA E., RANT W., 2008: Comparison of ewe milk production and lamb growth in Żelaźnieńska Sheep kept in a barn or under overhead shelter. *Ann. Anim. Sci*, Vol. 8, No 2 (2008), 175–183.
- NIEDZIÓŁKA R., PIENIAK-LENDZION K., 2005: Charakterystyka produktywności owiec rasy berrichone du cher w latach 1999–2002. *LXX Zjazd PTZ, Wrocław*, 125.
- RZEPECKI R., 1996: Intensywna produkcja jagniąt, *Fundacja Programów Pomocy dla Rolnictwa, Instytut Zootechniki*, 185.
- STANISZ M., GUT A., WAŻNA E., 1999: Wartość opasowa i rzezną jagniąt syntetycznej linii w typie owcy Berrichon du cher. *Prace Komisji Nauk Rolniczych i Komisji Nauk Leśnych*, 87, 179–185.

YILMAZ O., DENK H., BAYRAM D., 2007: Effects of lambing season, sex and birth type on growth performance in Norduz lambs. *Small Ruminant Research*, 68, 336–339.

Streszczenie: *Ocena przydatności do tuczu jagniąt białogłowej linii mięsnej utrzymywanej w gospodarstwie Ciosny w województwie łódzkim.* Na podstawie dokumentacji hodowlanej dokonano oceny użytkowości mięsnej 277 jagniąt urodzonych i odchowywanych w prywatnym gospodarstwie, w sześciu kolejnych latach. Przy użyciu pakietu statystycznego SPSS wersja 12.0 zostały oszacowane następujące parametry: masa ciała przy urodzeniu oraz w 28, 56 i 70 dniu życia jagniąt, wpływ płci, typu urodzenia, numeru wykotu matki i roku wykotu na tempo wzrostu jagniąt. Stwierdzono, że jagnięta tryczki uzyskały

istotnie wyższe przyrostyienne oraz masę ciała w porównaniu z jagniętami maciorkami. Brak różnic w przyrostach dziennych między jedynakami i bliźniętami w okresie od urodzenia do 28 dnia wskazuje na dobrą mleczność matek. Wyso-ko istotnie niższą masę ciała jagniąt zanotowano u pierwiastek. Uzyskane wyniki potwierdzają przydatność do tuczu jagniąt białogłowej linii syntetycznej zarówno tryczków, jak i maciorek.

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A comparison of polymorphism of DQA genes in European bison belonging to two genetic lines: Lowland and Lowland-Caucasian

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Abstract: A comparison of polymorphism of DQA genes in European bison belonging to two genetic lines: Lowland and Lowland-Caucasian. The currently living population of European bison (*Bison bonasus*) is divided into two genetic lines: Lowland line (LB) and Lowland-Caucasian line (LC). In order to compare the genetic diversity in two above mentioned lines the polymorphism of the major histocompatibility complex (MHC) DQA1 and DQA2 genes was analyzed. MHC genes are highly polymorphic and can therefore be used to assess genetic diversity in different species including endangered ones. The genetic polymorphism of exon 2 DQA1 and DQA2 genes was examined in 200 individuals of Lowland line and 56 individuals of Lowland-Caucasian line. The SSCP analysis revealed two alleles and two genotypes of exon 2 DQA1 gene and three alleles and four genotypes of exon 2 DQA2 gene. Frequency of genotypes of exon 2 DQA2 gene was significantly different (chi-square test $p \leq 0.05$) between two genetic lines of European bison. Four haplotype configurations of DQA genes – DQA1, DQA2, DQA1/DQA2 and DQA1/DQA2_2 were observed. DQA1 haplotype configuration was observed only in individuals belonging to LC and DQA1/DQA2_2 only in European bison from LB. Differences of frequency of haplotypes in two genetic lines were highly significant (chi-square test $p \leq 0.01$).

Key words: European bison, *Bison bonasus*, polymorphism, SSCP, DQA, MHC.

INTRODUCTION

Historically, the European bison was distributed through western, central and south-eastern Europe. In the beginning of twentieth century, free-ranging populations became extinct. Only 54 (29 males and 25 females) individuals with documented pedigrees survived in zoological gardens and reserves. The currently living population of European bison descended from only 12 founders (Olech, 2009) and is divided into two genetic lines: a Lowland (Białowieża) line and a Lowland-Caucasian line. The Lowland line i.e. pure lowland subspecies (*Bison bonasus bonasus*), originates from only 7 founders (4 males and 3 females). The gene pool of the Lowland-Caucasian line contains genes from all 12 founders including male “Kaukasus” originating from subspecies *caucasicus* (*Bison bonasus caucasicus*) (Slatis, 1960; Olech, 1987; 2003).

The species went through an extreme bottleneck and consequently, currently living populations of European bison are highly inbred. Previous studies using various markers: allozyme (Hartl and Pucek, 1994), blood groups (Sipko et al.,

1995), mitochondrial DNA (Tiedemann et al., 1998; Burzyńska et al., 1999) microsatellites (Gralak et al., 2004; Nowak and Olech, 2008), and highly polymorphic MHC genes (Udina et al., 1994a; 1994b; Udina and Shaikhaiev, 1998; Lopińska et al., 2003; Radwan et al., 2007) confirmed low genetic diversity in both genetic lines of European bison.

Studies of Major Histocompatibility Complex (MHC) genes in European bison are conducted based on existing knowledge about equivalent region in cattle. The bovine Major Histocompatibility Complex (MHC) called Bovine Lymphocyte Antigens (BoLA) is located on the short arm of chromosome 23 (BTA23) and contains three classes I, II and III (Andersson and Davies, 1994). Class III products have a wide range of activities from cell cytotoxicity (tumor necrosis factor TNF- α and TNF- β) to enzymatic activity (21 β -hydroxylase) (Andersson and Davies, 1994). The product of class III genes is also Heat Shock Protein (HSP70) (McShane et al., 2001). The genes of class I and class II encode cell-surface glycoproteins whose function is to recognize, bind in their antigen binding groove, and present antigenic peptides to T lymphocytes. The antigen binding groove is encoded by the second exon of the gene which demonstrates high polymorphism and this determines the capability of MHC products to bind to a wide range of peptides (Sharif et al., 1998). MHC genes are extremely polymorphic in cattle and other vertebrates.

The BoLA class II is divided by gene inversion event into two regions designated IIa and IIb. The IIa region contains genes DOA, DMA, DMB, DYA, DYB.

In IIb region are situated DRA, DRB, DQA and DQB genes which encode MHC class II molecules (Ballingall et al., 2004; Takeshima and Aida, 2006). A feature unique in cattle and sheep is certain variability in the number of DQ loci in their haplotypes. Each cattle class II haplotype expresses a single DR product and one or more DQ products due to duplication of the DQ genes and this duplication occurs in about half haplotypes (Ellis and Ballingall, 1999; Lewin et al., 1999; Russell, 2000). Cattle haplotypes carry either a single DQA1 locus, or single DQA1 locus together with a single DQA2 locus or two DQA2 loci (Gelhaus et al., 1999). All DQA loci in cattle and sheep are polymorphic with approximately 50 different alleles identified in both species (<http://www.projects.roslin.ac.uk/bola>, www.ncbi.nlm.nih.gov). Highly polymorphic genes of MHC are used as markers for cattle and other many species (Lewin et al., 1999) and also can be used to assess diversity in endangered species (Haig, 1998).

The aim of this study was to perform the first examination of variability in DQA loci in European bison and compare genetic diversity in exon 2 of DQA1 and DQA2 gene in two genetic lines: Lowland line and Lowland-Caucasian line.

MATERIALS AND METHODS

Samples (whole blood, liver and kidney tissue and hair roots) were collected from 200 European bison belonging to Lowland line (LB) and 56 from Lowland-Caucasian line (LC) came from free-ranging and captive herds from Poland and other European countries.

DNA isolation

The total DNA was extracted from whole blood using the standard phenol-chloroform extraction protocol (Sambrook et al., 1989), from soft tissues by treatment with proteinase K and precipitation by sodium chloride (<http://www.genomics.liv.ac.uk>), and from hair roots using the Chelex 100 (Sigma) method (Walsh et al., 1991).

Amplification

Exon 2 DQA1 gene

Exon 2 of DQA1 gene was amplified by PCR method with primers described by Snibson et al. (1998) primer Forward, and Zhou and Hickford (2004) primer Revers.

Amplification was carried out in 15 µl total volumes. The reaction mixture consisted of optimized concentrations of: 1xREDtaqPCRBuffer (Sigma), MgCl₂ (Polgen), dNTPs (Polgen), 8 pmol of each primers, 0.5 unit REDtaq DNA polymerase (Sigma), 100 ng DNA template and sterile water. The amplification profile consisted of 2 min at 94°C initial denaturation, followed by 30 cycles of 30 s at 94°C, 40 s at 58°C and 45 s at 72°C, with a final extension of 5 min at 72°C.

Exon 2 DQA2 gene

Exon 2 of DQA2 gene was amplified with polymerase chain reaction (PCR) using primers described by Scott et al. (1991). Amplification was carried out in 15 µl total volumes. The reaction mixture consisted of optimized concentrations of: 1xREDtaqPCRBuffer (Sigma), MgCl₂ (Polgen), dNTPs (Polgen), 8 pmol of each

primers, 0.5 unit REDtaq DNA polymerase (Sigma), 100 ng DNA template and sterile water. The amplification profile consisted of 2 min at 94°C initial denaturation, followed by 30 cycles of 30 s at 94°C, 40 s at 56°C and 45 s at 72°C, with a final extension of 5 min. at 72°C.

The PCR product was visualized in ethidium bromide stained with 1.5% agarose gels.

PCR-SSCP analysis

The amplified product of each sample was analyzed by Single Strand Conformation Polimorphism (SSCP) technique. PCR product was mixed with loading dye (Bromophenol blue, Xylene, Formamid, EDTA), denaturated at 90°C for 5 min and cooled on ice. Then product was resolved on 12% nondenaturing polyacrylamide gel. Electrophoresis was carried out on DCode™ Universal Mutation Detection System (BioRad) at constant conditions: 240 min. at 35 W, 10°C in 1XTBE buffer. Gels were stained with silver nitrate (AgNO₃) (Sambrook et al., 1989).

Cloning and sequencing

PCR products corresponding to different SSCP patterns were cloned and sequenced. Chosen PCR product was purified with QIAquick Gel Extraction (Qiagen) and cloned using pGEM™-TEasy vector (Promega). Purified PCR product was cloned into vector pGEM™-TEasy (Promega). Component cells of Escherichia coli JM109 (Promega) were transformed with recombinant plasmid and selected by ampicilin-blue/white screening. After growing in Luria-Bertani liquid

medium the positive clones (white) were prepared with Wizard®Plus Minipreps DNA Purification System (Promega) and sequencing in a reaction total volume 27 µl using Thermo Sequenase Cy5 Terminator Cycle Sequencing Kit (Amersham Bioscience). Nucleotide sequences were detected in automated sequenced ALFexpress II (Amersham Pharmacia Biotech).

Statistical and sequence analysis

The Popgen32 program (<http://cc.oulu.fi/~jaspi/popgen/popdown.htm>) was used to estimate the statistical parameters such as frequencies of genotypes and haplotypes.

Potential differences between two genetic lines of European bison were evaluated using chi-square test. The BLAST algorithm was used for the comparison of sequences obtained with homologous sequences from NCBI GenBank (<http://www.ncbi.nlm.nih.gov/BLAST>). A phylogenetic tree showing relationships was constructed from the matrix of sequences similarities calculated with the UPGMA method using MEGA program (Kumar et al., 2004).

RESULTS AND DISCUSSION

In PCR reaction used were primers described previously for amplification of exon 2 DQA1 gene (Snibson et al., 1998; Zhou and Hickford, 2004) and exon 2 DQA2 gene (Scott et al., 1991) in sheep. The European bison belongs to the family Bovidae so primers used for amplification of exon 2 of DQA1 and DQA2 genes in sheep, could be applied for amplification equivalent region in

European bison. The previous comparison analysis of the nucleotide sequence of exon 2 ovine DQA1 and DQA2 genes with equivalent fragments of cattle showed that the ovine sequences are more similar to their bovine counterparts than to each other (Snibson et al., 1998).

The second exon of DQA1 gene was successfully amplified by PCR reaction in 85 from 200 examined European bison belonging to Lowland line (LB) and in 39 from 56 in Lowland-Caucasian line (LC). The amplified fragment was 253 bp long. These primers did not amplified the analyzed fragment in 115 from LB and in 17 from LC.

The second exon of DQA2 gene was successfully amplified by PCR reaction in all animals from LB. In 12 animals belonging to LB amplified fragment of 282 bp was obtained and in 186 animals from this line product was 253 bp long.

Among European bison from LC PCR product 253 bp long was obtained in 43 from 56 examined, in the other 13 animals exon 2 DQA2 gene did not amplified.

On the basis of generated results, four different haplotypes were marked No1 (DQA1), No2 (DQA2), No3 (DQA1/DQA2) and No4 (DQA1/DQA2_2) in examined individuals. No4 haplotype occurred in animals with longer (282 bp) amplified fragment of exon 2 DQA2 gene. No1 haplotype was observed only in individuals from LC, and No4 haplotype was occurred only in LB line. Frequency of different haplotypes in two investigated genetic lines of European bison describes Table 1. Differences of frequency of haplotypes in two genetic lines were highly significant (chi-square test $p \leq 0.01$).

TABLE 1. Frequencies of observed haplotypes in European bison

Haplotype	LB	LC
No1 (DQA1)	0	0.232
No2 (DQA2)	0.425	0.429
No3 (DQA1/DQA2)	0.515	0.339
No4 (DQA1/DQA2_2)	0.060	0

Comparison of these results with previously described by other authors (Ballingall et al., 1997; Snibson et al., 1998; Hickford et al., 2000; 2004; Zhou and Hickford, 2004; Traul et al., 2005) showed that MHC class IIa haplotypes of European bison are akin to those identified in domestic cattle, sheep and American bison. Those haplotypes carry nonduplicated and duplicated DQA genes, animals have haplotypes with single DQA1 and duplicated DQAnull/DQA2 or DQA1/DQA2. In addition some haplotypes with duplicated DQA gene in cattle and American bison have also gene named DQA3 in configuration DQA2/DQA3 (Ballingal et al., 1997; Traul et al., 2005). The most common haplotype in European bison from LB is No2 (DQA2) which was identified in 57% examined animals. In LC, individuals with haplotype No3 (DQA1/DQA2) were in the majority (42.8%). In LB were no animals with single DQA1 haplotype.

Previous investigation revealed that most of sheep (82–89%) contain DQA1/DQA2 haplotype which is extremely polymorphic with 8 DQA1 alleles and 16 DQA2 alleles (Escayg et al., 1996). In American bison examined by Traul et al. (2005) 25% individuals had a haplotype with duplicated DQA gene.

Those studies showed also the presence of two unique SSCP patterns of

exon 2 DQA1 gene in both genetic lines of European bison. On the basis of obtained SSCP patterns, two alleles and two genotypes marked *a* and *b* were identified. Both of them were homozygous. The frequency of genotype *a* was 0.494 in LB and 0.513 in LC. Frequency of genotype *b* was respectively 0.506 and 0.487.

SSCP analysis detected three unique patterns of exon 2 DQA2 gene. On the basis of SSCP patterns, three alleles and four genotypes were identified. Identified genotypes were marked *c*, *d*, *e*, *f*. Three of them were homozygous (*c*, *d*, *e*), and one was heterozygous (*f*). Genotype *e* was observed in animals with longer PCR product (282 bp). Frequency of observed genotypes was respectively 0.540, 0.215, 0.060 and 0.185 in LB and 0.442, 0.186, 0.00, and 0.72 in LC. Frequency of genotypes of exon 2 DQA1 gene did not differ between two genetic lines of European bison (chi-square test $p = 0.981$). Whereas the frequency of genotypes of exon 2 DQA2 gene was significantly different (chi-square test $p \leq 0.05$).

More SSCP patterns of exon 2 DQA2 gene suggested highest polymorphism then in the exon 2 DQA1 gene in European bison. The analogous results described Snibson et al. (1998) in sheep. They stated that the second exon of the OLA-DQA2 gene is more polymorphic than the equivalent region of the OLA-DQA1.

Those studies revealed polymorphism of the second exon of the DQA1 and DQA2 gene in European bison. However polymorphism in this region is not high while compared to cattle and sheep. Lower number of DQA1 and DQA2 alleles in

European bison is probably resulting of extreme population bottleneck. Previous study of MHC genes including DQA genes in many wild bottlenecked species and populations showed low number of alleles in this region.

The nucleotide sequences of exon 2 DQA1 and DQA2 gene of European bison received in this study were submitted to the NCBI Gene Bank, and assigned the accession numbers EU153369.1 to EU153374.1. A comparison between obtained DQA sequences of European bison shows Figure 1. A UPGMA phylogenetic tree in Figure 2 was constructed from obtained nucleotide sequences of exon 2 DQA1, and DQA2 gene of European bison, and bovine and ovine nucleotide sequences published in the NCBI GenBank (<http://www.ncbi.nlm.nih.gov>). The tree revealed three clusters grouping sequences analyzed on two main branches. The cluster I contains sequences of exon 2 DQA1 and DQA2 gene of European bison, cattle and sheep. The similarity between two sequences of DQA2 and one of DQA1 gene from

European bison genome grouped in cluster I with equivalent ovine and bovine sequences was 97 to 100% (average 98%). One sequence of exon 2 DQA1 gene of European bison (EU153370.1) was clustered together with sequences of exon 2 DQA2 gene and average nucleotide homology between them was 98%. Comparison of two sequences from this cluster EU153369.1 and L139A2 showed 100% nucleotide homology although there were two different PCR reaction products: EU153369.1 – exon 2 DQA1 gene, L139A2 – exon 2 DQA2 gene. In cluster II were grouped sequences of exon 2 DQA2 and one sequence of exon 2 DQA1 gene with ovine sequences of exon 2 DQA2 gene. The average similarity between sequences within the cluster II was 98% (97–98%). The analyzed sequences of European bison from cluster I and cluster II showed 87 to 90% (average 88.83%) of nucleotide homology in the exon 2 DQA1 and DQA2 gene. The sequence number EU153371.1 was clustered on another tree branch into group III together with ovine exon 2

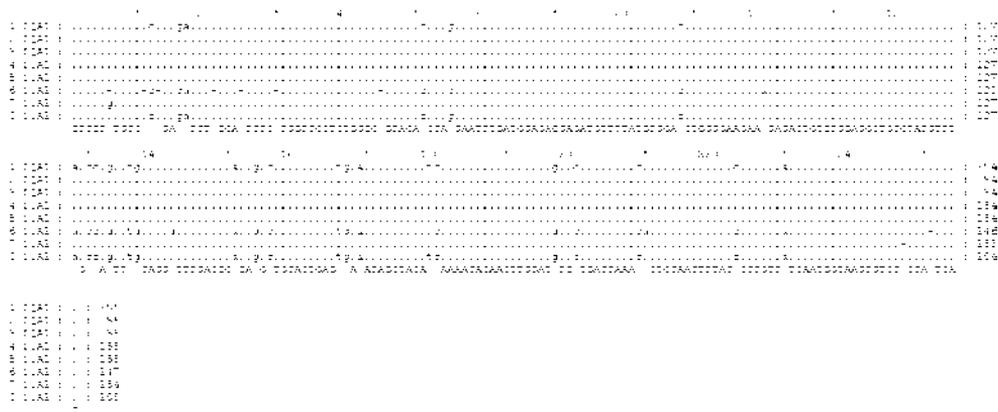


FIGURE 1. A comparison between nucleotide sequences of exon 2 DQA1 and DQA2 in European bison

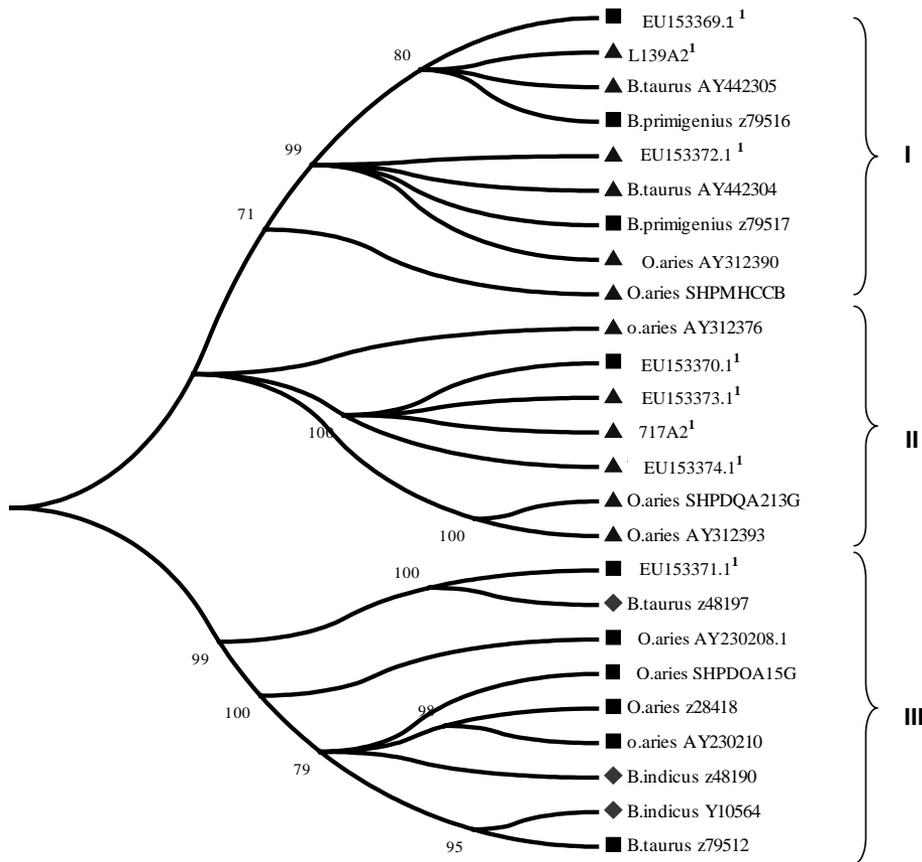


FIGURE 2. UPGMA tree constructed using DQA nucleotide sequences obtained and labeled with the symbols as follows; ■ – exon 2 of DQA1, ▲ – exon 2 of DQA2, ◆ – exon 2 DQA, ¹ – nucleotide sequences obtained. Tree is constructed using the MEGA program (Kumar et al., 2004). Bootstrap statistical analysis (Felsenstein, 1985) of tree topology is shown above branches. Branch lengths are proportional to genetic distance

DQA1, and cattle sequences described just like DQA.

Previous studies on the relationship between ovine and bovine DQA nucleotide sequences revealed two main branches of phylogenetic neighbor-joining tree clustered sheep DQA1 with cattle DQA1, whereas sheep DQA2 sequences clustered with cattle DQA2, DQA3 and DQA4 sequences (Hickford et al., 2004). In this study European bison DQA1

sequences were grouped with sequences of DQA1 and DQA2 bovine and sheep as well as DQA2 sequences. Moreover, bovine DQA1 and DQA2 sequences from NCBI Gen Bank, which were used for a comparison were grouped together in cluster I. This may suggest that the nature of DQA gene is not yet well understood and requires further research. It also indicates the pertinence of isolating further DQA loci.

REFERENCES

- ANDERSSON L., DAVIES C.J., 1994: The major histocompatibility complex. In: B.M.L. Goddeeris, W.I. Morrison, eds.: Cell-Mediated Immunity in Ruminants, 37–57, CRC Press, Boca Raton 1994.
- BALLINGALL K.T., LUYAI A., McKEEVER D.J., 1997: Analysis of genetic diversity at the DQA loci in African cattle: evidence for a BoLA-DQA3 locus. *Immunogenetics*, 46: 237–244.
- BALLINGALL K.T., ELLIS S.A., MACHUGH N.D., ARCHIBALD S.D., McKEEVER D.J., 2004: The DY genes of the cattle MHC: expression and comparative analysis of an unusual class II MHC gene pair. *Immunogenetics*, 55: 748–755.
- BURZYŃSKA B., OLECH W., TOPCZEWSKI J., 1999: Phylogeny and genetic variation of the European bison *Bison bonasus* based on mitochondrial DNA D-loop sequences. *Acta Theriologica*, 44 (3): 253–262.
- ELLIS S.A., BALLINGALL K.T., 1999: Cattle MHC: evolution in action? *Immunological Reviews*, 167: 159–168.
- ESCAYG A.P., HICKFORD J.G.H., MONTGOMERY G.W., DODDS K.G., BULLOCK D.W., 1996: Polymorphism at the ovine major histocompatibility complex class II loci. *Animal Genetics*, 27: 305–312.
- FELENSTEIN J., 1985: Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- GELHAUS A., FORESTER B., WIPPERN CH., HORSTMAN R.D., 1999: Evidence for an additional cattle DQA locus, BoLA-DQA5. *Immunogenetics*, 49: 321–327.
- GRALAK B., KRASIŃSKA M., NIEMCZEWSKI C., KRASIŃSKI Z.A., ŻURKOWSKI M., 2004: Polymorphism of bovine microsatellite DNA sequences in the lowland European bison. *Acta Theriologica*, 49 (4): 449–456.
- HAIG S.M., (1998): Molecular contribution to conservation. *Ecology*, 79: 413–425.
- HARTL G.B., PUCEK Z., 1994: Genetic Depletion in the European Bison (*Bison bonasus*) and the Significance of Electrophoretic Heterozygosity for Conservation. *Conservation Biology*, 8 (1): 167–174.
- HICKFORD J.G.H., RIDGWAY H.J., ESCAYG A.P., 2000: Evolution of the ovine MHC DQA region. *Animal Genetics*, 31: 200–205
- HICKFORD J.G.H., ZHOU H., SLOW S., FANG Q., 2004: Diversity of the ovine DQA2 gene. *Journal of Animal Science* 82: 1553–1563.
- KUMAR S., TAMURA K., NEI M., 2004: *MEGA 3*: Integrated software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. *Briefings in Bioinformatics* 5: 150–163.
- LEWIN H.A., RUSSEL G.C., GLASS E.J., 1999: Comparative organization and function of the major histocompatibility complex of domesticated cattle. *Immunological Reviews*, 167: 145–158.
- ŁOPIEŃSKA M., NOWAK Z., CHARON K.M., OLECH W., 2003: Estimation of polymorphism in chosen region of MHC in two genetic lines of European bison (*Bison bonasus* L.). *Applied Science Reports, Cattle Production and Breeding*, Polish Society of Animal Production, 68 (1): 17–24.
- McSHANE R.D., GALLAGER Jr. D.S., NEWKIRK H., TAYLOR J.F., BURZLAFF J.D., DAVIS S.K., SKOW L.C., 2001: Physical localization and order of genes in the class I region of the bovine MHC. *Animal Genetics*, 32: 235–239.
- NOWAK Z., OLECH W., 2008: Zmienność mikrosatelitarna w obrębie chromosomów płci u żubrów. *European Bison Conservation Newsletter* Vol. 1, 72–78.
- OLECH W., 1987: Analysis of inbreeding in European bison. *Acta Theriologica* 32: 373–387.
- OLECH W., 2003: The influence of individual and maternal inbreeding on calf survival in the European bison (Wpływ inbrodu osobniczego i inbrodu matki na przeżywalność cieląt żubra (*Bison bonasus*)). *Treatises and monographs*, Warsaw University of Life Sciences, pp. 78.
- OLECH W., 2009: The changes of founders' number and their contribution to the European bison population during 80 years of species' restitution *European Bison Conservation Newsletter* Vol. 2, 54–60.
- RADWAN J., KAWAŁKO A., WÓJCIK M., BABIK W., 2007: MHC-DRB3 variation in a free-

- living population of the European bison, *Bison bonasus*. *Molecular Ecology* 16: 531–540.
- RUSSEL G.C., 2000: Sequence duplication at the 3' end of BoLA-DQB genes suggests multiple allelic lineages. *Immunogenetics*, 52: 101–106.
- SAMBROOK J., FRITSCH E.F., MANIATIS T., 1989: *Molecular cloning: a laboratory manual*. Second edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.
- SCOTT P.C., GOGOLIN-EWENS K.J., ADAMS T.E., BRANDON M.R., 1991: Nucleotide sequence, polymorphism, and evolution of ovine MHC class II DQA genes. *Immunogenetics*, 34 (2): 67–79.
- SHARIF S., MALLARD B.A., WILKIE B.N, SARGEANT J.M., SCOTT H.M., DEKKERS J.C.M., LESLIE K.E., 1998: Associations of the bovine major histocompatibility complex DRB3 (BoLA-DRB3) alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle. *Animal Genetics*, 29: 185–193.
- SIPKO T.P., RAUTIAN G.S., UDINA I.G., UKAHANOV S.V., BERENDIAEVA Z.I., 1995: Изучение полиморфизма групп крови у зубров (*Bison bonasus*). *Genetica* 31 (1): 93–100.
- SLATIS H.M., 1960: An analysis of inbreeding in the European bison. *Genetics*, 45: 275–287.
- SNIBSON K.J., MADDOX J.F., FABB S.A., BRANDON M.R., 1998: Allelic variation of ovine MHC class II DQA1 and DQA 2 genes. *Animal Genetics*, 29: 356–362.
- TAKESHIMA S-N., AIDA Y., 2006: Structure, function and disease susceptibility of the bovine major histocompatibility complex. *Animal Science Journal*, 77: 138–150.
- TIEDEMANN R., NADLINGER K., PUCEK Z., 1998: Mitochondrial DNA-RFLP analysis reveals low levels of genetic variation in European bison (*Bison bonasus*). *Acta Theriologica*, Suppl. 5: 83–87.
- TRAUL D.L., BHUSHAN B., ELDRIDGE J.A., CRAWFORD T.B., Li H., DAVIES CHJ., 2005: Characterization of *Bison bison* major histocompatibility complex class IIa haplotypes. *Immunogenetics*, 57: 845–854.
- UDINA I.G., SOKOLOVA S.S., SIPKO T.P., SULIMOVA G.E., 1994a: Сравнительная характеристика полиморфизма ДНК локусов DQB и DRB главного комплекса гистосовместимости у представителей семейства BOVIDAE. *Genetica*, 30 (3): 356–360.
- UDINA I.G., SIPKO T.P., RAUTIAN G.S., BADAGUEVA Y.N., SULIMOVA G.E., 1994b: The study of DNA-polymorphism of European bison by PCR-analysis of kappa-casein gene and loci DQB and DRB of the major histocompatibility complex. 5th WCGALP/FAO Symposium: 145–150.
- UDINA I.G., SHAIKHAEV O., 1998: Restriction fragment length polymorphism (RFLP) of exon 2 of Mhc-DRB3 gene in European bison *Bison bonasus*. *Acta Theriologica*, Suppl. 5: 75–82.
- WALSH P.S., METZGER D.A., HIGUCHI R., 1991: Chelex 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material. *BioTechniques*, 10 (4): 506–513.
- ZHOU H., HICKFORD J.G.H., 2004: Allelic polymorphism in the ovine DQA1 gene. *Journal of Animal Science*, 82: 8–16.
<http://www.projects.roslin.ac.uk/bola>
<http://www.genomics.liv.ac.uk>
<http://www.ncbi.nlm.nih.gov/>
<http://www.ncbi.nlm.nih.gov/BLAST/>
<http://cc.oulu.fi/~jaspi/popgen/popdown.htm>

Streszczenie: Porównanie polimorfizmu genów DQA u żubrów należących do dwóch linii genetycznych: Białowieskiej i Białowiesko-kaukaskiej. Obecnie żyjąca populacja żubrów została podzielona na dwie linie genetyczne: białowiecką (LB) i białowiesko-kaukaską (LC). W celu porównania zmienności genetycznej u żubrów należących do dwóch linii genetycznych badano polimorfizm genów DQA należących do głównego kompleksu zgodności tkankowej (MHC). Geny MHC cechuje wysoki polimorfizm i w związku z tym mogą być one wykorzystywane do szacowania zmienności genetycznej różnych gatunków, w tym zagrożonych wyginięciem. Analiza polimorfizmu eksonu 2 genów DQA1 i DQA2 objęła 200 żubrów należących

do linii białowieskiej i 56 z białowiesko-kaukaskiej. Analiza SSCP ujawniła występowanie dwóch alleli i dwóch genotypów eksonu 2 genu DQA1 oraz trzech alleli i czterech genotypów eksonu 2 genu DQA2. Frekwencje genotypów eksonu 2 genu DQA2 różniły się istotnie (test chi-kwadrat $p \leq 0,05$) pomiędzy dwoma liniami genetycznymi żubrów. Zaobserwowano występowanie czterech haplotypów DQA – DQA1, DQA2, DQA1/DQA2 i DQA1/DQA2_2. Haplotyp DQA1 był obecny jedynie u osobników z LC a DQA1/DQA2_2 tylko u żubrów z LB. Analiza częstości występowania zidentyfikowanych ha-

plotypów w dwóch liniach genetycznych żubrów wykazała wysoko istotne statystyczne różnice (test chi-kwadrat $p \leq 0,01$).

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Section analysis of after born mason bee (*Osmia rufa* L.) material

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Abstract: *Section analysis of after born mason bee (Osmia rufa L.) material.* Experiment was performed at Apiculture Division, Warsaw University of Life Sciences – SGGW in 2005/2006 season. The aim of this study was to analyze the quality of mason bee pupae (*Osmia rufa* L.) originating from annual and biennial slots. Obtained cocoons were classified. Cocoons were divided into groups of healthy cocoons, empty cocoons, broken and infected. It was also examined and identified the presence of parasites praying on the mason bee cocoons. Research shows that in two-year old slots is more than 10 times less healthy cocoons and three times more damaged cocoons in comparison to the annual sockets. Species diversity of parasites in the biennial nests is wider than in the annuals. In annual material it was found only one parasite belonging to the species *Cacoxenus indagator* whereas biennial material contained species: *Cacoxenus indagator*, *Giraudiella inclusa*, *Trichodes apiarius* and *Sapyga quiquepunctata*. Research shows that the mason bee slots should be used once. Repeated use of slots increases the degree of infestation with parasites which reduces reproductive performance.

Key words: cocoons, mason bees, *Osmia rufa* L., nest parasites.

INTRODUCTION

Mason bee (*Osmia rufa* L.) belongs to the wild bees, which are increasingly used for pollination of plants in agriculture and horticulture. This bee is widespread

throughout the country. It visit more than 150 plant species (Teper, 2004). In the course of the evolution this insect has developed many of the characteristics that determine their adaptation to life under certain environmental conditions. A very important feature is the ability of that bees living under various climatic conditions, mode of reproduction, the use of different types of food base and a strategy of defense against parasites. An important factor limiting the number of mason bees is parasitism. (Wójtowski, 1971; Wójtowski and Wilkaniec, 1969; Bohart, 1955; Linsley, 1958; Medler, 1958; Stephen, 1958; 1962). The largest group of animals which parasites on mason bees are insects (Wójtowski and Szymaś, 1973). They inhabit a socket trap prepared for breeding bees. They destroy nests and cocoons. Associated parasites are most often common in places with high concentration of bee populations. Every year due to excessive density of bees increases the number of parasitic insects. Improper handling and lack of knowledge can cause invasive diseases emergence (eg caused by mites), parasitic species associated to mason bees were grouped into different categories: kleptoparasits, parasitoids, nest destroyers, predators, kleptobionts

and incidental nest residents (Krunic et al., 2005).

In breeding spreading of mason bee diseases is possible in several ways:

- by the contact of bees with flowers, where previously were infected insects,
- by using apiary equipment and tools not being disinfected,
- by reuse the old nests (the stems of plants or not sterilized artificial packets) (Flaga, 2002).

Krunic et al. (1995) and Stanisavljevič (1996) found that the most frequently occurring and highly restrictive limiting population of mason bee is Hymenoptera *Cacoxenus indagator* Loew. and mite *Chaetodactylus osmiaae* Dufour.

The disease caused by mites *Chaetodactylus osmiaae* affects both adult bees and brood (Flaga, 2002). Infection occurs most often while bees sit on the flowers. Females carry pollen with mites from the flowers to nest cells. Young females infected by mites have reduced reproductive ability. In Pisa area (Italy), a major parasite of mason bee is *Anthrax anthrax* Schrank. (Felicili, 2000).

To limit the number of parasites tin boxes used in the following years as slots place should be treated with a high temperature and 0.07% of endosulfanol solution. However the cane tubes should be burnt (Krunic et al., 2001).

MATERIALS AND METHODS

The work was performed at Apiculture Division of Warsaw University of Life Science – SGGW in 2005 and 2006. Research material were mason bee cocoons gained from annual and biennial trap slots. Observations were conducted

in perennial field of SGGW. In this place at the end of March 2005 was issued the slot material – annual traps (1028 cane tubes), biennial traps (1102 cane tubes) and 1400 cocoons of mason bee. This material was placed in two boxes hung at a height of two meters on the south wall of the outbuilding. Bees have been kept in this place from spring to early autumn. In the winter boxes with bees were moved to cool place (1–4°C). In the first week of April 2006 selection of cocoons was carried out. Reed socket were opened using a scalpel and then cocoons were removed from inside. Cocoons were segregated.

They were divided into 4 groups:

- full-shaped (ie, healthy),
- empty,
- damaged,
- infected.

During the selection the following parameters were evaluated:

1. Appearance of the cocoon:
 - Correct,
 - Damaged, struck with parasites,
 - Damaged from other unidentified causes.
2. Developmental stages (divided into pupae);
3. The presence of parasites (larvae, pupae, imago):
 - Large – Diptera (*Cacoxenus indagator* Loew.; *Giraudiella inclusa* Frauenfeld.); Coleoptera (*Trichodes apiarius* L.),
 - Small – Hymenoptera (*Sapyga* sp.).

During each assessment it was determined the number and percentage of healthy and infected cocoons, and the percentage of cocoons infested with parasites. In the evaluation there were also

taken into account abnormal and unusual changes in cocoons and in socket material (cane tubes). It was compare the usefulness of one year old and two year old cocoons in terms of all these parameters.

RESULTS

In the experiment it has been revised and classified 16 642 cocoons, in that number 7826 originated from one year old slots and 8816 originated from two-year old slots.

The number of healthy annual cocoons was 1618 (20.67%), see Figure 1. In the material of biennial cocoons healthy cocoons was only 157 which made less than 2%.

The number of cocoons infested with parasites in annual and biennial material was similar and it amounted 5114 (65.35%) and 5109 (57.96%) respectively.

The greatest differences were found in the cocoons assigned to the group with unidentified causes of damage, annual cocoons were 1094 and biennial were 3550 representing respectively 13.98% and 40.26%.

In the study it was also classified nest material (cane tubes), depending on the condition of cocoons found in tubes. There were evaluated totally 2130 cane tubes (Tab. 1). In the two years old material there was no tube containing only healthy cocoons. Tubes infected with parasites in the biennial nests was over

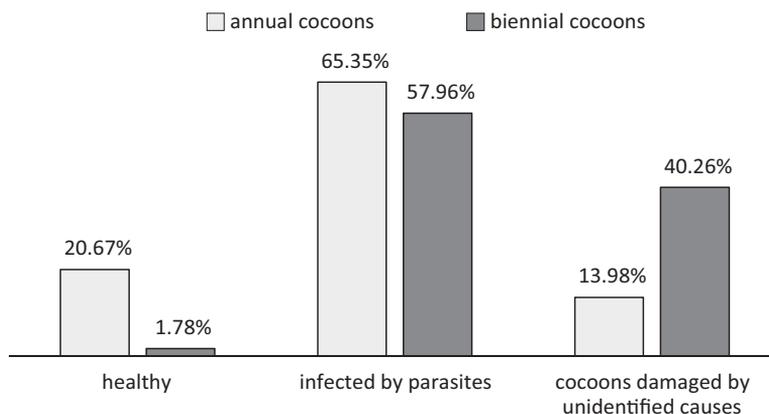


FIGURE 1. Percentage of classified cocoons

TABLE 1. Number of cane tubes originated from annual and biennial slots

Cane tubes \ Cocoon	Annual	Biennial	Total
Healthy	253	0	253
With parasites	257	945	1202
Other unidentified causes of damages	518	157	675
Total	1028	1102	2130
	2130		

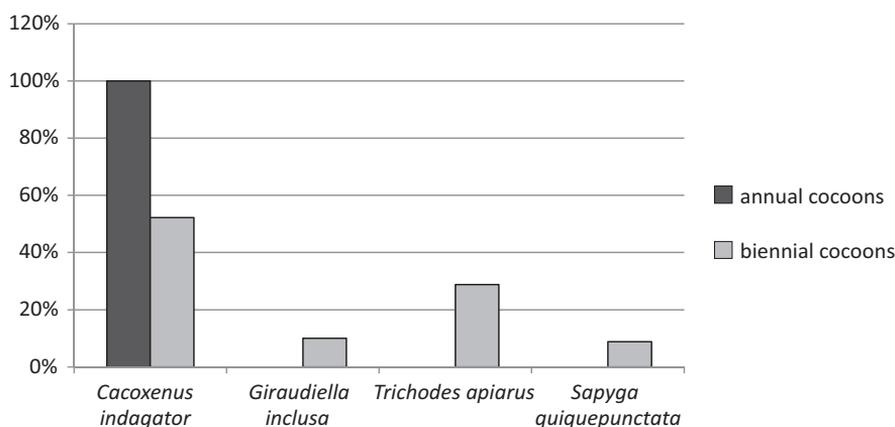


FIGURE 2. Percentage distribution of identified parasites in annual and biennial material

three times more than in the one-year slots. In biennial nest material was significantly less cocoons with damages of unidentified cause in comparison to the one year old slots. There were respectively 158 and 518 cocoons.

The parasite contamination level of the annual cocoons was similar to the contamination level of biennial parasite cocoons. The main and the only parasite that was present in the cocoons from annual nests was *Cacoxenus indagator* (100%). In biennial material was found presence of four types of parasites *Cacoxenus indagator* (52.2%), *Giraudiella inclusa* (10.03%), *Trichodes apiarus* (28.67%) and *Sapyga quiquepunctata* Fabricius (8.83%) (Fig. 2).

DISCUSSION

In recent years, due to the low profitability of beekeeping and the continuing phenomenon of bees dying decreased number of honey bee colonies. Therefore, the solitary bees are increasingly used to pollinate crops, garden and trees in orchards. Mason bees breeding, among

other, affects the quality and yield of onion (Wilkaniec et al., 2004; Wilkaniec et al., 2005; Biliński and Teper, 2004) and alfalfa (Wilkaniec et al., 2004). These authors found in their study that on the effectiveness of bee pollination the female mass of mason bees has a positive effect. The presence of nest parasites not only limits the number of bees that can emergence from cocoons, but also negatively impacts their individual development.

This study confirms the presence of one of the most common parasites found in socket material of mason bee which is *Cacoxenus indagator*. His presence also found Krunič et al. (1995) and Stanisavljević (1996) in their studies. The use of biennial nesting material did not affect the growth of the parasite invasion. The level of contamination with *Cacoxenus indagator* of the annual and biennial material was at similar level. In the socket material used twice in the second year there were appeared three other species of parasites: *Giraudiella inclusa*, *Trichodes apiarus* L. and *Sapyga quiquepunctata*. Their presence affected

very negatively for rearing mason bees. The number of healthy cocoons fell from 20% to less than 2% in such a situation it can be expected that in subsequent years without replacement of nesting material parasites could completely destroy the cocoons of mason bees. Therefore, one of the most important elements of rearing the bees is the use of a annual nesting material. Nest material used once should be destroyed such as burned (Krunič et al., 2001). In this way, we can limit the occurrence of the quantity and number of parasites species.

CONCLUSIONS

1. Healthy cocoons occurred only in the group of annual cocoons.

2. In both groups of cocoons, annual and biennial, were cocoons contaminated by parasites, and damaged by unidentified causes.

3. Sockets cane should be used once. Reuse slots increases the number of nest parasites species, and thereby reduces the number of healthy cocoons.

REFERENCES

- BILIŃSKI M., TEPER D., 2004: Chów i wykorzystanie murarki ogrodowej (*Osmia rufa* L.), (Hymenoptera, Megachilidae) do zapylania sadów. *J. Apic. Scien.* 48 (2): 69–74.
- BOHART G.E., 1955: Alkali bee vs. Drainage. *Farm and Home Science. Utah. Agr. Exp. Sta.* 16: 24, 39–40.
- FELICILI A., 2000: Le osmie, In: *Api e impollinazione* (Pincauti M., Ed) – Giunta Regionale Toscana, Firenze, 159–188.
- FLAGA S., 2002: Pszczoła Murarka ogrodowa. *Kraków*, 9–12; 31–35.
- KRUNIČ M., PINZAUTI A., FELICOLI A., STANISAVLLJEVIČ L.J., 1995: Further observations on *Osmia cornuta* Latr. And *Osmia rufa* L. as alternative fruit pollinators, domestications and utilization. – *Archives Biological Sciences Belgrade*, 47 (1–2): 59–66.
- KRUNIČ M., STANISAVLLJEVIČ L.J., BRAJKOVIČ M., TOMNOVIČ Z., RADOVIČ I., 2001: Ecological studies of *Osmia cornuta* (Latr.) (Hymenoptera, Megachilidae) populations in Yugoslavia with special attention to their diapause. *Acta Horticulturae*, 561: 297–301.
- KRUNIČ M., FELICOLI A., PINZAUTI M., Stanisavljevič L.J., 2005: The accompanying fauna *Osmia cornuta* and *Osmia rufa* and effective measures of protection, bulletin of insectology 58 (2): 141–152.
- LINSLEY G.E., 1958: The ecology of Solitary bees. *Hilgardia* 27 (19): 543–599.
- MEDLER J.T., 1958: Parasitism of bees in trap-nests by *Leucospis affinis* Sz. (Hym. Leucospidae). *Ent. News* 69: 21–24.
- STANISAVLLJEVIČ L.J., 1996: The impact of accompanying fauna of the populations of newly domesticated solitary bees *Osmia cornuta* (Latr.) and *Osmia rufa* L. (Hymenoptera, Megachilidae). *Master Thesis*, Faculty of Biology, University of Belgrade, p. 112.
- STEPHEN W.P., 1958: *Nomia melanderi* in Oregon. *Ent. Soc. Bull.* 15(3): 3.
- STEPHEN W.P., 1962: Propagation of the leaf cutter bees for alfalfa seed production. *Stat. Bull. Ore. Agric. Exp. Stat.* 586: 1–16.
- TEPER D., 2004: Dzika pszczoła. *Murarka ogrodowa. Pasięka* 3/2004, nr 3 (7): 52–54.
- WILKANIEC Z., GIEJDASZ K., FLISZKIEWIECZ M., 2004: The influence of food Mount consumer during the larval development imago of the red mason bee (*Osmia rufa* L., Megachilidae). *J. Apic. Scien.* 48 (1): 29–36.
- WÓJTOWSKI F., 1971: Bioekologiczne i techniczne problemy hodowli praktycznego użytkowania pszczół samotnic. *Wiad. Ekol.* 17 (1): 53–59.
- WÓJTOWSKI F., SZYMAŚ B., 1973: Entomofauna pasożytnicza i towarzysząca pszczołom samotniczym (*Apidea solitariae*) w pułapkach gniazdowych. *Roczniki AR w Poznaniu*, 171.
- WÓJTOWSKI F., WILKANIEC Z., 1969: Próby hodowli pszczół miesiarek i murarek (Hym. Apoidea, Megachilidae) w pułapkach gniazdowych. *Roczniki WSR w Poznaniu*, 42: 153–165.

Streszczenie: Analiza sekcyjna materiału powylęgowego pszczoły murarki ogrodowej (*Osmia rufa* L.). Doświadczenie wykonane w Pracowni Hodowli Owadów Użytkowych SGGW w Warszawie, w sezonie 2005/2006. Celem pracy było dokonanie analizy jakości poczwerek murarki ogrodowej (*Osmia rufa* L.) pochodzących z gniazd jednorocznych i dwuletnich. Uzyskane kokony poddano klasyfikacji. Wyróżniono grupy kokonów zdrowych, pustych, uszkodzonych i chorych. Zbadano i określono również obecność pasożytów na kokonach murarki. Z badań wynika, że w gniazdach dwuletnich jest ponad 10 razy mniej zdrowych kokonów i 3 razy więcej kokonów uszkodzonych, w porównaniu z gniazdami jednorocznymi. Zróżnicowanie gatunkowe pasożytów w gniazdach dwuletnich jest większe niż w jednorocznych. W materiale jednorocznym stwierdzono jedynie obecność pasożyta należącego do gatunku *Cacoxenus indagator*, natomiast

w materiale dwuletnim stwierdzono obecność czterech gatunków pasożytów: *Cacoxenus indagator*, *Gaudiella inclesa*, *Trichodes apiarius* L. i *Sapyga quiquepunctata* Fab. Badania dowodzą, że gniazda dla murarki ogrodowej powinny być wykorzystywane jednokrotnie. Wielokrotne wykorzystywanie gniazd zwiększa stopień zarażenia pasożytami, co wpływa na obniżenie wyników reprodukcyjnych.

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The survival and the awaking time of the worker bees after carbon dioxide anesthesia and gas treatment with different oxygen and nitrogen concentration

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Abstract: *The survival and the awaking time of the worker bees after carbon dioxide anesthesia and gas treatment with different oxygen and nitrogen concentration.* The experiment was performed at the Apiculture Division of Warsaw University of Life Sciences in 2009. The aim of this study was to investigate the bees awaking time and to check their survival rate during their waking up with the application of gas mixtures of different concentration of oxygen and nitrogen. In the experiment were examined 2000 worker bees (*Apis mellifera* L.). All bees were subjected anesthesia with carbon dioxide and awoken by air containing various proportions of oxygen and nitrogen. 241 bees were divided into 5 groups. The control group was awoken from anesthesia with the carbon dioxide in the atmospheric air. Experimental group were awoken in the air containing 50% of O₂ and 50% of N₂, 40% of O₂ and 60% of N₂, 30% of O₂ and 70% of N₂, 15% of O₂ and 85% of N₂. There was no repeatability of results between all examined groups in the first abdominal movements, individual walking bees and in all the bees awaking. Survival rate of groups of bees awoken in a mixture of 40% oxygen and 60% nitrogen was significantly higher in comparison to other groups. This experience was a prelude to further research on queen bees in order to accelerate the awakening from carbon dioxide anesthesia during insemination, and accelerate laying eggs.

Key words: honey bee, oxygen, nitrogen, carbon dioxide, survival rate.

INTRODUCTION

The first attempts to use carbon dioxide to anesthesia queen bees took Laidlaw in 1944. Jasiński (1998) found that before CO₂ began to be used for anesthesia in bees chloroform and ether were commonly used, substances far more harmful to humans. CO₂ anesthesia is used most often during the insemination of queen bees but also to anesthetize worker bees during wedding hives colonization for the better acceptance after queen bee addition (Wilkaniec and Maciejewska, 1995). After applying anesthesia with CO₂ during insemination queen bees were worse accepted by bees, as well as queen bees less frequent made flights from the hive and they often wandered (Wilde and Sobiechowski, 1992). Those authors also observed that queens after anesthesia with CO₂ begun laying eggs later and often incorrectly. Skowronek (1982) observed the development of the adjacent bodies (corpora allata) in worker bees after anesthesia with CO₂. He has found that in young bees (aged 10 days) increased activity of these structures, whereas in older bees, the effect was

the opposite (which was associated with a loss of ability to collect pollen, total or nearly total loss of production of wax). CO₂ narcosis also has a negative impact on the life span of bees (Austin, 1955). According to Chuda-Mickiewicz et al. (2002) carbon dioxide has a positive effect on the drones. This gas helps to complete the process of moving sperm from the testicles to the seminal vesicles and thus accelerates the maturation of drones.

According Konopacka (1989) similar effect to the carbon dioxide has a nitrous oxide (N₂O). After application of N₂O for anesthesia she found that the latency period (time from start of anesthesia to lying eggs by queen bees) and the number of sperm in the seminal reservoirs in queen bees was similar to that when using CO₂ narcosis. According to Skowronek et al. (1973) use of anesthesia with both: CO₂ and N₂O causes earlier initiation of lying eggs by the queen bee and increases the attractiveness of queens for the worker bees. The negative effects of worker bees anesthesia in pure nitrogen and NO₃ observed Ribbands (1954) and Simpson (1954). According to the authors use such a anesthetic reduced the flight activity of worker bees.

The aim of the experiment was to investigate the length of time of waking the bees and to check their survival after the application of different concentrations of oxygen and nitrogen in their wake. The results of this study provide a basis for future experiments on queen bees in order to accelerate the awakening from anesthesia with carbon dioxide during insemination and accelerate their lying eggs initiation.

MATERIALS AND METHODS

Experiments were conducted in July and August 2009 in the experimental apiary of Apiculture Division at Warsaw University of Life Sciences. 2000 worker bees at the age of 1–3 days were examined. In order to obtain the appropriate age of bee workers brood on emergence was insulated in isolators with a metal mesh in four maternal colonies. After this time the young bees were placed in 20 cages – 100 worker bees in each of them. Bees were kept in wooden cages at dimensions: height 17 cm, width 11 cm and depth of 6 cm. Each cage had two vents in the side walls, a closed plate opening through which bees were placed and food in the form of honey-sugar dough was given, and one hole at the bottom of the cage, which way the dead bees were removed. Front of the cage was glass, which enabled observation. On the cage drinker in the form of a syringe was placed. Inside the cage was a piece of honeycomb and utensil with honey-sugar dough. Tin such prepared cages bees were subjected to 3 minute of carbon dioxide anesthesia, and then wake up for 1 minute at various concentrations of oxygen and nitrogen (atmospheric air, 50% O₂ and 50% N₂, 40% O₂ and 60% N₂, 30% O₂ and 70% N₂, 15% O₂ and 85% N₂). Then the bees were observed and the time of successive stages of their recovery was recorded. After anesthesia bees were kept in cages at room temperature. Daily reported number of dead bees. Observation ended on the last dead bee in cage.

RESULTS AND DISCUSSION

Worker bees in the experiment were divided into five groups:

- a) control group (awaken in the air);
- b) 50% O₂ i 50% N₂;
- c) 40% O₂ i 60% N₂;
- d) 30% O₂ i 70% N₂;
- e) 15% O₂ i 85% N₂.

Four rounds (four repetitions for each of these groups) was done. The research based on the observation of two factors:

1. Recovery time for bees euthanized with carbon dioxide, which was measured in three stages. Time was measured from the moment of completion of treatment appropriate for the group – a mixture of oxygen and nitrogen to:
 - first reflexes of abdomen,
 - first steps of individual bees,
 - the wake of all the bees.

2. The survival of worker bees

In the first movements of the abdomen there was no repeatability of results. In each of the four rounds of the study

the first abdomen movements duration significantly differed within groups. Results averaging would not be a good reflection of the influence of test conditions (groups) on the time of recovery. In terms of single individuals walking time and all the bees awaking results were not repeatable. Some bees were observed moving in the time interval 4–12 minutes in the individual rounds. However, all the bees awake time interval was observed in 8–18 min. It can therefore conclude that the results are not affected by external conditions occurred during each round (Fig. 1).

The highest average survival rate was observed in the group anesthetize with gas mixture in a ratio of 40% oxygen and 60% of nitrogen and it was almost 18 days. In the next two groups awaking in a mixture of 30% oxygen and 70% nitrogen and 15% oxygen and

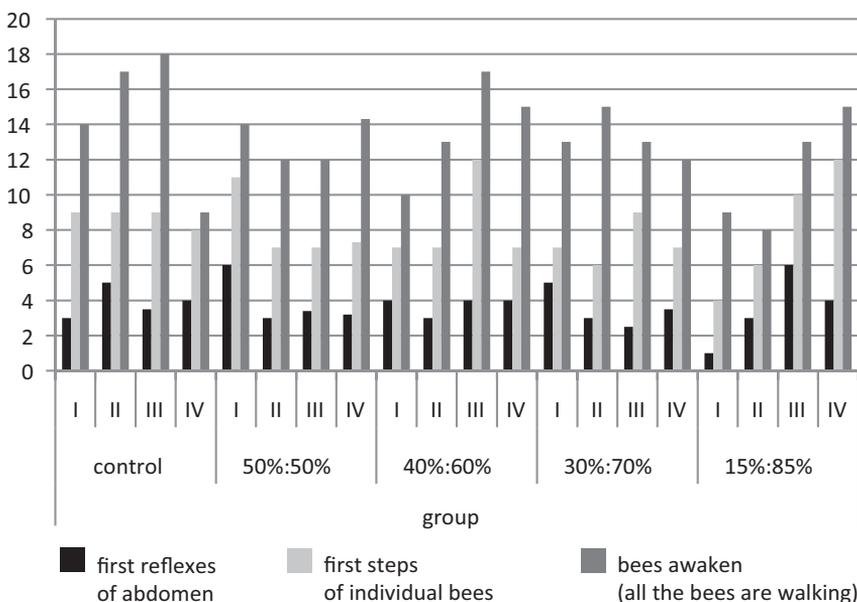


FIGURE 1. Recovery time of the bees in a mixture of different concentrations of oxygen and nitrogen

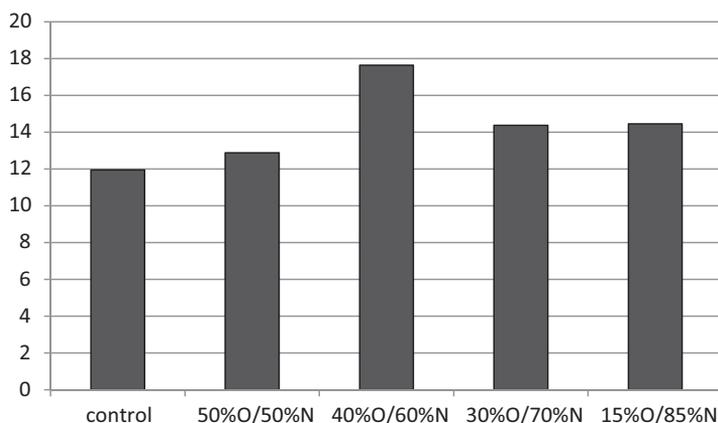


FIGURE 2. Average survival time for four rounds (days)

85% nitrogen, the average survival was lower and amounted up to 14.2 days. It is nearly four days shorter than in the group with the highest survival rates. In the group awoken in 50% oxygen and 50% nitrogen survival last 13 days. The lowest survival was found in bees awoken after general anesthesia in the atmospheric air (12 days). Multiple comparison tests (NIR) on bees survival rate showed that all differences between the two groups are statistically significant except the difference in group awoken in 30% oxygen and 70% nitrogen as well as 15% oxygen and 85% nitrogen. The study found a significantly lower mortality in the group awoken in 40% oxygen and 60% nitrogen compared with other groups (Fig. 2).

Madras-Majewska and Jasiński (2006) undertook studies to determine the time of worker bees awakening after anesthesia with CO₂ in different proportions of major air components oxygen and nitrogen. By examining the survival of bees authors found that the longest-

lived bees recovered in the air enriched with oxygen up to 40%, so in a mixture of 60% nitrogen and 40% oxygen. This study also confirms that the mixture of air used to bees awakening in a ratio of 60% nitrogen and 40% oxygen is the best proportion for the bees. This study provides a basis for future experiments on queen bees.

CONCLUSIONS

1. There was no repetition of worker bees awake time comparing the individual rounds of experience
2. The lowest average survival was observed in the group awoken from anesthesia in the atmospheric air.
3. The most preferred composition of the mixture, where the bees awake took place was 60% oxygen and 40% nitrogen. In this group the highest average survival time was observed (survival time was extended by 6 days compared to the group awoken in atmospheric air).

REFERENCES

- AUSTIN G.H., 1955: Effect of carbon dioxide anesthesia on bee behavior and expectation on life. *Bee World*. 36: 45–47.
- CHUDA-MICKIEWICZ B., PRABUCKI J., SAMBORSKI J., PERUŻYŃSKI G., 2002: Wpływ dwutlenku węgla na dojrzewanie i wartość rozrodczą trutni. *Pszczel. Zesz. Nauk*. 34: 16.
- JASIŃSKI Z., 1998: Naturalny i kontrolowany dobór u pszczoł. *Pszczelnictwo. Praca zbiorowa*. Wyd. Promocyjne „Albatros” Szczecin.
- KONOPACKA Z., 1989: Czynniki wpływające na wyniki sztucznego unasieniania matek pszczelich i ich jakość. *Rozprawa habilitacyjna*. Puławy.
- LIDLAW H.H., 1944: Artificial insemination of the queen bee *Apis mellifera* L. Morphological basis and results. *J. Morph.* 74, 3: 429–465.
- MADRAS-MAJEWSKA B., JASIŃSKI Z., 2006: Badanie wpływu różnych stężeń głównych składników powietrza tlenu i azotu na wybudzanie pszczoł robotnic. *Nauk. Konf. Pszczel. Puławy*.
- RIBBANDS C.R., 1954: Nitrous oxide anesthesia does not encourage re-orientation of honeybees. *Bee World*. 35, 5: 91–95.
- SKOWRONEK W., JAYCOX E.R., GYUNN G., 1973: Wpływ usypiania matek pszczelich na ich atrakcyjność dla pszczoł robotnic. *Pszczel. Zesz. Nauk*. 17: 11–15.
- SKOWRONEK W., 1982: Wpływ dwutlenku węgla na pszczoły. *Prace Instytutu Sadownictwa i Kwiaciarstwa. Seria D, nr 13. Monografie i Rozprawy*. Puławy.
- SIMPSON J., 1954: Effects some anesthetics on honeybees: nitrous oxide, carbon dioxide, ammonium nitrate smoker Fusem. *Bee World* 35, 8: 149–155.
- Statistical Product and Service Solution base version 17.0 for Windows. SPSS inc. USA 2009
- WILDE J., SOBIECHOWSKI K., 1992: Wpływ traktowania dwutlenkiem węgla na behawiorizm unasieniania matek pszczelich. *XXIX Nauk. Konf. Puławy*. s. 44–45.
- WILKANIEC Z., MACIEJEWSKA M., 1995: – Przeżywalność robotnic pszczoły miodnej (*Apis mellifera* L.) usypianych różnymi sposobami w badaniach laboratoryjnych. *Pszczel. Zesz. Nauk*. 1: 7–10.

Streszczenie: *Przeżywalność i czas wybudzania pszczoł robotnic po anestezji dwutlenkiem węgla i traktowanych mieszaniną gazową o różnych stężeniach tlenu i azotu. Doświadczenie zostało wykonane w Pracowni Hodowli Owadów Użytkowych SGGW w 2009 roku. Celem pracy było zbadanie czasu wybudzania się pszczoł oraz sprawdzenie ich przeżywalności po zastosowaniu różnych mieszanin gazów tlenu i azotu do ich wybudzania. W doświadczeniu zbadano 2000 pszczoł robotnic (*Apis mellifera* L.). Wszystkie pszczoły poddane narkozie z dwutlenku węgla były wybudzane w powietrzu zawierającym różne proporcje tlenu i azotu. Stworzono 5 grup. Grupa kontrolna wybudzana była z narkozy dwutlenku węgla w powietrzu atmosferycznym. Grupy doświadczalne wybudzane były w powietrzu zawierającym 50% O₂ i 50% N₂, 40% O₂ i 60% N₂, 30% O₂ i 70% N₂, 15% O₂ i 85% N₂. Nie stwierdzono powtarzalności wyników pomiędzy wszystkim badanymi grupami w zakresie pierwszych ruchów odwłokowych, pojedynczych chodzących osobników oraz wybudzenia się wszystkich pszczoł. Przeżywalność grupy pszczoł wybudzanej w mieszaninie 40% tlenu i 60% azotu była istotnie wyższa w porównaniu z pozostałymi grupami. Niniejsze doświadczenie było wstępem do dalszych badań, prowadzonych na matkach, w celu przyspieszenia wybudzania z narkozy dwutlenku węgla w czasie inseminacji oraz przyspieszenia ich czerwienia.*

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The influence of nests usage on mason bee (*Osmia rufa* L.) survival

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Abstract: *The influence of nests usage on mason bee (*Osmia rufa* L.) survival.* The research was performed at Apiculture Division of Warsaw University of Life Sciences – SGGW in 2004–2006. Main observations were carried in February and in March 2006. The aim of this study was to present the differences in mason bee (*Osmia rufa* L.) survival resulting from the nests usage. After opening all the tubes it was found that in annual nest quite healthy cocoons was 1618 (21%) and the loss of 6208 (79%). In the biennial nest profit was only 157 cocoons (2.5%) and the loss of up to 6075 cocoons (97.5%). Taking into account the number of healthy cocoons (profit) as well as representing a loss in both types of nests it can be noted that in the annual nest it could received more than 15 cocoons from the female. However, in the biennial nest, which can be expected more numerous population, it was achieved in one year only 11 cocoons from the female.

Key words: mason bees, *Osmia rufa* L., cocoons, nests.

INTRODUCTION

Mason bee has a high rate of reproduction and a wide food preferences. Tendency to artificial nests occupation, short period of adult forms emergence from cocoons, the ability to create clusters and colonies, as well as low workload are one of the ad-

vantages which does mason bee attractive as for a breeding species (Flaga, 2002).

Mason bee has developed several forms of protection of its species. One of them is a linear type of nest-building and appropriate gender distribution in each cell of cane tube. Fertilized eggs from which females are hatched, are placed in the further parts of the nest, and in cells located more outside are deposited unfertilized eggs. Males are more exposed to insect-eating birds or martens. Females inside the nests have bigger chances of survival (Flaga, 2002).

Another way of passive protection is to build by mason bees empty first cell in the nest tube. This is typical behavior of bees of the *Osmia* genus. Many Aculeata species build empty cells in a variable position and number (Krombein, 1967; Linsley, 1958; Stephen, 1969). Tactic of leaving the first cell empty or even the entire tube is designed to mislead the parasites, because the plug of larval cells does not differ from the closing caps of the blank cells (or the whole tube). An empty cell is present in more than 80% of nest traps (Balfour-Browne, 1925; Bayer-Helms, 1933; Brechtel, 1986, Jacob-Remacle, 1976).

In terms of nesting material selection mason bees are very plastic. They can inhabit the holes from knots in a tree, hollow brick slots, holes in the bricks, space between the planks and in the straw covering the roofs. Wilkaniec and Giejdasz (2001) found that mason bee can also nest in the paper tubes, plastic straws for drinking and in shrinkable insulating covers. According to their study, only the tube of cane were settled at 100%.

The most important task of rearing mason bee is to get as many populations for pollination of crops as possible (Wilkaniec et al., 2004). Suitable nesting material selection can affect the reproductive performance of these insects.

MATERIALS AND METHODS

The study was performed at the Apiculture Division of Warsaw University of Life Science – SGGW. The experience lasted from March 2004 till April 2006. Research material was mason bee cocoons risen in annual and biennial cane nest traps. In March 2004, 1200 cocoons of mason bee were brought from the Institute of Arboriculture and Floriculture in Puławy, which after hatching colonized previously prepared cane nest tube. These tubes were used for two seasons (from spring to autumn 2004 and from spring to autumn 2005) without the selection of cocoons and cane tubes. Nests were built of closely bind the common cane stalks, tied in bundles and loaded in the plastic cover. An average package consisted of 60–80 tubes of 18–20 cm in length and diameter of 6–8 mm. In 2004 it was prepared nest made of 1028 tubes and in 2005 made of 1102 cane tubes. So

constructed nests was placed in special boxes. These boxes have been suspended at the height of 2 m on the outer wall of the building and an additional safety net covered them as the protection from birds. In the spring of 2005 from ISiK (IAF) in Puławy brought another 1200 cocoons, and bees after emergence colonized prepared for them new common reed stalks used that time only one season (from spring 2005 to autumn 2005). All the research material of two experimental years (cocoons used one season and used for two seasons) in October 2005 was transferred to a cool place (1–4°C) for overwintering. In April 2006 selection of cocoons was performed. Cocoons were removed from the tubes using a scalpel and gently cutting the tube along its axis so not to damage the inside of the cocoons. After opening nests cocoons were sorted for profit (healthy cocoons, normal) and loss (mechanically damaged cocoons, dead larvae of bees and tubes that have not been settled). The material was assessed taking into account the following parameters for the appearance of cocoons: normal (healthy) and damaged (infected).

RESULTS

After opening all the tubes it was found that in annual nest quite healthy cocoons was 1618 (21%) and the loss of 6208 (79%). In the biennial nest profit was only 157 cocoons (2.5%) and the loss of up to 6075 cocoons (97.5%) (Tab. 1). The loss consisted of empty cells, infected, moldy and mechanically damaged cocoons and also dead larvae of mason bee.

In the one-year old material, where the amount of all losses of cocoons was

TABLE 1. Number of cocoons in both types of nests

	Annual nests	Biennial nests
Profit	1618	157
Loss	6208	6075
Total	7826	6232

TABLE 2. Type of loss in annual and biennial nests

Nest type Type of loss	Annual	Biennial
Infected cocoons	5114	2037
Empty cocoons	311	488
Damaged cocoons	23	1812
Dead larvae	760	1738

assigned to 6208, up to 5114 cocoons (82%) were infected. There were 311 empty cocoons (5%) and mechanically damaged 23 cocoons (0.4%). Died larvae of mason bee were 760 (12.24%) (Tab. 2).

In two years material on the loss consisted of 2037 infected cocoons (33.5%),

488 empty cocoons (8%) and as much as 1812 damaged cocoons (30%) that were badly formed or mechanically damaged by other emergence from the nest bees. Dead larvae were 1718 (28.5%) – Figure 1.

Knowing the exact number of tubes in both types of nests and the average number of eggs laid by mason bee females in a single tube it could be counted the expected number of cocoons, which should be found in a nest at the end of the season. Assuming that the female lays 15 eggs, inhabiting two tubes following results were received (Tab. 3).

The total number of cocoons – 7710 in the case of annual nest and 8265 in the case of biennial nest, are presumed numbers, ie the profit that could be obtained if all the cells were occupied by healthy cocoons. In the case of two year-rearing cocoons from 1200, which initiated established breeding approximately 600 individuals would be a female. From

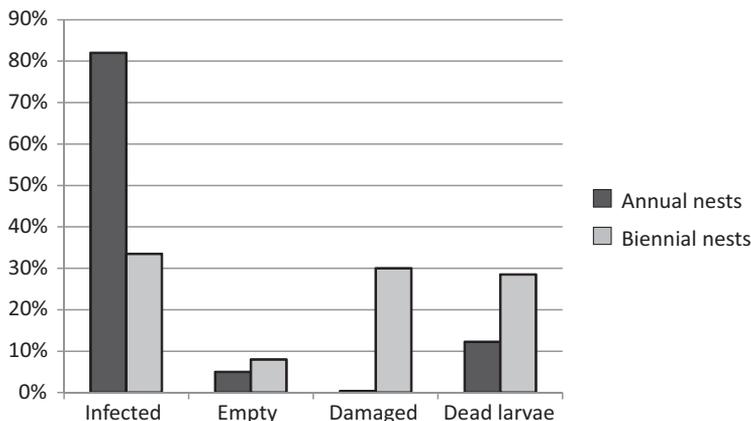


FIGURE 1. Comparison of cocoons losses in annual and biennial nests – in percentage (%)

TABLE 3. Estimated number of cocoons

Annual nests	Biennial nests
1028 tubes × 7.5 cocoons = 7710 cocoons	1102 tubes × 7.5 cocoons = 8265 cocoons

TABLE 4. Actual number of cocoons in tubes

Annual nests	Biennial nests
1618 (profit) + 6208 (loss) = 7826 cocoons 1028 tubes x = 7.61 cocoons/tube	157 (profit) + 6075 (loss) = 6232 cocoons 1102 tubes x = 5.6 cocoons/tube

one female can be gained average of 15 cocoons, giving 9000 individuals that could be hatched in the spring of 2005. Assuming that 4500 of 9000 bees are females, each of which again lays an average of 15 eggs, in the second year of usage new generation should amount 67 500 individuals. Knowing the exact number of healthy cocoons (profit) and infected cocoons (loss) in both types of nests it was ascertained the actual number of cocoons (Tab. 4).

The Table 4 shows that the actual number of cocoons in annual nest is 7.61 of cocoons per one nest tube, and in the biennial nest it was only 5.6 cocoons per one tube. Taking into account the number of healthy cocoons (profit) as well as representing a loss in both types of nests it can be noted that in the annual nest it could received more than 15 cocoons from the female. However, in the biennial nest, which can be expected more numerous population, it was achieved in one year only 11 cocoons from the female.

DISCUSSION

In recent years, due to the low profitability of beekeeping and the continuing phenomenon of bees dying decreased number of honey bee colonies. Therefore, the solitary bees are increasingly used to pollinate crops, garden and trees in orchards (Flaga, 2002).

Mason bee survival in annual nests is much higher (21%) than in biennial nests (2.5%). In annual nests infected cocoons represented significantly greater loss (82%) than in biennial nests (33.5%).

Lack of cocoon selection was the cause of population decrease in the second year of nest usage. The survival of mason bee was mainly determined by cleanliness and order appliance, because the sanitary conditions have a direct impact on the viability and number of offspring.

In such situation it can be supposed that in subsequent years without replacement of nesting material the mason bee cocoons losses would be even greater. Therefore one of the most important elements of rearing the bees is the use of a annual nesting material. Nest material used once should be destroyed such as burned (Krunič et al., 2001). In this way, we can increase the probability of healthy cocoons appearance and increase the mason bee population needed to effective pollination of crops (Wilkaniec et al., 2004).

CONCLUSIONS

1. In annual as well as biennial nests there are healthy cocoons (profit) and infected cocoons (loss).
2. Mason bee survival in annual nests is significantly higher (21%) than in biennial nests (2.5%).

3. In annual nests infected cocoons represented significantly greater loss (82%) than in biennial nests (33.5%).

4. Lack of cocoon selection was the cause of population decrease in the second year of nest usage.

5. The survival of mason bee was mainly determined by cleanliness and order appliance, because the sanitary conditions have a direct impact on the viability and number of offspring.

REFERENCES

- BALFOUR-BROWNE F., 1925: Concerning the Habitats of Insects, Univ. Press, Cambridge.
- BAYER-HELMES F., 1933: Untersuchungen zum Fortpflanzungsverhalten solitär lebender Wildbienen an den Beispflanzen *Osmia rufa* und *Megachile versicolor*. Diplomarbeit, Göttingen.
- BRECHTEL F., 1986: Die Stechimmenfauna des Bienienwaldes und seiner Randbereiche (Stüpfplatz) unter besonderer Berücksichtigung der Ökologie kunstnestbewohnender Arten, Pollichia, Bad Dürkheim.
- FLAGA S., 2002: Pszczoła Murarka ogrodowa. Kraków, 9–12; 31–35.
- JACOB-REMACLE A., 1976: Une operations nichoirs artificiels pour Hymenoptères dans trios jardins de Liège, Bull. Ann. Soc. R. Entomol. Belgique.
- KROMBEIN K.V., 1967: Trap-nesting Wasp and Bees: Life History, Nest and Associates, Smithsonian Press, Washington.
- KRUNIĆ M., STANISAVLJEVIĆ L.J., BRAJKOVIĆ M., TOMNOVIĆ Z., RADOVIĆ I., 2001: Ecological studies of *Osmia cornuta* (Latr.) (Hymenoptera, Megachilidae) populations in Yugoslavia with special attention to their diapause. – Acta Horticulturae, 561: 297–301.
- LINSLEY G.E., 1958: The ecology of Solitary bees. Hilgardia 27 (19): 543–599.
- STEPHEN W. P., BOHART G.E., TORCHO P.F., 1969: The Biology and External Morphology of Bees. Oregon St. Univ. Agric. Exp. Stat., Corvallis, Oregon.
- WILKANIEC Z., GIEJDASZ K., FLISZKIEWICZ M., 2004: The influence of food Mount consumer during the larval development imago of the red mason bee (*Osmia rufa* L., Megachilidae) J. Apic. Scien. 48 (1): 29–36.
- WILKANIEC Z., GIEJDASZ K., 2001: Wpływ średnicy rurek gniazdowych na wielkość oprzędów i płeć pokolenia potomnego pszczoły murarki ogrodowej *Osmia rufa* L. AR. im A. Cieszkowskiego w Poznaniu. 119–120.

Streszczenie: Wpływ sposobu użytkowania gniazd na przeżywalność murarki ogrodowej (*Osmia rufa* L.). Pracę wykonano w Pracowni Hodowli Owadów Użytkowych SGGW w Warszawie w latach 2004–2006. Główne obserwacje prowadzono w lutym i marcu roku 2006. Celem pracy było przedstawienie różnic w przeżywalności murarki ogrodowej (*Osmia rufa* L.) wynikającej ze sposobu użytkowania gniazd. Po otwarciu wszystkich badanych rurek stwierdzono, że w gnieździe jednorocznym kokonów całkiem zdrowych było 1618 sztuk (21%), a strata wynosiła 6208 sztuk (79%). W gnieździe dwuletnim zysk wynosił jedynie 157 kokonów (2,5%), a strata aż 6075 sztuk (97,5%). Na stratę składały się komórki puste, kokony chore i spleśniałe, uszkodzone mechanicznie oraz martwe larwy murarki. Biorąc pod uwagę liczbę kokonów zdrowych (zysk), jak i stanowiących stratę w obu typach gniazd, zauważyć można, że w gnieździe jednorocznym można było uzyskać więcej niż 15 sztuk kokonów od jednej samicy. Natomiast w gnieździe dwuletnim, w którym spodziewać się można liczniejszej populacji, uzyskano zaledwie 11 kokonów od samicy w jednym roku.

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Effect of the initial body weight of Ross 308 chicken broilers on the rate of growth

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Abstract: *Effect of the initial body weight of Ross 308 chicken broilers on the rate of growth.* The results of broilers' rearing are greatly dependent on the quality of chicks which is affected by age and health state of reproduction stock, weight of hatching egg and body weight of the hatched chicks. The aim of the conducted experiment was to compare the effect of body weight of the chicks on their first day of life on the growth rate of chickens and their body weight on 42 day of rearing. The studies were conducted on 728 Ross 308 chicks. The birds were kept on a litter, in compartment, being at random divided into 8 cubicles. One-day chicks were marked with chick marks. The birds were individually weighed each week, i.e. on 1, 8, 15, 22, 29, 36 and 42 day of life. For statistical calculations, the collected data were divided into three groups, 91 pcs in each group. Body weight of one-day chick was taken as criterion: group 1 – lower than, or equal to 39 g; group 2 – within interval of 40–42 g; group 3 – higher than 42 g. On the ground of the conducted studies it may be concluded that Ross 308 chicken broilers with body weight higher than 40 g should be selected for rearing because smaller chicks, in spite of the highest growth rate, reach lower body weight on 42 day of life as compared to heavier chickens.

Key words: Ross 308 broilers, body weight, growth rate.

INTRODUCTION

The results of broilers' rearing are greatly dependent on the quality of chicks

which is affected by age and health state of reproduction stock, weight of hatching egg and body weight of the hatched chicks (Meijerhof, 2005; Mendes et al., 2007; Wolanski et al., 2007). Visual score, Tona or Pascar score and day-old chick weight are commonly used for measuring chick quality (Tona et al., 2003; Tona et al., 2005; Meijerhof 2006). Chick weight is the most widely used indicator for day-old chick quality assessment (Decuyper et al., 2002). The length of the chicken is also related with the size of the egg, as is body weight, but to a lesser extent. The total length increases about 5% (from 19 to 20 cm) for breeders from 25 to 60 weeks of age. Length at 18 days can also be used as an indicator for the efficiency of an in-ovo injection process, as it is correlated with the place where the vaccine is administered (allantios, amnion, breast muscle or neck) (Meijerhof, 2005). However, recent research (Joseph et al., 2006) has shown that differences for hatch weight among treatments are largely explained by variations in residual yolk mass. Objective definition of chick quality needs probably a combination of several factors such as day old chick weight, chick

physical aspects and others, etc. (Tona et al., 2005).

The aim of the conducted experiment was to compare the effect of body weight of the chicks on their first day of life on the growth rate of chickens and their body weight on 42 day of rearing.

METHODS AND MATERIALS

The studies were carried out in the experimental farm of SGGW, on 728 broiler chickens of Ross 308, the birds were managed on the litter, in standard zoohygienic conditions, in accordance with the standards, recommended by Ross company. The density rate was equal to 11.4 birds/m². The chickens were fed the diets from Reguły company (Tab. 1). The birds were kept on a litter, in compartment, being at random divided into 8 cubicles. One-day chicks were marked with chick marks. The birds were individually weighed each week, i.e. on 1, 8, 15, 22, 29, 36 and 42 day of life. For statistical calculations, the collected data were divided into three groups, 91 pcs in each group. Body weight of one-day chick was taken as criterion: group 1

– lower than, or equal to 39 g; group 2 – within interval of 40–42 g; group 3 – higher than 42 g. The obtained results were statistically developed by variance analysis, calculated by the least square method, using computer program SPSS 14.0 PL for Windows.

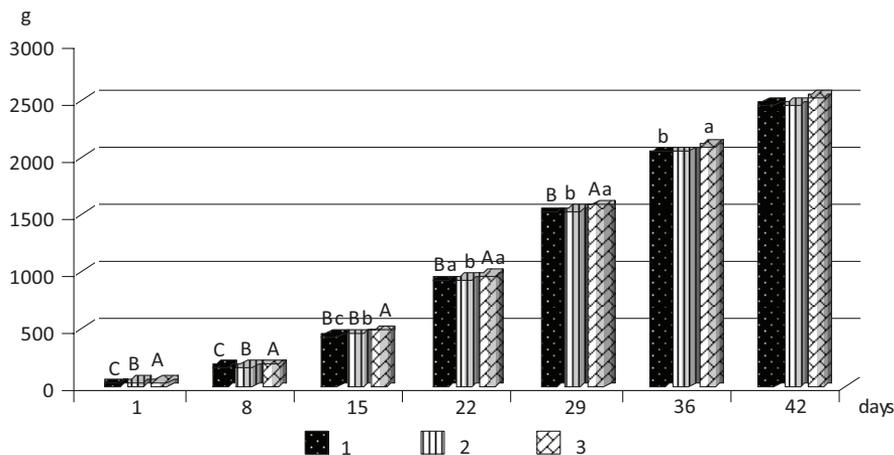
RESULTS AND DISCUSSION

Statistically significant differences ($P \leq 0.01$ and $P \leq 0.05$) in body weight of one-day chickens and during the successive days of rearing (8, 15, 22 and 29) between all groups of chickens, were found. Significance of differences ($P \leq 0.05$) was also confirmed on 36 day of life but only between group 1 and 3. On 42 day of life, differences in body weight of the birds were insignificant although broilers from group 3 reached higher body weight by 57 g in average as compared to body weight of broilers from group 1 (Fig. 1).

Between 8 and 36 day of life, the highest growth rate was characteristic of the smallest chickens (group 1), significantly lower values of this trait were recorded for chickens from group 2 and

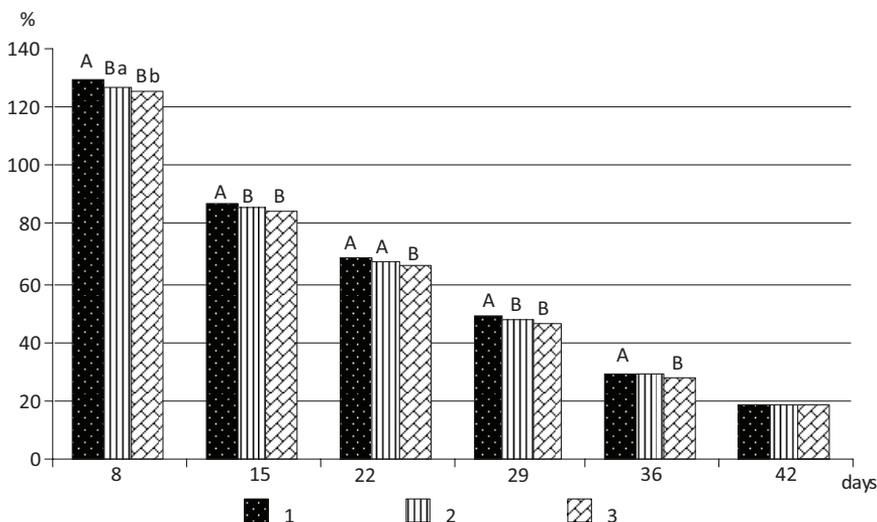
TABLE 1. Feed composition

Raw material	Starter	Grower	Finisher
Wheat	27.3	25.5	36.0
Maize 8.5%	29.7	32.7	26.8
Soybean meal 46	32.0	31.6	27.2
Soybean oil	3.8	5.6	5.8
Limestone	1.4	0.6	0.2
Premix	6.0	4.0	4.0
Analysis			
ME (MJ/kg)	13.70	14.16	14.25
Crude protein (%)	22.10	20.44	19.10
Fat (%)	5.94	7.73	7.81
Fibre (%)	3.10	2.97	2.93



A, B, C – Means with the different subscripts differ significantly at $P \leq 0.01$
 a, b, c – Means with the different subscripts differ significantly at $P \leq 0.05$

FIGURE 1. Body weight of broiler chickens (g)



A, B, C – Means with the different subscripts differ significantly at $P \leq 0.01$
 a, b, c – Means with the different subscripts differ significantly at $P \leq 0.05$

FIGURE 2. Growth rate of broiler chickens (%)

the lowest values were demonstrated for group 3. Differences in growth rate between group 2 and 3 were significant on 22 day of observations. On 42 day of life, the growth rate was the same for all groups (Fig. 2).

Tona et al. (2005) suggested that though there are still some unknown factors that can be involved in chick quality definition, quantitative (weight or length) and qualitative assessment of day-old chick quality are relevant and related to

performance. In this study no significant difference ($p > 0.05$) was observed in the body weight of the day-old chicks from the different sources. Though body weight is an easy and highly objective measurement, the value is relative. Day-old chick weight is highly correlated with egg weight but does not give good indication for chick development (Meijerhof, 2005). This is because chick weight contains the real chick weight so the amount of egg that is transformed into the chick tissue and remaining yolk residue. Embryos use the fat in the yolk as fuel for their development and therefore the deviation between real chick weight (without remaining yolk sac) and yolk residue is an indicator for development. If a lot of yolk is left over, then less should be considered lower. However, this does not show in day-old chicks weight (Meijerhof, 2005).

Tona (2000) and Tona et al. (2005) established the correlations between several of the qualitative parameters that been included in determining chick quality. Interestingly, most parameters are highly correlated with the conditions of the navel area, amount of retracted yolk and chick activity indicating that these parameters alone may be sufficient for sorting day-old chicks into quality groups.

CONCLUSION

On the ground of the conducted studies it may be concluded that Ross 308 chicken broilers with body weight higher than 40 g should be selected for rearing because smaller chicks, in spite of the highest growth rate, reach lower body weight on 42 day of life as compared to heavier chickens.

REFERENCES

- DECUYPERE E., TONA K., BAMELIS F., CAR-EGHI C., KEMPS B., DE KETELAERE B., DE BAERDEMAKER J., BRUGGEMAN V., 2002: Broiler breeders and egg factors interacting with incubation conditions for optimal hatchability and chick quality. *Arch. Geflügelk Special Issue* 66, 56–57.
- GEIDAM Y.A., KUMSHE H.A., BUKAR-KOLO M.Y., GULANI I.A., MARGIMARI Z.N., 2008: Quality assessment of layer day-old chicks supplied to Maiduguri, North-Eastern Nigeria. *Asian Journal of Animal and Veterinary Advances* 3 (1): 24–29.
- JOSEPH N.S., LOURENS A., MORAN JR E.T., 2006: The effects of suboptimal eggshell temperature during incubation on broiler chick quality. *Poult. Sci* 85, 932–938.
- MEIJERHOF R., 2005: Defining and measuring quality in day old broilers. *Int. Hatch. Prac.* 19, 7.
- MEIJERHOF R., 2006: Chick size matters. *World Poult.* 22, 30–31.
- MENDES M., DINCER E., ARSLAN E., 2007: Profile analysis and growth curve for body mass index of broiler chickens reared under different feed restrictions in early age. *Arch. Tierz.* 50, 403–411.
- SPSS 2006. SPSS, 14.0 for Windows user's guide, 2006, by SPSS Ins. USA.
- TONA K., BAMELIS F., DE KETELAERE B., BRUGGEMAN V., MORAES V.M.B., BUYSE J., ONAGBESAN O., DECUYPERE E., 2003: Effects of egg storage time on spread of hatch, chick quality and juvenile growth. *Poult. Sci.* 82, 736–741.
- TONA K., BRUGGEMAN V., ONAGBESAN O., BAMELIS F., GBEASSOR M., MERTENS K., DECUYPERE E., 2005: Day-old chick quality: relationship to hatching egg quality, adequate incubation practice and prediction of broiler performance. *Avian Poult. Biology Rev.* 16, 109–119.
- WOLAŃSKI N.J., RENEMA R.A., ROBINSON F.E., CARNEY V.L., FANCHER B.L., 2007: Relationship among egg characteristics, chick measurements and early growth traits in ten broiler breeder strains. *Poult. Sci.* 86, 1784–1792.

Streszczenie: *Wpływ początkowej masy ciała kurcząt brojlerów kurzych Ross 308 na tempo wzrostu. Wyniki odchowu brojlerów w dużej mierze zależą od jakości piskląt, na którą mają wpływ: wiek i stan zdrowotny stada reprodukcyjnego, masa jaja wylęgowego oraz masa ciała wykłutych piskląt. Celem przeprowadzonego doświadczenia było porównanie wpływu masy ciała piskląt w pierwszym dniu życia na tempo wzrostu kurcząt oraz ich masę ciała w 42 dniu odchowu. Badania przeprowadzono na 728 kurczętach Ross 308. Ptaki utrzymywano na ściółce, w pomieszczeniu podzielonym losowo na 8 przedziałów. Jednodniowe pisklęta zaznaczono znaczkami pisklęcymi. Kurczęta były co tydzień indywidualnie ważone, tj. w 1, 8, 15, 22, 29, 36 i 42 dniu życia. Do obliczeń statystycznych zebrane dane podzielono na trzy grupy, liczące po 91 sztuk. Jako kryterium podziału przyjęto masę ciała jed-*

nodniowego pisklęcia: grupa 1 – niższa lub równa 39 g; grupa 2 – w przedziale od 40 do 42 g; grupa 3 – większa niż 42 g. Na podstawie uzyskanych wyników stwierdzono, że do odchowu kurcząt brojlerów Ross 308 należy wybierać pisklęta o masie ciała większej niż 40 g, ponieważ mniejsze pisklęta, mimo najszybszego tempa wzrostu, osiągają niższą masę ciała w 42. dniu w porównaniu z pisklętami cięższymi (o 57 g).

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Effect of the age of the first calving on milk performance and inter-calving period of Polish Holstein-Friesian cows

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Abstract: *Effect of the age of the first calving on milk performance and inter-calving period of Polish Holstein-Friesian cows.* The aim of the work was to determine the effect of the age of the first calving on milk performance in the 1st, 2nd and 3rd lactation, the sum of yield of milk, fat and protein for three complete lactations and the amount of the mentioned components as calculated per one day of life. The age of the first calving had a significant influence on milk yield only in the first lactation. The percentage fat content did not differ significantly between the groups of the age of calving whereas the studied factor affected significantly the protein content in milk of the cows after the second and third calving. The obtained results indicate that the most favorable economically mating of heifers takes place until the 27th month of life as such animals give the greatest quantities of milk, fat and protein, as calculated per one day of life.

Key words: age of the first calving, milk performance, milk, fat and protein amount as calculated per one day of life.

INTRODUCTION

The period of heifer rearing has a great effect on the later productivity of dairy cows, and the abbreviation of the mentioned period causes the improvement of production profitability. Most of the studies suggest abbreviation of the rearing period; the other ones indicate the higher

productivity of animals, being calved at the later age. According to Wilson (2006) and Plate-Church (2006), a heifer which does not become pregnant until the 15th month of life, brings losses in the quantity of ca. 100 USD per month. In the opinion of Heinrich (1993), the rearing of the heifers constitutes 15–20% of all costs in cattle breeding. Many researchers (Mourits et al., 1997; Gardner et al., 1988; Lin et al., 1988; Meyer et al., 2005 and Harel et al., 2006) stated that it was justifiable to lower the age of calving even to 22 months. It does not cause big losses in productivity and, simultaneously, lowers the costs of nutrition. The authors suggest, however, that such early commencement of performance is possible only with the correctly conducted rearing so as the heifers reach the appropriate body weight and height in sacrum at such a young age. Powell (1985), Nilforooshan and Edriss (2004) and Harel et al. (2006) express the opinion that about half of the heifers in the USA are calved at the age of 23–27 months. In the countries where cattle breeding and milk production is found on a high level, we may observe lowering of the age of the first parturition. Also, in Poland there is a small decline of the age of the first

calving of heifers. In 2010, the mean for the discussed parameter in the herds covered with milk recording was equal to ca. 27 months (Polish Federation of Cattle Breeders and Milk Producers, 2011).

MATERIAL AND METHODS

The studies were conducted with 319 high-yielding cows of Polish Holstein-Friesian breed in Central Poland. The analysis included the cows which had three full lactations completed. The data for analysis derived from breeding documentation (card of cow-heifer). The animals were kept in loose housing barns and fed TMR system.

The aim of the experiment was to determine the effect of the age of the first calving on milk performance in the 1st, 2nd and 3rd (305-day) lactation, sum of the yield of milk, fat and protein for three full lactations and the quantity of milk, fat and protein as calculated per one day of life.

The cows were classified into four groups in respect of the age of the first calving:

1. Below 24 months,

2. 24–27 months,

3. 27–30 months,

4. Above 30 months.

The groups consisted of 27, 179, 64 and 49 individuals, respectively. The average age of the first parturition in the examined population was 26.6 months and it was comparable with the mean in the herds covered with milk recording in Poland, which was equal to ca. 27 months (Polish Federation of Cattle Breeders and Milk Producers, 2011).

The obtained data were statistically developed, using single-factor variance analysis. The program SPSS Statistics 17.0 was employed.

RESULTS AND DISCUSSION

The mean milk yields during 3 first lactations in relation to age of calving are given in Table 1.

Milk yield in the first lactation increased together with the age of calving, excluding the group of heifers which were calved at the age of 27–30 months. The cows which were calved at the latest time, i.e. above the 30th month of life, produced the highest quantities of milk (7649.6 kg).

TABLE 1. Milk yield in 3 first lactations (kg)

Age of calving (months)	Number of animals	LSM SE	Lactation 1	Lactation 2	Lactation 3
< 24	27	LSM	6339.70 ^{ABa}	8149.30	8530.20
		SE	70.68	90.85	95.10
24–27	179	LSM	7362.90 ^A	8199.80	8581.50
		SE	7.45	8.29	8.68
27–30	64	LSM	7203.60 ^a	8037.90	8270.50
		SE	28.25	31.52	32.44
> 30	49	LSM	7649.60 ^B	8281.40	8347.20
		SE	43.87	47.49	47.87

Values of figures in the same columns, marked with the same letters, differ significantly; big letters^{AA} – $P \leq 0.01$; small letters^{aa} – $P \leq 0.05$.

The lowest one was produced by the primiparous cows whose age of calving was found below 24 months (6339.7 kg). The differences between the groups were statistically confirmed ($P \leq 0.01$; $P \leq 0.05$).

The increase of yield was also indicated by Lee (1976), Brzozowski et al. (1985), Hibner et al. (1993), Pirlo et al. (2000) and Nilforooshan and Edriss (2004) and Sawa (1998).

The considerably lower productivity of primiparas, calved below 2 years of life, was indicated by Litwińczuk and Borkowska (1987), Gnyp and Litwińczuk (1997) and Czerniawska-Piątkowska et al. (2005). The results, as being presented in Table 2, differ from the results of the studies of Dobkowski (1965) and Wójcik (2001) who stated that after calving at the age of 33–34 months, the lowering of yield had place. The studies of Guliński et al. (2003) showed that calving after 26th month of life caused decline of production. In the opinion of Fisher et al. (1983) the age of calving did not affect significantly the level of production of primiparous cows.

In the second lactation, the differences in yield were already not as high as during the first one and they were statistically insignificant (Tab. 1). The earlier calved cows compensate the losses caused by earlier parturition and the yield becomes equalized.

A small differentiation in respect of the yield in the second lactation was also indicated by Swanson (1961), Brzuski et al. (1988) and Sawa (1998) who indicates, however, that the higher production level was obtained by the cows which were calved between 25 and 27 month of life. The results of own studies are different than those ones obtained by

Litwińczuk and Borkowska (1987), Gnyp and Litwińczuk (1997) and also, Piech and Tarkowski (2003) who stated that the heifers calved between the 27th and 30th month of life obtained the highest performance. The examined population includes high-yielding dairy cows and it may be a reason for different results. The experiment conducted by Wójcik (2001) indicates a constant increase of yield together with the age.

The analysis of milk yield in the third lactation (Tab. 1) revealed that the greatest quantities of milk were produced by the cows from 2 first categories of the calving age. The differences between the groups were not statistically confirmed.

The obtained results were similar to those ones obtained by Swanson (1961) and Sawa (1998) who stated that the cows in the third lactation, as being calved earlier for the first time, reached the higher yield. Wójcik (2001) as well as Piech and Tarkowski (2003) do not report on the meaningful effect of the age of calving on the yield after the third parturition. In the studies of the recent authors, however, the cows calved above the 30th month of life had a significantly lower yield as compared to the remaining ones. Different tendencies were obtained by Czerniawska-Piątkowska et al. (2005). Their experiment showed that the highest yield was found in the animals calved at the latest time.

The data concerning the fat and protein content in the milk of the examined cows are given in Table 2.

Fat content in the milk of cows in the first lactation was decreased together with the increase of the age of the first calving but differences between the groups were insignificant.

TABLE 2. Fat and protein content in milk of the cows in 3 first lactations

Age of calving	Number of animals	LSM	Lactation 1	Lactation 2	Lactation 3
		SE			
Fat					
< 24	27	LSM	4.34	4.25	4.40
		SE	0.048	0.047	0.049
24–27	179	LSM	4.31	4.36	4.43
		SE	0.005	0.005	0.005
27–30	64	LSM	4.22	4.27	4.32
		SE	0.017	0.017	0.017
> 30	49	LSM	4.21	4.30	4.40
		SE	0.024	0.025	0.025
Protein					
< 24	27	LSM	3.35	3.38	3.3
		SE	0.037	0.038	0.037
24–27	179	LSM	3.37	3.41 ^a	3.38 ^a
		SE	0.003	0.003	0.003
27–30	64	LSM	3.33	3.40 ^b	3.37 ^b
		SE	0.013	0.013	0.013
> 30	49	LSM	3.32	3.31 ^{ab}	3.31 ^{ab}
		SE	0.019	0.019	0.019

Values of figures in the same columns, marked with the same letters, differ significantly – $P \leq 0.05$.

Similar results were obtained by Piech and Tarkowski (2003) and Petraškiene et al. (2007). The analysis of the results, as being presented by the authors, indicates the decline of fat content together with the prolongation of the non-productive period. Completely different results were obtained by Swanson (1961), Gnyp and Litwińczuk (1997), Pirlo et al. (2000), Wójcik (2001) and Nilforooshan and Edriss (2004). In the opinion of the mentioned researchers, fat content in primiparas was increasing together with the age of the first delivery.

During the second lactation, the highest fat content was obtained by the cows which were calved at the age of 24–27 months (4.36%). The youngest animals had the lowest quantities of the discussed component in their milk; it was

equal to 4.25%. Any significant differences between the means in the particular groups were not found.

When analyzing the percentage fat content in milk in the third lactation, we may state that any significant differences between the particular age groups were not found, similarly as in two first lactations. Except for the third age group of calving (27–30 months) where the lowest fat content was found, the milk of cows from the remaining groups contained practically the same quantities of fat.

The similar results were also reported by other authors. Sawa (1998), Wójcik (2001) and Czerniawska-Piątkowska et al. (2005) did not observe a high differentiation between the means in the age groups.

Protein content in the milk of primiparous cows was inconsiderably decreasing together with the age. The highest content was found in the group of 24–27 months: 3.37%. A similar tendency was observed also by Petraškiene et al. (2007). The mentioned authors stated that the prolongation of the non-productive period caused a decrease of the content of protein in milk. Similar results were obtained by Pirlo et al. (2000). A reverse situation was found in the studies of Piech and Tarkowski (2003) – the primiparas, who commenced milk performance at the earliest time, reached the lowest protein content and those calved at the latest period – reached the highest one.

From the data presented in Table 2, it may be concluded that the age of calving affects significantly protein content in the milk of cows after the second calving. The highest (3.41%) value of the discussed parameter was found in the group of 24–27 months and the lowest one (3.31%) – in the group calved at the age of more than 30 months. The differences between the groups were statistically confirmed ($P \leq 0.05$).

The results obtained in the third lactation are similar as those in the second one ($P \leq 0.05$). The highest protein level was stated in the groups of 24–27 and 27–30 months. They amounted to 3.38% and 3.37%, respectively. The lower yield was found in the oldest and the youngest group (3.31% and 3.3%, respectively). Completely different results were demonstrated by Wójcik (2001) who stated that the age of calving did not affect significantly the protein content in the third lactation.

Table 3 shows the sum of kilograms of milk obtained by cows in three full

lactations in the particular age categories of the first calving. The lowest milk yield was observed in the animals which were calved at the earliest time, i.e. before completing the second year of life (25 641 kg). The cows which had their first parturition between the 24th and 27th month of life had the best results in respect of the examined parameter.

Their milk production was equal to 27 277 kg. In the successive age categories, milk yield amounted to 26 207 kg and 27 067 kg, respectively. Any statistically significant differences between the groups were not, however, found.

The similar results were obtained by Sawa (1998). In the opinion of the author, the greatest life performance was recorded for the cows which were calved between the 25th and 27th month of life. The animals, which were calved earlier or later, reached lower milk yield. The results of the experiment of the author (in contrary to the results given in Table 3) indicate that the cows, being calved above the 30th month of life reach the lowest sum of the yield. They produce also significantly lower quantities of milk as compared to the group at the age of 25–27 months. The results, presented by Piech and Tarkowski (2003) differ from the results of own studies. The highest productivity was revealed by the cows which were calved before the 25th month of life and the lowest one – above the 30th month. The lowest yield of the animals calved after completion of the 30th month of life was also indicated by Gnyp and Litwińczuk (1997). The best values for the discussed parameter (similarly as in Table 3) were obtained by the cows, whose parturition occurred between the 24th and 27th month. Somewhat

TABLE 3. Sum of the milk yield from three full lactations (kg)

Age of calving	Number of animals	LSM SE	Sum of kg of milk	Sum of kg of fat	Sum of kg of protein
m < 24	27	LSM	25.641	1.098	866
		SE	285.850	12.241	9.654
24–27	179	LSM	27.277	1.192 ^a	934
		SE	27.582	1.205	0.944
27–30	64	LSM	26.207	1.120 ^a	893
		SE	102.781	4.393	3.502
> 30	49	LSM	27.067	1.165	905
		SE	155.221	6.681	5.190

Values of figures in the same columns, marked with the same letters, differ significantly – $P \leq 0.05$.

different results were presented by Nilforooshan and Edriss (2004) and by Petraškiene et al. (2007). The mentioned researchers report that the prolongation of rearing the heifers leads to lowering of life milk performance.

From the data contained in Table 3, it is followed that the greatest quantities of fat during 3 lactations were produced by the cows which were calved at the age of 24–27 months (1192 kg). The weakest results in this respect were obtained by the cows which were calved at the youngest age – 1098 kg but the differences between the groups were statistically insignificant. On the other hand, the significance of differences between the means in the groups 2 and 3 was revealed ($P \leq 0.05$). The oldest age category obtained inconsiderably less fat than in case of the cows calved between the 24th and 27th month.

The maximum fat production by the cows calved at the age of 24–27 months was also indicated by Gnyp and Litwińczuk (1997). The mentioned authors stated that the worst results in relation to this trait were obtained in case of the cows the parturition of which was delayed above 30th month of life. Dickerson

and Chapman represented another opinion (1940); they stated that the sum of fat yield was increasing gradually with the increase of the age of calving. The contrary results were obtained by Petraškiene et al. (2007) who showed that the prolongation of the period from birth to calving caused a decline of life fat production. The similar results are presented in the paper of Meyer et al. (2005).

The results, contained in Table 3, illustrate how the age of calving affected the quantity of the obtained protein. Again, the highest performance was obtained by the cows which were calved the first time at the age of 24–27 months (934 kg) and the lowest amount was found in case of the cows calved at the earliest time (866 kg). The differences were not, however, statistically confirmed.

Milk fat and protein yield, as being calculated per one day of life, is a significant factor from the economical point of view (Tab. 4).

The results presented in Table 3 indicate that the cows calved until 27 months produced the greatest quantities of milk as calculated per one day of life (13.56 and 13.61 kg).

Similar results were also obtained by Gnyp and Litwińczuk (1997). They stated that the cows in the groups below 24 and 24–27 months had identical production and the means for these groups were significantly higher as compared to later-calved cows. Similar results were presented by Gnyp et al. (2006). They indicated that the cows, which were calved before 2 years of life, produced inconsiderably greater quantities of milk. The mentioned authors report also that production of the animals which had their parturition before the 27th month of life was significantly higher as compared to the later-calved ones. Also, Lin et al.

(1988) stated that earlier calving caused increase of production as calculated per one day of life.

The results, contained in Table 4 indicate that production of fat as well as of protein was found on the highest level in cows which were calved the first time before the 27th month of life. The differences between the groups were statistically significant ($P \leq 0.01$; $P \leq 0.05$).

In Table 5, the mean inter-calving period for the cows, covered with the studies, is given.

The results, contained in Table 5 indicate that in all groups the inter-calving period was higher than the optimal one.

TABLE 4. Cow performance as calculated per one day of life (kg/day)

Age of calving	Number of animals	LSM SE	Milk yield	Fat yield	Protein yield
< 24	27	LSM	13.560 ^a	0.580 ^a	0.460 ^a
		SE	0.151	0.006	0.005
24–27	179	LSM	13.610 ^{B,C}	0.590 ^{B,C}	0.470 ^{B,C}
		SE	0.014	0.001	0.000
27–30	64	LSM	12.770 ^C	0.550 ^C	0.440 ^C
		SE	0.050	0.002	0.002
> 30	49	LSM	12.480 ^{Ba}	0.540 ^{Ba}	0.420 ^{Ba}
		SE	0.072	0.003	0.002

Values in the same columns, marked with the same letters differ significantly – big letters ^{AA} $P \leq 0.01$; small letters ^{aa} $P \leq 0.05$.

TABLE 5. The mean inter-calving period (days)

Age of calving	Number of animals	LSM SE	Inter-calving period
< 24	27	LSM	429
		SE	4.783
24–27	179	LSM	436 ^a
		SE	0.441
27–30	64	LSM	418 ^a
		SE	1.639
> 30	49	LSM	422
		SE	2.420

Values in the same columns, marked with the same letters differ significantly $P \leq 0.05$.

The best (the shortest) period between the parturition was found for the cows which were the first time calved between the 27th and 30th month of life (418 days). On the other hand, the weakest results were obtained for the cows which were the best in respect of production (24–27 months) – 436 days. The differences between the groups were statistically significant ($P \leq 0.05$). In relation to the mentioned parameter, the youngest cows obtained considerably higher value as compared to the oldest animals (429 and 422 days, respectively).

Dickerson and Chapman (1940) stated that the cows being calved before the 25th month of life had the highest value of inter-calving period. The best results in this respect were demonstrated by the cows which were calved between the 26th and 30th month of life. Different results were obtained by Gnyp and Litwińczuk (1997). In the opinion of the mentioned authors, the group calved at the age of 24–27 months was the best one and the group which was calved at the age of 27–30 months was the weakest one. The studies of Szulc and Radzik (1977) showed that the inter-calving period was maintained on the constant level (excluding the cows which were calved before the 29th month of life). A considerable increase of the length of the examined parameter together with the delay of the first parturition was indicated in the studies of Hibner (1982). The mentioned author states that the younger animals have the better fertility. The differences between the results obtained in own studies and those ones, presented by other authors may result from the fact that the exami-

ned population consists of high-yielding dairy cows.

CONCLUSIONS

The age of the first calving affected significantly the milk yield only during the first lactation.

The greatest quantities of milk were produced by the cows which were calved at the latest period, i.e. above the 30th month of life. During the successive lactations, any significant differences in productivity of different age groups were not found and the cows calved as younger animals compensated their losses connected with the younger age at parturition and produced a similar and even higher milk quantity.

The highest total milk yield for the first 3 lactations was obtained by the cows, calved for the first time at the age of 24–27 months but except for fat yield; the differences between the mentioned group and the remaining ones were insignificant. This fact indicates the justness of the universally practiced recommendation for the breeders that the optimum age of calving for Polish HF heifers is the age of 24–27 months.

The percentage fat content did not differ significantly between the age groups of calving. On the other hand, the age of calving affected significantly protein content in milk of the cows in the second and third lactation. Significant differences in milk, fat and protein production as calculated per one day of life were found. The best results in this respect were stated for the cows calved until the 27th month of life. Delaying of the first parturition caused the decline of the discussed indicators.

REFERENCES

- BRZOZOWSKI P., MISZTALI I., RUDZIŃSKI F., 1985: Zależność pomiędzy sezonem i wiekiem pierwszego ocielenia a produktywnością pierwiastek w hodowli zarodowej. Zesz. Probl. Post. Nauk Rol., 300, 91–95.
- BRZUSKI P., SZAREK J., PARZELSKI S., 1988: Wpływ czynników środowiskowych na wydajność krów rasy ncb i mieszańców ncbxhf. Acta Agr. et Silv., Zootechnika, 27, 3–14.
- CZERNIAWSKA-PIĄTKOWSKA E., KAMIEŃECKI H., WÓJCIK J., RZEWUCKA E., SZEWCZUK M., PILARCZYK R., 2005: Wpływ wieku pierwszego ocielenia na produktywność krów czarno-białych z różnym udziałem genów rasy holsztyńsko-fryzyskiej. Folia Univ. Stetin. Zootechnika, 243 (47), 25–32.
- DICKERSON G.E., CHAPMAN A.B., 1940: Butterfat production, reproduction, growth and longevity in relation to age at first calving. J. Anim. Sci., 1940, 76–81.
- DOBKOWSKI A., 1965: Wydajność mleka w pierwszej laktacji w zależności od wieku i ciężaru przy pierwszym zacieleniu. Prz. Hod., 5, 21–22.
- FISHER L.J., HALL J.W., JONES S.E., 1982: Weight and age at calving and weight change related to first lactation milk yield. J. Dairy Sci., 66, 2167–2172.
- GARDNER R.W., SMITH L.W., PARK R.L., 1988: Feeding and management of dairy heifers for optimal lifetime productivity. J. Dairy Sci., 71, 996–999.
- GNYP J., JANKOWSKI P., PIECH M., 2006: Wpływ wieku krów w dniu pierwszego wycielenia na ich późniejszą wydajność mleczną, płodność i zdrowotność. LXXI Zjazd PTZ, Streszczenia, Z. 1, s. 1.
- GNYP J., LITWIŃCZUK Z., 1997: Efektywność użytkowania krów w zależności od wieku przy pierwszym wycieleniu. Med. Weter., 53 (7), 415–418.
- GULIŃSKI P., GIERSZ B., NIEDZIAŁEK G., MŁYNEK K., 2003: Kształtowanie się wieku pierwszego wycielenia i jego znaczenie dla użytkowości mlecznej pierwiastek utrzymywanych w gospodarstwach wschodniego Mazowsza w latach 1977–2000. Acta Sci. Pol., Zootechnika, 2 (2), 21–40.
- HAREL E., NORMAN H.D., WRIGHT J.R., 2006: Trends in Calving Ages and Calving Intervals for Dairy Cattle Breeds in the United States. J. Dairy Sci., 89, 365–370.
- HEINRICH A.J., 1993: Raising dairy replacement to meet the need of the 21st century. J. Dairy Sci., 76, 3179–3187.
- HIBNER A., 1982: Zależność między wiekiem przy pierwszym wycieleniu a długością życia i użytkowania oraz płodnością krów. Prz. Hod. 7, 44–46.
- HIBNER A., ZIEMIŃSKI R., SAKOWSKI T., 1993: Próba określenia optymalnego terminu rozpoczęcia użytkowania mlecznego krów – mieszańców F₁ (cbxhf). Pr. Mater. Zootech., 44, 71–75.
- LEE A.J., 1976: Relationship between milk yield and age at calving in first lactation. J. Dairy Sci., 59, 1794–1801.
- LIN C.Y., MCALLISTER A.J., BATRA T.R., LEE A.J., 1988: Effects of early and late breeding of heifers on multiple lactation performance of dairy cows. J. Dairy Sci., 71, 2735–2743.
- LITWIŃCZUK Z., BORKOWSKA D., 1987: Wpływ wieku pierwszego wycielenia na produktywność, płodność oraz długość użytkowania krów. Zesz. Probl. Post. Nauk Rol., 332, 242–245.
- MEYER M.J., EVERETT R.W., VanAmburgh M.E., 2000: Reduced age at first calving: Effects on lifetime production, longevity and profitability. http://ag.arizona.edu/extension/dairy/az_nm_newsletter/2005/july.pdf.
- MOURITS, M.C.M., DIJKHUIZEN A.A., HURINE R.B.M., GALLIGAN D.T., 1997: Technical and economic models to support heifer management decisions: basic concepts. J. Dairy Sci., 80, 1406–1415.
- NILFOROOSHAN M.A., EDRISS M.A., 2004: Effect of age at first calving on some productive and longevity traits in Iranian Holsteins of the Isfahan Province. J. Dairy Sci., 87, 2130–2135.
- PETRAŠKIENĖ R., GIRSKIENĖ B., PALECKAITIS M., 2007: Influence of age at first calving on production traits in lithuanian black-and-white cattle population. Veterinarija Ir Zootechnika, T. 40 (62), 67–72.
- PIECH M., TARKOWSKI J., 2003: Wpływ wieku pierwszego ocielenia na produktywność

- krów czarno białych z różnym udziałem genów rasy holsztyńsko-fryzyjskiej. Zesz Zesz. Nauk. Prz. Hod., 68 z. 1, 153–160.
- PIRLO G., MIGLIOR F., SPERONI M., 2000: Effect of age at first calving on production traits and on difference between milk yield returns and rearing costs in Italian Holsteins. J. Dairy Sci., 83, 603–608.
- PLATE-CHURCH A., 2006: Determining Optimal Age at First Calving. <http://genex.crinet.com/page638/DeterminingOptimalAgeAtFirstCalving>.
- POLSKA FEDERACJA HODOWCÓW BYDŁA I PRODUCENTÓW MLEKA, 2011: Wyniki oceny wartości użytkowej krów mlecznych. <http://www.pfhb.pl>.
- POWELL R.L., 1985: Trend of age at first calving. J. Dairy Sci., 68, 768–772.
- SAWA A., 1998: Genetyczne i środowiskowe uwarunkowania użyteczności krów w poszczególnych okresach życia. Rozprawy Akademii techniczno-Rolnicza w Bydgoszczy, nr 88, 31–35.
- SWANSON E.W., 1961: Milk production and growth of identical twin heifers calving for the first time at two and three years of age. J. Dairy Sci., 44, 2027–2034.
- SZULC T., RADZIK W., 1977: Wpływ wieku pierwszego wycielenia na wartość niektórych cech użytkowych krów. Prz. Hod., 19, 16–18.
- WILSON R., 2006: Age at first calving: The dollars and sense. <http://genex.crinet.com/page438/AgeAtFirstCalvingTheDollarsAndSense>.
- WÓJCIK P., 2001: Wpływ wieku pierwszego wycielenia na produktywność krów rasy czarno-białej. Roczn. Nauk. Zoot., T. 28, z. 1, 189–198.

Streszczenie: *Wpływ wieku pierwszego ocielenia na użyteczność mleczną i okres międzyocieleniowy krów rasy polskiej holsztyńsko-fryzyjskiej.* Celem pracy było określenie wpływu wieku pierwszego wycielenia na: użyteczność mleczną w 1., 2. i 3. laktacji; sumę wydajności mleka, tłuszczu i białka za trzy pełne laktacje oraz ilość tych składników w przeliczeniu na 1 dzień życia. Wiek pierwszego ocielenia istotnie wpływał na wydajność mleka tylko w pierwszej laktacji. Procentowa zawartość tłuszczu nie różniła się istotnie między grupami wieku wycielenia, natomiast badany czynnik wpływał istotnie na zawartość białka w mleku krów po drugim i trzecim wycieleniu. Otrzymane wyniki wskazują, że najkorzystniejsze, pod względem ekonomicznym, jest takie krycie jałówek, aby ocielenie nastąpiło do 27. miesiąca życia, gdyż od takich zwierząt uzyskuje się najwięcej mleka, tłuszczu i białka w przeliczeniu na jeden dzień życia.

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Comparison of body condition score (BCS) and muscularity level results of Charolaise cows

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Abstract: *Comparison of body conditions score (BCS) and muscularity level results of Charolaise cows.* The aim of the study was to calculate the correlation between body condition score (BCS) and musculature score of Charolaise cows. All the correlation coefficients of BCS measured in the particular body parts were high (from 0.861 to 0.898) and statistically significant ($P \leq 0.01$). All the above mentioned coefficients examined on the front of neck appearance were not significant in comparison to those examined on the other parts of body. The dependence between BCS in the particular parts of body and the final BCS result was proved ($P \leq 0.01$). The highest correlation coefficient ($r = 0.898$) was found for the final BCS examined in the ischiadic tubers. All of the obtained correlation coefficients between cow BCS and cow muscularity were high ($p \leq 0.01$). The highest correlation coefficient ($r = 0.501$) between BCS and the final musculature score was noticed for BCS measured at shoulder. All of the obtained correlation coefficients between the particular notes for cow musculature were high and statistically significant ($p \leq 0.01$). The highest correlation ($r = 0.978$) was observed between rump convexity and the final musculature grade.

Key words: beef cattle, BCS, musculature score.

INTRODUCTION

The most important factors determining beef cattle herd economy are the number

of good quality calves born each year and feeding costs of breeding herd. The number of calves born depends on fertility results influenced by calving course depending on many factors, among which BCS and muscularity score are ones of the most important. Cow BCS is caused mainly by health status, maintenance system including feeding quality etc. (Selk et al., 1986; Jasiorowski et al., 1996; Dickerson, 1978). There are not enough papers (especially by Polish authors) concerning influence of BCS and muscularity level in the beef herds.

The aim of presented study was to determine correlation coefficients between BCS and muscularity examination of Charolaise beef cattle breed. BCS scale is mainly used to estimate energetic reserves of cow organism. The level of muscularity development (muscles width and profiles) is used for commercial assessment of slaughter quality. Since both above mentioned investigations are done at the same body parts, it would be worth to know the correlations between them. Higher than usually obtained coefficients could indicate, that the overfed cows are unjustly classified as the best muscular ones, whereas in practice the

assessments in question are very similar, if not the same (Beverly, 1985; Dadati et al., 1985; McKirnan, 2000; Morris and Wilton, 1976; Selk et al., 1986; Whitman et al., 1975; Wiltbank, 1983).

MATERIAL AND METHODS

The investigations were carried out at 4 breeding farms housing purebred Charolaise cattle associated in Polish Association of Beef Cattle Breeders & Producers. The records for 155 purebred Charolaise cows were taken for statistical analysis.

Charolaise cows' Body Condition Score (BCS) was examined according to Richard's 9-point scale (Richards et al., 1986). Simultaneously muscularity grade was estimated according to Przysucha and Grodzki, 2004; Przysucha, 2009. BCS was examined at: back, tail base, ischiadic tubers, hip tubers, shoulder and neck front (dewlap). During muscularity grade assessment the following traits were considered: (1) shoulder convexity, (2) loins width, (3) thigh convexity, (4) thigh profile, (5) buttocks depth as well as (6) rump convexity. Each above mentioned feature was estimated from 1 to 5 points. Basing on that ranking muscularity grade of each body part was scored as: (1) very weak, (2) weak, (3) average, (4) good, (5) very good (Przysucha, 2009).

On the basis of individual BCS score measured at 6 (the most important) body parts and cow muscularity grade the following correlation coefficients were calculated:

- between BCS determined in different body parts;
- between BCS and muscularity grade of different body parts;

- between muscularity grade of Charolaise cows determined in different body parts.

RESULTS AND DISCUSSION

In Table 1 correlation coefficients (r) between BCS examined in the different body parts of Charolaise cows are presented. All correlation coefficients between total BCS points assessed at cow's: back, ischiadic tubers, hip tubers, tail base and shoulder were very high (from 0.861 to 0.898) and statistically significant ($p \leq 0.01$).

All correlation coefficients between BCS assessed at cow's dewlap and on the basis of other parts of the body were much lower, but statistically significant.

Significant ($p \leq 0.01$) dependence between BCS measured in particular body parts and the final BCS was noticed. The highest correlation coefficient between final BCS ($r = 0.898$) and the condition measured at ischiadic tubers was observed.

Table 2 shows correlation between BCS and muscularity grade of different body parts of Charolaise cows. All correlation coefficients between BCS assessed at cow's back, tail base, shoulder and ischiadic tubers and hip tubers and muscularity grade estimated basing on shoulder convexity, loin width, thigh convexity, thigh profile as well as rump convexity were high and statistically significant ($p \leq 0.01$). Cow BCS examined on base of dewlap had the lower and not statistically significant influence on cow muscularity score. The highest coefficient between the BCS and the final muscularity score was found for BCS examined at shoulder ($r = 0.501$). It should be stated,

TABLE 1. Correlation coefficients (r) between BCS determined in different body parts of Charolaise cows (n = 140)

		Cow BCS						
		Back	Tail base	Ischiadic tubers	Hip tubers	Shoulder	Dewlap	Total
Cow BCS	Back	1.000	0.794 AA	0.799 AA	0.783 AA	0.807 AA	0.312 AA	0.861 AA
	Tail base		1.000	0.868 AA	0.793 AA	0.816 AA	0.322 AA	0.892 AA
	Ischiadic tubers			1.000	0.843 AA	0.818 AA	0.344 AA	0.898 AA
	Hip tubers				1.000	0.769 AA	0.259 AA	0.869 AA
	Shoulder					1.000	0.377 AA	0.893 AA
	Dewlap						1.000	0.356 AA

AA – dependence significant at $p \leq 0.01$.

TABLE 2. Correlation coefficients (r) between BCS and muscularity grade of different body parts of Charolaise cows (n = 140)

		Cow muscularity grade								
		Shoulder convexity	Loins width	Total front	Thigh convexity	Thigh profile	Buttocks depth	Rump convexity	Total rear	Total
Cow BCS	Back	0.282 AA	0.279 AA	0.284 AA	0.375 AA	0.346 AA	0.327 AA	0.331 AA	0.362 AA	0.383 AA
	Tail base	0.347 AA	0.353 AA	0.349 AA	0.462 AA	0.449 AA	0.436 AA	0.435 AA	0.458 AA	0.470 AA
	Ischiadic tubers	0.274 AA	0.287 AA	0.281 AA	0.427 AA	0.401 AA	0.402 AA	0.414 AA	0.421 AA	0.433 AA
	Hip tubers	0.288 AA	0.306 AA	0.297 AA	0.364 AA	0.325 AA	0.339 AA	0.344 AA	0.350 AA	0.371 AA
	Shoulder	0.409 AA	0.404 AA	0.407 AA	0.493 AA	0.449 AA	0.447 AA	0.451 AA	0.477 AA	0.501 AA
	Dewlap	0.184 (*)	0.164	0.179 (*)	0.101	0.074	0.040	0.056	0.075	0.094
	Total	0.377 AA	0.377 AA	0.379 AA	0.477 AA	0.453 AA	0.442 AA	0.448 AA	0.467 AA	0.486 AA

AA – dependence significant at $p \leq 0.01$.

that the dependence between final BCS scoring and the total muscularity assessment was slightly lower ($r = 0.486$).

Correlation between muscularity score measured at the particular body

parts are shown in Table 3. All of the coefficients were statistically significant ($p \leq 0.01$), but the highest influence on the final muscularity score had rump convexity ($r = 0.978$) as well as the total

TABLE 3. Correlation coefficients (r) between muscularity grade of Charolaise cows determined in different body parts (n = 140)

		Cow muscularity grade								
		Shoulder convexity	Loins width	Total front	Thigh convexity	Thigh profile	Buttocks depth	Rump convexity	Total rear	Total
Cow muscularity grade	Shoulder convexity	1.000	0.977 AA	0.997 AA	0.622 AA	0.638 AA	0.606 AA	0.609 AA	0.635 AA	0.620 AA
	Loins width		1.000	0.986 AA	0.649 AA	0.638 AA	0.634 AA	0.637 AA	0.649 AA	0.635 AA
	Total front			1.000	0.632 AA	0.642 AA	0.616 AA	0.619 AA	0.642 AA	0.642 AA
	Thigh convexity				1.000	0.926 AA	0.919 AA	0.928 AA	0.968 AA	0.940 AA
	Thigh profile					1.000	0.958 AA	0.949 AA	0.981 AA	0.965 AA
	Buttocks depth						1.000	0.990 AA	0.979 AA	0.978 AA
	Rump convexity							1.000	0.978 AA	0.978 AA
	Total rear								1.000	0.970 AA

AA – dependence significant at $p \leq 0.01$.

note for total rear (thigh convexity, thigh profile, buttocks depth, rump convexity) ($r > 0.970$).

CONCLUSIONS

- All the correlation coefficients of BCS measured in the particular body parts were high (from 0.861 to 0.898) and statistically significant ($P \leq 0.01$).
- All the above mentioned coefficients examined on the front of neck (dewlap) appearance were much lower and not significant.
- The dependence between BCS in the particular parts of body and the final BCS result was proved ($P \leq 0.01$).
- The highest correlation coefficient ($r = 0.898$) was found for the final BCS examined in the ischiadic tubers.

- All of the obtained correlation coefficients between cow BCS and cow muscularity were high ($p \leq 0.01$).
- The highest correlation ($r = 0.501$) was observed between shoulder and the final musculature grade.

REFERENCES

- BEVERLY J.R., 1985: Reproduction In beef cattle as related to nutrition and body condition. Kentucky roundup of reproductive efficiency in beef cattle, p. 1–2.
- DADATI F.B., KENNEDY B.W., BURNSIDE E.B., 1985: Relationships between conformation and reproduction in Holstein cows: type and calving performance. *J. Dairy Sci.* 68, 2639–2646.
- DICKERSON G.E., 1978. Animal size and efficiency: basic concepts. *Anim. Prod.* 27, 367–379.
- JASIOROWSKI H., KIJAK Z., POCZYNAJŁO S., WAJDA S., 1996: Program rozwoju

- hodowli bydła mięsnego w Polsce, Fundacja „Rozwój SGGW”, 5–67.
- McKIRNAN B., 2000: Frame scoring of beef cattle. Agfact A2.3.4. Department of Primary Industries, State of New South Wales, Australia.
- MORRIS C.A., WILTON J.W., 1976: The influence of body size on the biological efficiency of cows: A review. *Can. Anim. Sci.* 56: 613–647.
- PRZYSUCHA T., 2009: Osobnicze uwarunkowania przebiegu oścień krów oraz umięśnienia i żywotności cieląt pochodzących po buhajach rasy piemontese użytkowanych w Polsce i we Włoszech. Rozprawa habilitacyjna. Wyd. SGGW, 1–136.
- PRZYSUCHA T., GRODZKI H., 2004: Przydatność różnych ras bydła mięsnego do chowu w czystości rasy i krzyżowania towarowego. *Mat. Konf. „Hodowla i produkcja bydła mięsnego”*. Międzynarodowe Targi Poznańskie POLAGRA FARM, Poznań, 63–73.
- RICHARDS M.W., SPITZER J.C., WARNER M.B., 1986: Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. *J. Anim. Sci.* 62: 300–306.
- SELK G.E., WETTEMANN R.P., LUSBY K.S., RASBY R.J., 1986: The importance of body condition at calving on reproduction in beef cows. *OSO Agric. Exp. Sta. Publ.* 118: 3163–3169.
- WHITMAN R.W., REMMENG A.E., WILTBANK J.N., 1975: Weight change, condition and beef cow reproduction. *J. Anim. Sci.* 41: 387.
- WILTBANK J.N., 1983: Maintenance of high level of reproductive performance in beef cow herd. *Vet. Clin. N. Am. Large Anim. Proc.* 5: 41–57.
- Streszczenie:** Porównanie wyników oceny kondycji (BCS) i oceny stopnia umięśnienia krów rasy Charolaise. Celem pracy było określenie korelacji między wynikami oceny kondycji, a wynikami oceny stopnia umięśnienia krów mięsnej rasy charolaise. Wszystkie współczynniki korelacji między ocenami kondycji mierzonej w różnych partiach ciała były wysokie (od 0,861 do 0,898) i statystycznie istotne ($p \leq 0,01$). Wszystkie współczynniki korelacji między oceną kondycji określaną wyglądem podgardla, a określaną na podstawie innych partii ciała były znacznie niższe, ale istotne. Stwierdzono istotną ($p \leq 0,01$) zależność między ocenami kondycji w poszczególnych partiach ciała (poza podgardlem), a ostateczną (zbiorną) oceną kondycji krowy. Najwyższy współczynnik korelacji z oceną ostateczną kondycji ($r = 0,898$) odnotowano dla kondycji ocenianej w guzach kulszowych. Wszystkie współczynniki korelacji między kondycją krowy ocenianą w różnych partiach ciała a stopniem umięśnienia krowy ocenianym na podstawie wypukłości barku, szerokości łędźwi, wypukłości i profilu uda oraz wypukłości zadu były wysokie i statystycznie istotne ($p \leq 0,01$). Najwyższy współczynnik korelacji między oceną kondycji, a ostateczną oceną stopnia umięśnienia odnotowano dla kondycji ocenianej na barku zwierzęcia ($r = 0,501$). Wszystkie współczynniki korelacji między szczegółowymi ocenami stopnia umięśnienia krowy były wysokie i statystycznie istotne ($p \leq 0,01$). Najwyższą korelację z ostateczną oceną stopnia umięśnienia miała ocena wypukłości zadu ($r = 0,978$).
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Comparison of body condition score (BCS) and muscularity level results of Limousine cows

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Abstract: *Comparison of body conditions score (BCS) and muscularity level results of Limousine cows.* The aim of the study was to calculate the correlation between body condition score (BCS) and musculature score of Limousine cows. All the correlation coefficients of BCS measured in the particular body parts were high (from 0.742 to 0.884) and statistically significant ($P \leq 0.01$). All the above mentioned coefficients examined on the front of neck appearance were not significant in comparison to those examined on the other parts of body. The dependence between BCS in the particular parts of body (except front of the neck) and the final BCS result was proved ($P \leq 0.01$). The highest correlation coefficient ($r = 0.920$) was found for the final BCS examined in the ischiadic tubers. All of the obtained correlation coefficients between cow BCS and cow muscularity were high ($p \leq 0.01$). The highest correlation coefficient ($r = 0.631$) between BCS and the final musculature score was noticed for BCS measured at the ischiadic tubers. All of the obtained correlation coefficients between the particular notes for cow musculature were high and statistically significant ($p \leq 0.01$). The highest correlation ($r = 0.967$) was observed between rump profile and the final musculature grade.

Key words: beef cattle, BCS, musculature score.

INTRODUCTION

The most important factors determining beef cattle herd economy are the number

of good quality calves born each year and feeding costs of breeding herd. The number of calves born depends on fertility results influenced by calving course depending on many factors, among which BCS and muscularity score are ones of the most important. Cow BCS is caused mainly by health status, maintenance system including feeding quality etc. (Selk et al., 1986; Jasiorowski et al., 1996; Dickerson, 1978). There are not enough papers (especially by Polish authors) concerning influence of BCS and muscularity level in the beef herds.

The aim of presented study was to determine correlation coefficients between BCS and muscularity examination of Limousine beef cattle breed. BCS scale is mainly used to estimate energetic reserves of cow organism. The level of muscularity development (muscles width and profiles) is used for commercial assessment of slaughter quality. Since both above mentioned investigations are done at the same body parts, it would be worth to know the correlations between them. Higher than usually obtained coefficients could indicate, that the overfed cows are unjustly classified as the best muscular ones, whereas in practice the

assessments in question are very similar, if not the same (Beverly, 1985; Dadati et al., 1985; McKirnan, 2000; Morris and Wilton, 1976; Selk et al., 1986; Whitman et al., 1975; Wiltbank, 1983).

MATERIAL AND METHODS

The investigations were carried out at 4 breeding farms housing purebred Limousine cattle associated in Polish Association of Beef Cattle Breeders & Producers. The records for 635 purebred Limousine cows were taken for statistical analysis.

Limousine cows' Body Condition Score (BCS) was examined according to Richard's 9-point scale (Richards et al., 1986). Simultaneously muscularity grade was estimated according to Przysucha and Grodzki, 2004; Przysucha, 2009. BCS was examined at: back, tail base, ischiadic tubers, hip tubers, shoulder and neck front (dewlap). During muscularity grade assessment the following traits were considered: (1) shoulder convexity,

(2) loins width, (3) thigh convexity, (4) thigh profile, (5) buttocks depth as well as (6) rump convexity. Each above mentioned feature was estimated from 1 to 5 points. Basing on that ranking muscularity grade of each body part was scored as: (1) very weak, (2) weak, (3) average, (4) good, (5) very good (Przysucha, 2009).

On the basis of individual BCS score measured at 6 (the most important) body parts and cow muscularity grade the following correlation coefficients were calculated:

- between BCS determined in different body parts;
- between BCS and muscularity grade of different body parts;
- between muscularity grade of Limousine cows determined in different body parts.

RESULTS AND DISCUSSION

In Table 1 correlation coefficients (r) between BCS examined in the different body parts of Limousine cows are

TABLE 1. Correlation coefficients (r) between BCS determined in different body parts of Limousine cows ($n = 635$)

		Cow BCS						
		Back	Tail base	Ischiadic tubers	Hip tubers	Shoulder	Dewlap	Total
Cow BCS	Back		0.771 AA	0.811 AA	0.788 AA	0.777 AA	-0.061	0.856 AA
	Tail base			0.884 AA	0.829 AA	0.742 AA	0.005	0.876 AA
	Ischiadic tubers				0.879 AA	0.783 AA	0.023	0.920 AA
	Hip tubers					0.759 AA	-0.006	0.890 AA
	Shoulder						-0.006	0.842 AA
	Dewlap							-0.029

AA – dependence significant at $p \leq 0.01$.

presented. All correlation coefficients between BCS assessed at cow's back, ischiadic tubers, hip tubers, tail base and shoulder were very high (from 0.742 to 0.884) and statistically significant ($p \leq 0.01$).

All correlation coefficients between BCS assessed at cow's dewlap and on the basis of other parts of the body were much lower and statistically not significant.

Significant ($p \leq 0.01$) dependence between BCS measured in particular body parts and the final BCS was noticed. The highest correlation coefficient between final BCS ($r = 0.920$) and the condition measured at ischiadic tubers was observed.

Table 2 shows correlation between BCS and muscularity grade of different body parts of Limousine cows. All correlation coefficients between BCS

assessed at cow's back, tail base, shoulder and ischiadic tubers and hip tubers and muscularity grade estimated basing on shoulder convexity, loin width, thigh convexity, thigh profile as well as rump convexity were high and statistically significant ($p \leq 0.01$). Cow BCS examined on base of dewlap had the lower and not statistically significant influence on cow muscularity score. The highest coefficient between the BCS and the final muscularity score was found for BCS examined at ischiadic tubers ($r = 0.631$). It should be stated, that the dependence between BCS and the total muscularity assessment was even higher ($r = 0.648$).

Correlation between muscularity score measured at the particular body parts are shown in Table 3. All of the coefficients were statistically significant ($p \leq 0.01$), but the highest influence on the final muscularity score had thigh

TABLE 2. Correlation coefficients (r) between BCS and muscularity grade of different body parts of Limousine cows (n = 635)

		Cow muscularity grade								
		Shoulder convexity	Loins width	Total front	Thigh convexity	Thigh profile	Buttocks depth	Rump convexity	Total rear	Total
Cow BCS	Back	0.458 AA	0.455 AA	0.460 AA	0.559 AA	0.559 AA	0.554 AA	0.556 AA	0.566 AA	0.578 AA
	Tail base	0.443 AA	0.452 AA	0.448 AA	0.610 AA	0.597 AA	0.589 AA	0.596 AA	0.609 AA	0.614 AA
	Ischiadic tubers	0.452 AA	0.458 AA	0.457 AA	0.625 AA	0.611 AA	0.614 AA	0.621 AA	0.627 AA	0.631 AA
	Hip tubers	0.443 AA	0.445 AA	0.446 AA	0.581 AA	0.584 AA	0.570 AA	0.571 AA	0.590 AA	0.592 AA
	Shoulder	0.481 AA	0.481 AA	0.484 AA	0.561 AA	0.561 AA	0.549 AA	0.552 AA	0.568 AA	0.586 AA
	Dewlap	0.104 AA	-0.069	-0.097	0.04	-0.027	-0.007	0.009	-0.002	-0.030
	Total	0.488 AA	0.486 AA	0.491 AA	0.630 AA	0.625 AA	0.618 AA	0.621 AA	0.637 AA	0.648 AA

AA – dependence significant at $p \leq 0.01$.

TABLE 3. Correlation coefficients (r) between muscularity grade of Limousine cows determined in different body parts (n = 635)

		Cow muscularity grade								
		Shoulder convexity	Loins width	Total front	Thigh convexity	Thigh profile	Buttocks depth	Rump convexity	Total rear	Total
Cow muscularity grade	Shoulder convexity		0.962 AA	0.998 AA	0.606 AA	0.618 AA	0.597 AA	0.578 AA	0.620 AA	0.736 AA
	Loins width			0.976 AA	0.624 AA	0.620 AA	0.603 AA	0.598 AA	0.626 AA	0.736 AA
	Total front				0.614 AA	0.623 AA	0.602 AA	0.586 AA	0.625 AA	0.741 AA
	Thigh convexity					0.944 AA	0.949 AA	0.954 AA	0.977 AA	0.959 AA
	Thigh profile						0.972 AA	0.967 AA	0.987 AA	0.967 AA
	Buttocks depth							0.984 AA	0.982 AA	0.961 AA
	Rump convexity								0.981 AA	0.958 AA
	Total rear									0.979 AA

AA – dependence significant at $p \leq 0.01$.

profile ($r = 0.967$) as well as the total note for total rear (thigh convexity, thigh profile, buttocks depth, rump convexity) ($r = 0.979$).

CONCLUSIONS

- All the correlation coefficients of BCS measured in the particular body parts were high (from 0.742 to 0.884) and statistically significant ($P \leq 0.01$).
- All the above mentioned coefficients examined on the front of neck (dewlap) appearance were not significant.
- The dependence between BCS in the particular parts of body and the final BCS result was proved ($P \leq 0.01$).
- The highest correlation coefficient ($r = 0.920$) was found for the final BCS examined in the ischiadic tubers.

- All of the obtained correlation coefficients between cow BCS and cow muscularity were high ($p \leq 0.01$).
- The highest correlation ($r = 0.967$) was observed between thigh profile and the final musculature grade.

REFERENCES

- BEVERLY J.R., 1985: Reproduction In beef cattle as related to nutrition and body condition. Kentucky roundup of reproductive efficiency in beef cattle, p. 1–2.
- DADATI F.B., KENNEDY B.W., BURNSIDE E.B., 1985: Relationships between conformation and reproduction in Holstein cows: type and calving performance. J. Dairy Sci. 68, 2639–2646.
- DICKERSON G.E., 1978: Animal size and efficiency: basic concepts. Anim. Prod. 27, 367–379.
- JASIOROWSKI H., KIJAK Z., POCZYNAJ-ŁO S., WAJDA S., 1996: Program rozwoju

- hodowli bydła mięsnego w Polsce, Fundacja "Rozwój SGGW", 5–67.
- McKIRNAN B., 2000: Frame scoring of beef cattle. Agfact A2.3.4. Department of Primary Industries, State of New South Wales, Australia.
- MORRIS C.A., WILTON J.W., 1976: The influence of body size on the biological efficiency of cows: A review. *Can. Anim. Sci.* 56: 613–647
- PRZYSUCHA T., 2009: Osobnicze uwarunkowania przebiegu oścień krów oraz umięśnienia i żywotności cieląt pochodzących po buhajach rasy piemontese użytkowanych w Polsce i we Włoszech. Rozprawa habilitacyjna. Wyd. SGGW, 1–136.
- PRZYSUCHA T., GRODZKI H., 2004: Przydatność różnych ras bydła mięsnego do chowu w czystości rasy i krzyżowania towarowego. *Mat. Konf. „Hodowla i produkcja bydła mięsnego”*. Międzynarodowe Targi Poznańskie POLAGRA FARM, Poznań, 63–73.
- RICHARDS M.W., SPITZER J.C., WARNER M.B., 1986: Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. *J. Anim. Sci.* 62: 300–306.
- SELK G.E., WETTEMANN R.P., LUSBY K.S., RASBY R.J., 1986: The importance of body condition at calving on reproduction in beef cows. *OSO Agric. Exp. Sta. Publ.* 118: 3163–3169.
- WHITMAN R.W., REMMENG A.E.E., WILTBANK J.N., 1975: Weight change, condition and beef cow reproduction. *J. Anim. Sci.* 41: 387.
- WILTBANK J.N., 1983: Maintenance of high level of reproductive performance in beef cow herd. *Vet. Clin. N. Am. Large Anim. Proc.* 5: 41–57.
- Streszczenie:** Porównanie wyników oceny kondycji (BCS) i oceny stopnia umięśnienia krów rasy Limousin. Celem pracy było określenie korelacji między wynikami oceny kondycji a wynikami oceny stopnia umięśnienia krów mięsnej rasy limousin. Wszystkie współczynniki korelacji między ocenami kondycji mierzonej w różnych partiach ciała były wysokie (od 0,742 do 0,884) i statystycznie istotne ($p \leq 0,01$). Wszystkie współczynniki korelacji między oceną kondycji określanej wyglądem podgardla, a określanej na podstawie innych partii ciała, były bliskie zeru i nieistotne. Stwierdzono istotną ($p \leq 0,01$) zależność między ocenami kondycji w poszczególnych partiach ciała (poza podgardlem) a ostateczną (zbiorną) oceną kondycji krowy. Najwyższy współczynnik korelacji z oceną ostateczną kondycji ($r = 0,920$) odnotowano dla kondycji ocenianej w guzach kulszowych. Wszystkie współczynniki korelacji między kondycją krowy ocenianą w różnych partiach ciała a stopniem umięśnienia krowy ocenianym na podstawie wypukłości barku, szerokości łędźwi, wypukłości i profilu uda oraz wypukłości zadu były wysokie i statystycznie istotne ($p \leq 0,01$). Najwyższy współczynnik korelacji między oceną kondycji a ostateczną oceną stopnia umięśnienia odnotowano dla kondycji ocenianej w guzach kulszowych ($r = 0,631$). Wszystkie współczynniki korelacji między szczegółowymi ocenami stopnia umięśnienia krowy były wysokie i statystycznie istotne ($p \leq 0,01$). Najwyższą korelację z ostateczną oceną stopnia umięśnienia miała ocena profilu uda ($r = 0,967$).
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Comparison of body condition score (BCS) and muscularity level results of Hereford cows

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Abstract: *Comparison of body condition score (BCS) and muscularity level results of Hereford cows.* The aim of the study was to calculate the correlation between body condition score (BCS) and musculature score of Hereford cows. All the correlation coefficients of BCS measured in the particular body parts were high (from 0.825 to 0.905) and statistically significant ($P \leq 0.01$). All the above mentioned coefficients examined on the front of neck (dewlap) appearance were not significant in comparison to those examined on the other parts of body. The dependence between BCS in the particular parts of body and the final BCS result was proved ($P \leq 0.01$). The highest correlation coefficient ($r = 0.926$) was found for the final BCS examined in the ischiadic tubers. All of the obtained correlation coefficients between cow BCS and cow muscularity were high ($p \leq 0.01$). The highest correlation coefficient ($r = 0.687$) between BCS and the final musculature score was noticed for BCS measured at the ischiadic tubers. All of the obtained correlation coefficients between the particular notes for cow musculature were high and statistically significant ($p \leq 0.01$). The highest correlation ($r = 0.975$) was observed between rump convexity and the final musculature grade.

Key words: beef cattle, BCS, musculature score.

INTRODUCTION

Hereford breed is the most popular beef cattle breed in the world. High fertility,

extraordinary maternal ability as well as particularly low demand regarding environment and fodder quality cause that this breed is housed successfully all over the world. In the commercial crossing Hereford cows, because of their average body weight, excellent fertility, relatively good milking performance and calving ease and docility are often used as a maternal breed. In the Polish beef cattle recording scheme Herefords are bred for beef herd development (heifers for herd replacement) and to produce pedigree bulls for mass commercial crossing with Black & White cows. Breeding goal in Herefords comes down to retain body weight of mature cows on the level of 550–600 kg, and 900–1000 kg for bulls as well as calving ease and high level of maternal traits (Dickerson, 1978; Fitzhugh, 1978; Jasiorowski et al., 1996).

The aim of presented study was to determine correlation coefficients between BCS and muscularity examination of Hereford beef cattle breed. BCS scale is mainly used to estimate energetic reserves of cow organism. The level of muscularity development (muscles width and profiles) is used for commercial

assessment of slaughter quality. Since both above mentioned investigations are done at the same body parts, it would be worth to know the correlations between them. Higher than usually obtained coefficients could indicate, that the overfed cows are unjustly classified as the best muscular ones, whereas in practice the assessments in question are very similar, if not the same (Beverly, 1985; Dadati et al., 1985; McKirnan, 2000; Morris and Wilton, 1976; 1977; Selk et al., 1986; Whitman et al., 1975; Wiltbank, 1983).

MATERIAL AND METHODS

The investigations were carried out at 3 breeding farms housing purebred Hereford cattle associated in Polish Association of Beef Cattle Breeders & Producers. The records for 635 purebred Hereford cows were taken for statistical analysis. Hereford cows' Body Condition Score (BCS) was examined according to Richard's 9-point scale (Richards et al., 1986). Simultaneously muscularity grade was estimated according to Przysucha, 1998; Przysucha et al., 2002; Przysucha, 2009. BCS was examined at: back, tail base, ischiadic tubers, hip tubers, shoulder and neck front (dewlap). During muscularity grade assessment the following traits were considered: (1) shoulder convexity, (2) loins width, (3) thigh convexity, (4) thigh profile, (5) buttocks depth as well as (6) rump convexity. Each above mentioned feature was estimated from 1 to 5 points. Basing on that ranking muscularity grade of each body part was scored as: (1) very weak, (2) weak, (3) average, (4) good, (5) very good (Przysucha, 2009).

On the basis of individual BCS score measured at 6 (the most important) body

parts and cow muscularity grade the following correlation coefficients were calculated:

- between BCS determined in different body parts;
- between BCS and muscularity grade of different body parts;
- between muscularity grade of Hereford cows determined in different body parts.

RESULTS AND DISCUSSION

In Table 1 correlation coefficients (r) between BCS examined in the different body parts of Hereford cows are presented. All correlation coefficients between BCS assessed at cow's back, ischiadic tubers, hip tubers, tail base and shoulder were very high (from 0.742 to 0.884) and statistically significant ($p \leq 0.01$).

All correlation coefficients between BCS assessed at cow's dewlap and on the basis of other parts of the body were much lower, but statistically significant.

Significant ($p \leq 0.01$) dependence between BCS measured in particular body parts and the final BCS was noticed. The highest correlation coefficient between final BCS ($r = 0.926$) and the condition measured at ischiadic tubers was observed.

Table 2 shows correlation between BCS and muscularity grade of different body parts of Hereford cows. All correlation coefficients between BCS assessed at cow's back, tail base, shoulder and ischiadic tubers and hip tubers and muscularity grade estimated basing on shoulder convexity, loin width, thigh convexity, thigh profile as well as rump convexity were high and statistically significant ($p \leq 0.01$). Cow BCS

TABLE 1. Correlation coefficients (r) between BCS determined in different body parts of Hereford cows (n = 626)

		Cow BCS						
		Back	Tail base	Ischiadic tubers	Hip tubers	Shoulder	Dewlap	Total
Cow BCS	Back	1.000	0.836 AA	0.848 AA	0.848 AA	0.835 AA	0.285 AA	0.892 AA
	Tail base		1.000	0.902 AA	0.838 AA	0.835 AA	0.308 AA	0.905 AA
	Ischiadic tubers			1.000	0.862 AA	0.847 AA	0.316 AA	0.926 AA
	Hip tubers				1.000	0.825 AA	0.289 AA	0.895 AA
	Shoulder					1.000	0.326 AA	0.899 AA
	Dewlap						1.000	0.315 AA

AA – dependence significant at $p \leq 0.01$.

TABLE 2. Correlation coefficients (r) between BCS and muscularity grade of different body parts of Hereford cows (n = 626)

		Cow muscularity grade								
		Shoulder convexity	Loins width	Total front	Thigh convexity	Thigh profile	Buttocks depth	Rump convexity	Total rear	Total
Cow BCS	Back	0.548 AA	0.557 AA	0.556 AA	0.601 AA	0.592 AA	0.594 AA	0.615 AA	0.617 AA	0.630 AA
	Tail base	0.574 AA	0.571 AA	0.577 AA	0.631 AA	0.619 AA	0.622 AA	0.651 AA	0.650 AA	0.650 AA
	Ischiadic tubers	0.607 AA	0.606 AA	0.612 AA	0.658 AA	0.649 AA	0.649 AA	0.687 AA	0.682 AA	0.687 AA
	Hip tubers	0.587 AA	0.571 AA	0.584 AA	0.625 AA	0.619 AA	0.616 AA	0.639 AA	0.643 AA	0.635 AA
	Shoulder	0.595 AA	0.598 AA	0.600 AA	0.655 AA	0.644 AA	0.646 AA	0.669 AA	0.672 AA	0.670 AA
	Dewlap	0.259 AA	0.260 AA	0.261 AA	0.312 AA	0.303 AA	0.307 AA	0.302 AA	0.310 AA	0.310 AA
	Total	0.614 AA	0.615 AA	0.620 AA	0.676 AA	0.666 AA	0.668 AA	0.688 AA	0.693 AA	0.691 AA

AA – dependence significant at $p \leq 0.01$.

examined on base of dewlap had the lower, but statistically significant influence on cow muscularity score. The highest coefficient between the BCS and the final muscularity score was

found for BCS examined at ischiadic tubers($r = 0.687$). It should be stated, that the dependence between BCS and the total muscularity assessment was even higher ($r = 0.691$).

TABLE 3. Correlation coefficients (r) between muscularity grade of Hereford cows determined in different body parts (n = 626)

		Cow muscularity grade								
		Shoulder convexity	Loins width	Total front	Thigh convexity	Thigh profile	Buttocks depth	Rump convexity	Total rear	Total
Cow muscularity grade	Shoulder convexity	1.000	0.951 AA	0.990 AA	0.825 AA	0.842 AA	0.837 AA	0.795 AA	0.827 AA	0.870 AA
	Loins width		1.000	0.983 AA	0.834 AA	0.843 AA	0.839 AA	0.822 AA	0.841 AA	0.843 AA
	Total front			1.000	0.837 AA	0.851 AA	0.846 AA	0.812 AA	0.840 AA	0.834 AA
	Thigh convexity				1.000	0.978 AA	0.979 AA	0.930 AA	0.975 AA	0.971 AA
	Thigh profile					1.000	0.992 AA	0.925 AA	0.975 AA	0.970 AA
	Buttocks depth						1.000	0.929 AA	0.976 AA	0.969 AA
	Rump convexity							1.000	0.980 AA	0.980 AA
	Total rear								1.000	0.975 AA

AA – dependence significant at $p \leq 0.01$.

Correlation between muscularity score measured at the particular body parts are shown in Table 3. All of the coefficients were statistically significant ($p \leq 0.01$), but the highest influence on the final muscularity score had rump convexity ($r = 0.980$) as well as the total note for total rear (thigh convexity, thigh profile, buttocks depth, rump convexity) ($r = 0.975$).

CONCLUSIONS

- All the correlation coefficients of BCS measured in the particular body parts were high (from 0.892 to 0.905) and statistically significant ($P \leq 0.01$).
- All the above mentioned coefficients examined on the front of neck (dewlap) appearance were not significant in comparison to those examined on the other parts of body.

- The dependence between BCS in the particular parts of body and the final BCS result was proved ($P \leq 0.01$).
- The highest correlation coefficient ($r = 0.926$) was found for the final BCS examined in the ischiadic tubers.
- All of the obtained correlation coefficients between cow BCS and cow muscularity were high ($p \leq 0.01$).
- The highest correlation ($r = 0.975$) was observed between rump convexity and the final musculature grade.

REFERENCES

- BEVERLY J.R., 1985: Reproduction In beef cattle as related to nutrition and body condition. Kentucky roundup of reproductive efficiency in beef cattle, p. 1–2.
- DADATI F.B., KENNEDY B.W., BURNSIDE E.B., 1985: Relationships between conformation and reproduction in Holstein cows: type

- and calving performance. *J. Dairy Sci.* 68, 2639–2646.
- DICKERSON G.E., 1978: Animal size and efficiency: basic concepts. *Anim. Prod.* 27, 367–379.
- FITZHUGH H.A., 1978: Animal size and efficiency, with special reference to the breeding female. *Anim. Prod.* 27, 393–401.
- JASIOROWSKI H., KIJAK Z., POCZYNAJ-ŁO S., WAJDA S., 1996: Program rozwoju hodowli bydła mięsnego w Polsce, Fundacja „Rozwój SGGW”, 5–67.
- McKIRNAN B., 2000: Frame scoring of beef cattle. Agfact A2.3.4. Department of Primary Industries, State of New South Wales, Australia.
- MORRIS C.A., WILTON J.W., 1976: The influence of body size on the biological efficiency of cows: A review. *Can. Anim. Sci.* 56: 613–647.
- MORRIS C.A., WILTON J.W., 1977: The influence of body size on the economic efficiency of cows. A review. *Anim. Breed. Abstr.* 45, 39–153.
- PRZYSUCHA T., 1998: Hodowla rodzimych ras bydła mięsnego we Włoszech. Wyd. SGGW, Warszawa.
- PRZYSUCHA T., 2009: Osobnicze uwarunkowania przebiegu ocieleni krów oraz umięśnienia i żywotności cieląt pochodzących po buhajach rasy piemontese użytkowanych w Polsce i we Włoszech. Rozprawa habilitacyjna. Wyd. SGGW, 1–136.
- PRZYSUCHA T., GRODZKI H., FAROPPA V., STOPYRA R., ZDZIARSKI K., 2002: Wzorce oceny umięśnienia cieląt pochodzących z krzyżowania krów czarno-białych z buhajami włoskiej rasy piemontese. *Przegląd Hodowlany* 2, 12–14.
- RICHARDS M.W., SPITZER J.C., WARNER M.B., 1986: Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. *J. Anim. Sci.* 62: 300–306.
- SELK G.E., WETTEMANN R.P., LUSBY K.S., RASBY R.J., 1986: The importance of body condition at calving on reproduction in beef cows. *OSO Agric. Exp. Sta. Publ.* 118: 3163–3169.
- WHITMAN R.W., REMMENG A.E., WILTBANK J.N., 1975: Weight change, condition and beef cow reproduction. *J. Anim. Sci.* 41: 387.
- WILTBANK J.N., 1983: Maintenance of high level of reproductive performance in beef cow herd. *Vet. Clin. N. Am. Large Anim. Proc.* 5: 41–57.
- Streszczenie:** Porównanie wyników oceny kondycji (BCS) i oceny stopnia umięśnienia krów rasy hereford. Celem pracy było określenie korelacji między wynikami oceny kondycji, a wynikami oceny stopnia umięśnienia krów mięsnej rasy hereford. Wszystkie współczynniki korelacji między ocenami kondycji mierzonej w różnych partiach ciała były wysokie (od 0,892 do 0,905) i statystycznie istotne ($p \leq 0,01$). Wszystkie współczynniki korelacji między oceną kondycji określanej wyglądem podgardla, a określanej na podstawie innych partii ciała były znacznie niższe, ale istotne. Stwierdzono istotną ($p \leq 0,01$) zależność między ocenami kondycji w poszczególnych partiach ciała (poza podgardlem), a ostateczną (zbiorną) oceną kondycji krowy. Najwyższy współczynnik korelacji z oceną ostateczną kondycji ($r = 0,926$) odnotowano dla kondycji ocenianej w guzach kulszowych. Współczynniki korelacji między kondycją krowy ocenianą w różnych partiach ciała, a stopniem umięśnienia krowy ocenianym na podstawie wypukłości barku, szerokości lędźwi, wypukłości i profilu uda oraz wypukłości zadu były wysokie i statystycznie istotne ($p \leq 0,01$). Najwyższy współczynnik korelacji między oceną kondycji, a ostateczną oceną stopnia umięśnienia odnotowano dla kondycji ocenianej w guzach kulszowych ($r = 0,687$). Wszystkie współczynniki korelacji między szczegółowymi ocenami stopnia umięśnienia krowy były wysokie i statystycznie istotne ($p \leq 0,01$). Najwyższą korelację z ostateczną oceną stopnia umięśnienia miała ocena wypukłości zadu ($r = 0,980$).

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The coat characteristic of yak maintained in conditions of the Gdańsk zoological garden

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Abstract: *The coat characteristic of yak maintained in conditions of the Gdańsk zoological garden.* The investigation concerned the analysis of chosen coat features of yak maintained in totally different environment than they naturally exists. The study has been carried out on all adult yak individuals (4 females and 4 males) kept in Gdańsk zoological garden. The coat samples have been taken in autumn from middle of the left side of animal to determine the proportion of hair fractions, length and fibre thickness within fraction, fibre surface features and cross section. The fractions were separated based on the length of the fibre within sample. Proportion of fraction was calculated from the fibre number within it. Fibre diameter of each fraction has been measured using microprojection method. The fraction length was determined measuring fibre bunches with accuracy to 0.5 cm. The scales on the fibre surface as well as shape of cross-section were observed using scanning microscope. Two fibre fractions were determined: coarse hair (25%) and mid-type fibres (75%). The down fibres has not been found. The diameter of coarse hair and mid-type fraction averaged out 58.21 μm and 36.52 μm respectively. The coarse hair thickness in males was greater than in females ($P \leq 0.01$). Fibres belonging to mid-type fraction were characterized by clear scales and more regular, circular shape. In coarse hair disappearance of scales, medullation and elliptical cross section has been recorded. The coarse hair fraction was much longer than mid-type fibres ($P \leq 0.01$). There was not statistical difference in fraction length depending on sex of animals.

Key words: yak, environment, coarse hair, mid-type fibres, fibre thickness, fibre length.

INTRODUCTION

The yak (*Peophagus grunniens* or *Bos grunniens*) are found on the plateau of western China in alpine and subalpine regions, at altitudes from 2000 to 5000 m with a cold, semi-humid climate. The greatest density of the yak appears in Qinghai-Tibetan Plateau (Miller, 1990).

The population of those animals in Chinese territories is estimated to exceed 13 million. The majority of them are domesticated. As wild animals they occur in Tibetan highland. About 1-million yaks exists in other Asiatic countries (Wiener et al., 2003). However, they are also present in North America in zoological parks and in many European countries in zoological gardens.

The yak are very good adapted to extreme environmental conditions like: very low temperature, high altitude with low oxygen content of the air, high solar radiation and cyclical nutrition with short growing season for herbage. The compact conformation, large lungs, heart and rumen relative to yak body size

are useful to this adaptability. The most important factor determining distribution of yak is temperature. They perform adequately when annual mean temperature is below 5°C and in the hottest months is not above 13°C. They can survive also at temperatures -40°C and below (Xu and Wu, 1984; Wiener *et al.*, 2003).

Except compact conformation, heat conservation is effected also by coat structure (Ouyang, 1985). The coat consists three types of fibre: coarse, long hair, down fibres and mid-type fibres. The composition of the coat and hair growth change with season. As temperature falls, down fibre grow densely among coarser hairs. As air temperature rises, down fibres begin shed from the fleece. In spite of that, yak are characterized by low tolerance to high temperature. It is result of poorly developed of sweat glands (Wang and Ouyang, 1984).

The finest and most dense coat is found in calves. The proportion of down fibres declines with age (Wiener *et al.*, 2003). In the coat structure of yak, the length of fibres is also very considerable. Long hair, especially occurrent in lower parts of yak's body, perfectly isolate them from cold, snow-covered ground.

The purpose of presented study was analysis of chosen coat features of yak maintained in totally different conditions, than they naturally exists. The fleeces of yak from Gdańsk zoological garden have been indicated.

MATERIAL AND METHODS

The study has been carried out on all adult yak individuals kept in Gdańsk zoological garden. There were 4 males, 4 females and 2 calves in the flock. The calves' coat

was excluded from analysis because of low number of animals in that group. The animal has been kept all year long on 300 m² paddock with roofed feed rack.

Green forages and tree branches fed the animals during the summer and hay in winter. Additionally, they obtained root crops and grain meal. Free access to water and mineral licks was also provided.

The coat samples have been taken in autumn from middle of the left side of animal to determine the proportion of hair fractions, length and fibre thickness within fraction, fibre surface features and cross section.

The analysed samples had the same weight. They were divided on fractions based on the length of the fibre within sample. All fibres from each fraction were counted, what has allowed indicating their proportion in sample.

Fibre diameter of each fraction has been measured using microprojection method according to PN-72/P-04900, modified by measurement of all fibres on the slide area.

The fraction length was determined measuring fibre bunches with accuracy to 0.5 cm.

The scales on the fibre surface as well as shape of cross-section were observed using scanning microscope (SEM) Joel type JSM 35C.

Statistical analysis of the data was performed using the SPSS 14.0 software (2003). To determine level of significance the t-Student test has been used.

RESULTS AND DISCUSSION

On the base of difference on fibre length two hair fractions were separated in investigated yak coat. The average share

of longer coarse hair was about 25%, while shorter mid-type fibre fraction 75%. Although differences did not show statistical significance, the proportion of mid-type fibres in females was 11% higher than in males (Figs 1, 2).

The diameter of coarse hair and mid-type fraction averaged out 58.21 µm and 36.52 µm respectively.

According to Xue and Yu (1981) and Wang and Ouyang (1984) fibre thickness of coarse hair is above 52.5 µm, while mid-type fibres contained between 25 µm – 52.5 µm, what was also confirmed

in present study (Tab. 1). The coat of investigated yak did not contain down fibres, average thickness of which should not exceed 25 µm, but usually is at the level between 16–18 µm (Ji et al., 2001). The lack of down fibres could testify the adaptation of these animals to different climatic conditions. It belongs to remember, that yak exhibits worse tolerance on temperature above 22°C (Li, 1985).

The coarse hair thickness in males was of 50% ($P \leq 0.01$) greater than in females (Tab. 2). Mid-type fibres did not show that large differentiation. The sex influ-

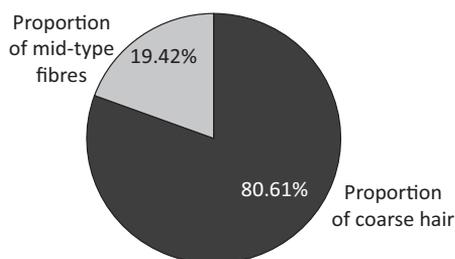


FIGURE 1. The distribution of fibre fractions in yak females (%)

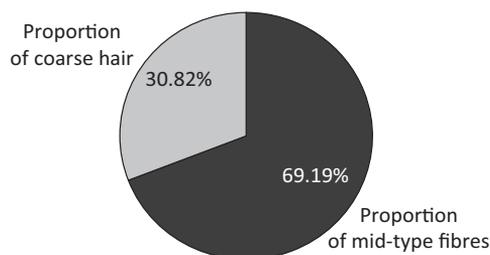


FIGURE 2. The distribution of fibre fractions in yak males (%)

TABLE 1. The characteristic of the different types of fibre in studied yaks

Specification	Mid-type fibre	Coarse hair	Standard error	Statistical significance
Fraction proportion (%)	74.90	25.10	6.19	**
Fibre thickness (µm)	36.52	58.21	4.18	**
Variability (%)	40.79	35.77	2.21	ns
Fibre length (cm)	4.81	8.34	0.38	**

ns – not significant effect; *significance at $P \leq 0.05$; **significance at $P \leq 0.05$.

TABLE 2. The effect of sex on diameter and length of the different types of fibre

Specification	Females	Males	Standard error	Statistical significance
Coarse hair thickness (µm)	46.01	70.41	4.61	**
Mid-type fibre thickness (µm)	34.58	38.47	3.04	ns
Coarse hair length (cm)	7.75	8.94	0.61	ns
Mid-type fibre length (cm)	5.00	4.63	0.44	ns

ns – not significant effect; *significance at $P \leq 0.05$; **significance at $P \leq 0.05$.

ence on this trait has been confirmed also by Zhang (1977) and Xue and Yu (1981).

The variability of fibre thickness was similar in both separated fractions. But mid-type fibres had worse equalization in

comparison to coarse hair (Tab. 1). It indicates the difficulty in proper separation of fibres, what demonstrate dispersion curve of fibre thickness (Figs 3, 4). If not difference in length between fractions mid-

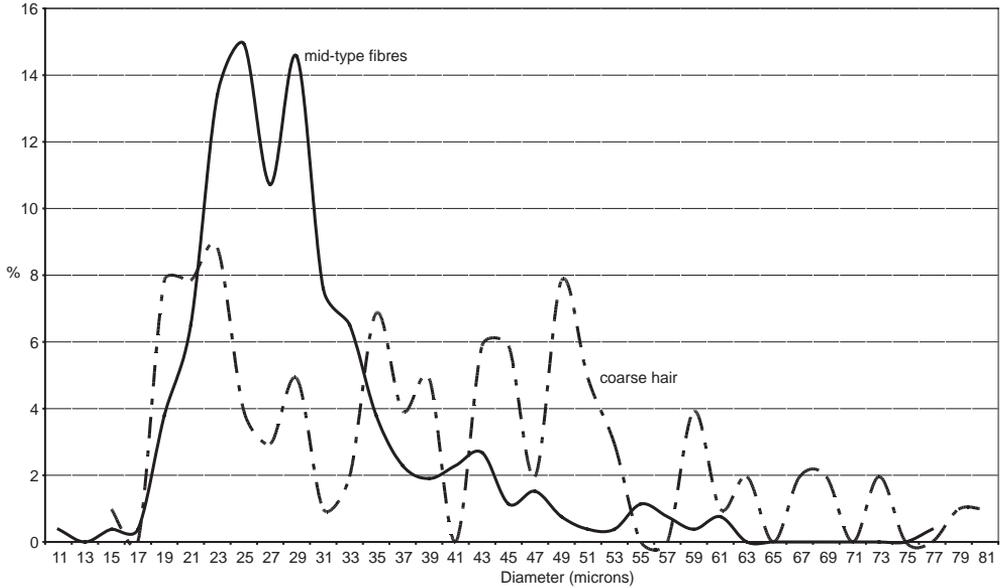


FIGURE 3. Variation in fibre diameter within studied fractions

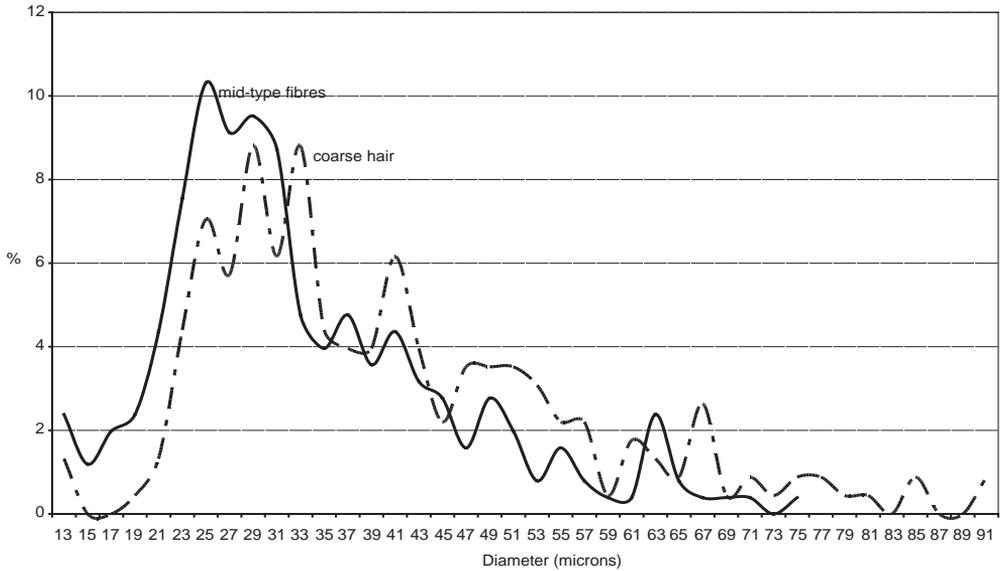


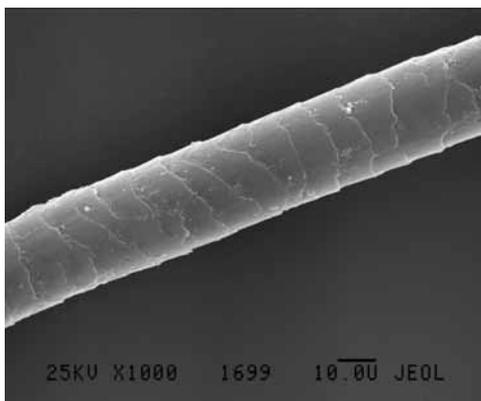
FIGURE 4. Variation in fibre diameter within studied fractions

-type fibres, based only on their thickness, could be included to coarse hair as well.

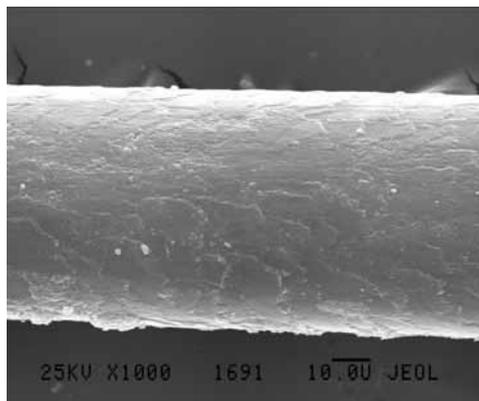
However, appurtenance of fibres to mid-type fraction was verified by analysis of fibres surface and their cross section. Fibres belonging to that fraction were characterized by clear, regular scales, occurrent on whole its length. While the surface image of coarse hair showed unclear acutance and disappearance of scales (Phot. 1, 2). It is in accordance with Wang (1984), which considered that the angle between scale and hair shaft in

yak is very small so that scale virtually sticks to the shaft, what gives poor felting qualities of yak hair.

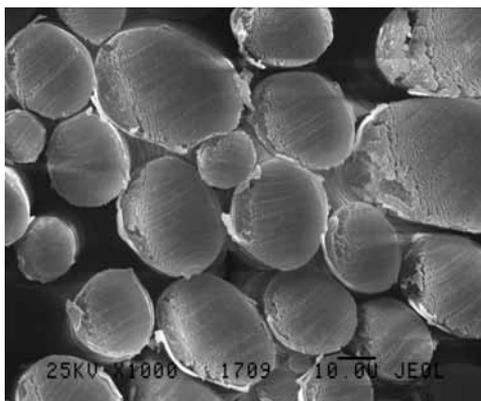
The analysis of cross section of investigated fractions indicates more regular, circular shape of mid-type fibres and elliptical of coarse hair. The presence of medulla was stated also only in coarse hair fraction (Phot. 3, 4). The occurrence of medullation in thickest fibres was confirmed by Wang (1984) in investigations on the ultramicroscopic structure of the yak coat.



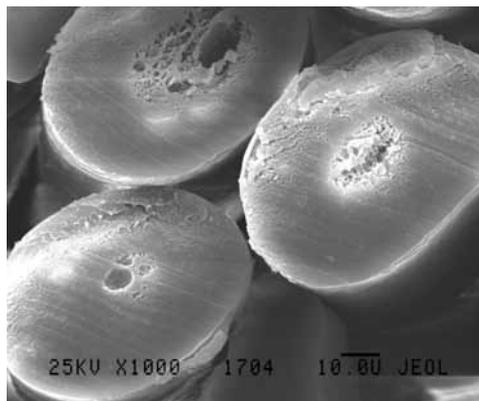
PHOTOGRAPH 1. The scale on the surface of the mid-type fibre (phot. D. Makowiecka)



PHOTOGRAPH 2. The scale on the surface of the coarse hair (phot. D. Makowiecka)



PHOTOGRAPH 3. Cross-section of mid-type of fibres (phot. D. Makowiecka)



PHOTOGRAPH 4. Cross-section of coarse hair with medulla (phot. D. Makowiecka)

The coarse hair fraction was about 57% longer than mid-type fibres ($P \leq 0.01$), (Tab. 1). There was not statistical difference in fraction length depending on sex of animals (Tab. 2).

The differences in hair length are mainly related to fibre type and body parts. According to Zhang (1989) the length of coarse hair varying from 8.9 cm to 21.1 cm and mid-type fibres from 5.3 to 13 cm, with the longest in both cases on the belly and the shortest on the shoulder. The similar dependences concern down fraction also, but it has not been found of studied yak coat.

CONCLUSIONS

1. In the coat of yak maintained in Gdańsk zoological garden the coarse hair (25%) and mid-type fibre (75%) fractions has been separate. The occurrence of down fraction has not been stated.

2. The average thickness of coarse hair fraction reached 58.21 μm , while mid-type fibres 36.52 μm . Coarse hair fraction was significantly thicker in males. The diameter of mid-type fibre was similar in both sexes.

3. The appurtenence to coarse or mid-type fraction was confirmed by surface and cross section analysis of fibres. Coarse hair showed medullation and disappearance of scales.

4. The coarse hair were almost twice as much longer than mid-type fibres.

REFERENCES

- JI Q., PU Q., DALVA Y., CIREN D., DAWA Q., 2001: Studies on wool-producing performance and main physical traits of underwool of 3 main groups of yak in Tibet. *Chinese Journal of Animal Science* 37(4), 29–30.
- LI S., 1985: The observation on yak's heat resistance. *Journal of China Yak*, 1: 10–13.
- MILLER D.J., 1990: Grassland of the Tibetan Plateau. *Rangelands* 12, 159–163.
- NORMA PN-72/P-04900, Metody badań surowców włókienniczych. Wełna.
- OUYANG X., WANG Q., 1984: An observation on adoption of calf yak. A research on utilization and exploitation in the northwestern part of Sichuan province. Sichuan National Publishing House, 159–161.
- OUYANG X., 1985: Studies on the cold resistance of yak. *Journal of Southwest Nationalities Collage (Animal Husbandry and Veterinary Science Edition)* 4, 28–34.
- WANG J., 1984: Observation on the ultramicroscopic structure of the hair fibre of yak. *Journal of Southwest Nationalities Collage (Animal Husbandry and Veterinary Science Edition)* 4, 9–14.
- WANG J., OUYANG X., 1984: Physical features of yak hair. *Journal of Southwest Nationalities Collage (Animal Husbandry and Veterinary Science Edition)* 1, 25–29.
- WIENER G., JIANLIN H., RUIJUN L., 2003: The yak. *FAO Regional Office for Asia and the Pacific*.
- XU R., WU Z., 1984: A test report about physiologic indices of yaks in Dari Area. *Journal of China Yak* 1, 1–5.
- XUE J., YU Z., 1981: The property and utilization of yak's down hair. *Journal of China Yak* 1, 1–5.
- ZHANG R., 1977: The hair quality of white yak on Zhuaxixiulong grassland of Tianzhu country. *Journal of Gansu Agriculture University* 2, 42–47.
- ZHANG R., 1989: China: the yak. *Gansu Scientific and Technology Press, Lanzhou, China*, 386.

Streszczenie: *Charakterystyka okrywy włosowej jaków utrzymywanych w warunkach gdańskiego ogrodu zoologicznego. Badania dotyczyły analizy wybranych cech okrywy włosowej jaków utrzymywanych w środowisku zupełnie odmiennym od naturalnych warunków ich bytowania. Badaniem objęto wszystkie osobniki dorosłe (4 samice i 4 samce) stada jaków utrzymywanych w gdańskim ZOO. Próby okrywy pobrano jesienią ze środka boku. Posłużyły one do określenia udziału frakcji włosów, grubości i długości*

w obrębie frakcji oraz cech powierzchni włosów i ich przekrojów. Frakcje włókien wydzielono na podstawie różnic w długości. Udział frakcji wyznaczono z ilości włókien w jej obrębie. Grubość włókien określono metodą mikroprojekcyjną. Długość frakcji mierzono z dokładnością do 0,5 cm. Powierzchnię włosów oraz przekroje poprzeczne analizowano na podstawie obrazów ze skaningowego mikroskopu elektronowego. Wyodrębniono dwie frakcje włosów: zewnętrzną (25%) i pośrednią (75%). W okrywie badanych jaków nie znaleziono frakcji puchowej. Grubość frakcji zewnętrznej i pośredniej wynosiła odpowiednio 58,21 i 36,52. Zarejestrowano różnice w grubości frakcji zewnętrznej ($P < 0,01$) pomiędzy samcami i samicami. Włókna frakcji pośredniej charakteryzowały się wyraźnym

ołoskowaniem i bardziej regularnymi przekrojami poprzecznymi. We włoskach grubych zarejestrowano zanik ołoskowania i występowanie rdzenia, a przekrój poprzeczny był eliptyczny. Włókna frakcji zewnętrznej były znacznie dłuższe niż frakcji pośredniej. Nie stwierdzono istotnych różnic długości włókien w obrębie frakcji pomiędzy samicami i samcami.

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The chemical composition and fatty acid profile in milk of Polish Mountain Sheep and Polish Merino

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Abstract: *The chemical composition and fatty acid profile in milk of Polish Mountain Sheep and Polish Merino.* The chemical composition and fatty acid profile of milk of Polish Mountain Sheep (PMS) and Polish Merino (PM) were investigated. The study was carried out on 20 suckling PMS ewes from the flock of Podhale region and on 20 PM ewes from the flock located in central part of Poland. The animals were fed winter diet. The milk samples have been collected from ewes at the age of 3–4 years and in the 4th week of lactation. Milk chemical composition of PMS and PM ewes was similar. The differences in fatty acids profile in both tested breeds have been observed. In milk of PMS the portion of medium chain fatty acids (MCFA) was higher ($P \leq 0.01$) than PM ewes. In the group of long chain fatty acids (LCFA), the C18:0, C18:1 *c*7 ($P \leq 0.01$), C18:1 *c*9 ($P \leq 0.05$) content was higher for PM, but C18:3, C20:4, C20:3, C20:5 and C22:5 portion ($P \leq 0.01$) was greater for PMS. The milk of Polish Mountain Sheep was characterized by higher proportion of PUFA n-3, lower n-6/n-3 ratio ($P \leq 0.01$) as well as higher content ($P \leq 0.01$) of conjugated linoleic acid *c*9, *t*11.

Key words: sheep, milk, chemical composition, fatty acids.

INTRODUCTION

Milk is one of the product rich in nutritive components and biologically active

substances, thus play an important role in human nutrition. An anticarcinogenic and antiantherogenic property has been attributed to the conjugated linoleic acid C18:2 *c*9, *t*11 isomer and linolenic acid (C18:3 *c*9, *c*12, *c*15) is also of interest in cardiovascular problems. Other fatty acids (FA) have been related to human health because of their monounsaturated and polyunsaturated nature (Collomb et al., 2006; Mesa et al., 2007).

Milk from sheep is widely used in the Mediterranean and Balkan regions for making hard and soft cheese, yoghurt and other dairy products. In Poland the only one breed, Polish Mountain Sheep is milked. The milk from this breed is used to produce “oszcypek” cheese, which is protected by PDO, but it should be also pointed out, that the second very important product obtained from this sheep are slaughter lambs. At present, the lamb meat from “Podhale” is registered as a regional product. This lambs are obliged to slaughter up to 16 kg of body weight and till then stay with their dams and have to produce only in the Podhale region.

The Polish Merino is the most numerous dual-purpose breed in Poland.

The lambs of this breed are usually fattened to low body weight, then the quality of ewe's milk does matter in their nutrition. The Polish Merino flocks are mostly located in the western region of Poland, although they can also be found in other parts of the country, except the mountain area.

The earlier study on milk quality of both breeds indicated the differences in basic chemical composition as well as in bioactive components content (Bonczar et al., 2009; Ciuryk et al., 2004; Pakulski and Pakulska, 2009; Patkowska et al., 2005). Because sheep milk is a basis of lambs diet may influence carcass quality in regard to health beneficial value, particularly in light lambs (Scerra et al., 2007; Radzik-Rant, 2005).

The aim of present study was the analysis of the chemical composition and fatty acid profile of milk for Polish Mountain Sheep and Polish Merino in place of their maintenance, at early stage of lactation during the winter-feeding.

MATERIAL AND METHODS

Animal treatments

Milk samples has been collected from 20 suckling Polish Mountain Sheep (PMS) ewes from the flock of Podhale region and from 20 suckling Polish Merino (PM) ewes from Experimental Farm of the Institute of Genetic and Animal Breeding in central Part of Poland.

The animals were fed winter diet (October to May). The PMS ewes received haylage (4.84 MJ EN/kg DM (dry matter); 5.5% CP(crude protein)/kg DM) and wheat bran (6.83 MJ EN/kg DM; 15.86 CP/kg DM). The PM ewes were fed diet containing: grass hay (5.76 MJ EN/kg DM; 12.68 CP/kg DM), oat meal (6.76 MJ EN/kg DM; 14.73 CP/kg DM) and corn silage (5.98 MJ EN/kg DM; 2.52 CP/kg DM).

The samples of feed were analysed for dry matter, crude protein, crude fibre (CF) and ether extract (EE). The results are given in Table 1. The fatty acid concentration in feed was also determined (Tab. 2).

TABLE 1. Chemical composition of feed consumed by ewes

Feed	Dry matter (%)	Crude protein (%)	Ether extract (%)	Crude fibre (%)
Oat meal	89.51	14.73	1.96	6.29
Hay	90.99	12.68	2.60	26.09
Corn silage	31.25	2.52	0.82	9.21
Haylage	51.20	5.51	0.64	17.30
Wheat bran	89.93	15.86	3.45	7.93

TABLE 2. Fatty acid concentration in feed consumed by ewes ($\mu\text{g/g}$ DM feed)

Fatty acid	Oat meal	Corn silage	Hay	Wheat bran	Haylage
C14:0	20.1	40.4	13.4	18.9	10.3
C16:0	1430.5	1685.8	192.6	1718.2	208.4
c9C16:1	17.2	18.5	7.6	13.4	13.4
C18:0	545.5	720.8	31.4	307.6	29.6
c11C18:1	1662.2	2269.4	78.7	1413.1	28.1
c9c12C18:2	3096.4	5489.4	175.0	5848.8	211.2
c9c12c15C18:3	274.7	294.1	204.8	387.3	430.6

All sampled sheep were at the age of 3–4 years and in the 4th week of lactation. Two hours before milk collection, the lambs were separated from their dams, then ewes were hand milked and 100 ml samples for further analysis have been taken. Each milk sample was divided in two parts, one for chemical analysis, the second for fatty acid determination.

Milk and feed composition

Each milk sample was analysed for fat, protein, lactose, total solids (TS) with IR spectrometry using Milkoscan FT-120.

Chemical composition of fodder were analysed according to procedure AOAC (1990).

Fatty acid analysis in milk and feed samples

Milk fat was extracted according to the Rose-Gottlieb method and fatty acid methyl esters were prepared by the trans esterification method (AOAC, 1990). Separation and quantification of the methyl esters were carried out using a gas chromatograph Hewlett Packard 5890, equipped with capillary column DD-23 (length 60 m, internal diameter 0.25 mm, film thickness 0.25 μm). Operation conditions were: helium flow 20 cm/s; a FID detector at 240°C; a split-splitless injector at 220°C. The temperature program of column was: 1 min at 130°C, a increase to 210°C at 10°C/min; held at this temperature for 25 min, then increased to 230°C at 2.5°C/min and held for 18 min at 230°C. The identification of fatty acids was conducted based on retention time

relatively to palmitic acid (16:0). For all acids and their isomers quantitative analysis by external calibration was performed using standards of chosen acids (Sigma and Supleco). Results are expressed as g/100 g esterified FA.

The feed samples to analysis of fatty acids were prepared according to Czuderna et al. (2009). Gas chromatograph analysis was performed using Hewlett Packard 6890N, equipped with capillary column CP7489 (100 m, i.d. 0.25 mm, film thickness 0.2 μm). The temperature program of column was: 4 min at 70°C, a increase to 150°C at 12°C/min; held at this temperature for 6 min, to 168°C at 8°C/min; held for 27 min; to 190°C at 0.75°C; held for 10 min; to 210°C at 1.8°C/min; held for 15 min; to 234°C at 6°C/min; held for 4 min and to 136°C at 6°C/min and held for 24 min.

FAME identification was validated based on electron ionization spectra of FAME and compared with authentic FAME standards and NIST 2007 reference mass spectra library.

The data of fatty acid composition was processed to compute the content of short-chain fatty acids (SCFA; C4:0-C10:0), medium-chain fatty acids (MCFA; C12:0-C16:1), long-chain fatty acids (LCFA; C17:0-C22:6), saturated fatty acids (SFA) monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Moreover the ratio of rumenic acid (CLA) to vaccenic acid (TVA) and C14:1/C14:0 (Δ^9 desaturase activity index) were calculated. Finally, the atherogenicity index was estimated as follows: (C12:0+4x C14:0+C16:0)/(MUFA+PUFA) (Marino et al., 2008).

Statistical analysis

Statistical treatment of the data of chemical composition of milk and fatty acid composition of milk was performed using the SPSS 14.0 software (2003) based on a linear model that included the effect of breed. All effects were tested against residual mi- squares to determine level of significance.

RESULTS AND DISCUSSION

The milk chemical composition is shown in Table 3. The milk of Polish Merino ewes were characterized by higher fat, protein, lactose, total solids content than milk of Polish Mountain Sheep, although significant differences were observed only in TS ($P \leq 0,05$).

The fat content in milk of PM was higher (7.82% vs. 4.80%) compared to results obtained for this breed in second month of lactation by Ciuryk et al. (2004) but was similar to merino sheep in Italy (Pulina et al., 2005). Wherease, Pakulski and Pakulska (2009) for coloured Polish Merino strain obtained higher portion of milk fat. According to Wohlt et al. (1981) in milk of Dorset ewes, the breed that like Polish Merino has not been improved for milk yield and quality, fat content was much higher (12.6%). The fat content in milk of Polish Mountain

Sheep did not differ from dairy sheep breeds maintained in Mediterranean and Balkan regions (Pulina et al., 2005; Addis et al., 2005; Ploumi et al., 1988; Mihaylova et al., 2005).

The milk protein content of PMS and PM ewes (4.36% and 4.72% respectively) was lower in relation to Italian dairy breeds and crossbred sheep (Tsigai \times Blackhead Plaren) (Pulina et al., 2005; Mihaylova et al., 2005). Bonczar et al. (2009) Konieczny (2009); and Molik et al. (2008) also reported higher average milk protein content for PMS ewes but in the differet stage of lactatiom and during pasture period.

Compered to present study the higher portion of lactose in milk of Polish Mountain Sheep was reported by Konieczny (2009), while Molik et al. (2008) registered lower content of this componet.

In Tables 4 and 5, the results of milk fatty acid composition in both studied sheep breeds are summarized. The differences in short-chain fatty acids (C4:0-C10:0) portion in milk fat between analysed breeds were not statistically significant. The proportion of SCFA in milk of PM and PMS ewes was 9.44 and 9.03 g/100 g respectively (Tab. 5). The similar results were obtained during the grazing period in milk of Tsigai (9.21 g/100 g) by Gerchev and Michay-

TABLE 3. Least square means (LSM) and their standard errors (SE) for milk chemical composition of Polish Merino and Polish Mountain Sheep

Item	Polish Merino		Polish Mountain Sheep		P value
	LSM	SE	LSM	SE	
Fat (%)	7.82	0.38	7.04	0.31	0.12
Protein (%)	4.72	0.15	4.36	0.12	0.08
Lactose (%)	5.13	0.05	5.01	0.04	0.06
Total solids (%)	18.24	0.42	16.98	0.35	0.03

TABLE 4. Concentration of the individual fatty acids of the milk fat of studied sheep breeds (g/100 g of total fatty acid methylesters)

Fatty acids	Polish Merino	Polish Mountain Sheep	SE	Statistical significance
	LSM	LSM		
C4:0	1.98	1.81	0.09	NS
C6:0	1.48	1.24	0.11	NS
C8:0	1.49	1.48	0.12	NS
C10:0	4.50	4.51	0.40	NS
C10:1	0.10	0.19	0.05	NS
C12:0	2.81	2.84	0.27	NS
C12:1	0.11	0.11	0.01	NS
C14:0	7.74	9.30	0.48	*
C14:1	0.36	0.44	0.03	NS
C15:0	0.87	1.29	0.07	**
C15:1	0.30	0.27	0.02	NS
C16:0	22.37	25.56	0.33	**
C16:1	0.34	0.28	0.03	NS
C16:1_izo	0.79	0.78	0.05	NS
C17:0	1.07	1.35	0.03	**
C17:1	0.47	0.56	0.03	*
C18:0	14.64	10.97	0.46	**
C18:1 t11	1.77	2.11	0.14	NS
C18:1 c9	28.59	23.26	1.42	*
C18:1 c7	0.55	0.40	0.02	**
C18:2 n-6	2.30	2.11	0.09	NS
C18:3 n-6	0.21	0.21	0.02	NS
C18:3 n-3	0.49	2.06	0.05	**
C18:2 c9, t11 (CLA)	0.56	0.90	0.05	**
C20:1	0.07	0.12	0.01	**
C20:4 n-6	0.12	0.22	0.02	**
C20:3 n-3	0.07	0.17	0.01	**
C20:5 n-3	0.05	0.12	0.01	**
C22:5 n-3	0.07	0.14	0.01	**
C22:6 n-3	0.03	0.04	0.01	NS

LSM – least square mean; SE: standard error; NS: not significant effect; *P ≤ 0.05. **P ≤ 0.01.

lova (2009). Atti et al. (2006) reported higher value of those acids (12.5%) for Scilo-Serde ewes, which received feedlot diet. Almost twice as much garter portion of SCFA has been determined in other study for PMS and for dairy sheep in Mediterranean area. The dif-

ferences concerned, especially of C4:0, C6:0 as well as C10:0 (Bonczar et al., 2009; De La Fuente et al., 2009; Addis et al., 2005; Carta et al., 2008). The portion of SCFA was also higher (about 17 g/100 g) in milk of coloured Polish Merino (Pakulski and Pakulska, 2009).

TABLE 5. The concentration of group of fatty acids in milk fat of studied sheep breeds (g/100 g of total fatty acid methylesters)

Fatty acids	Polish Merino	Polish Mountain Sheep	SE	Statistical significance
	LSM	LSM		
SCFA C4-C10	9.44	9.03	0.61	NS
MCFA C12-C16:1	35.70	40.87	1.12	**
LCFA C17-C22:6	51.04	44.74	1.78	*
SFA	58.95	60.34	1.35	NS
MUFA	33.46	28.52	1.45	*
PUFA	3.88	5.97	0.13	**
UFA	37.34	34.49	1.53	NS
PUFA n-3	0.69	2.52	0.05	**
PUFA n-6	2.63	2.55	0.08	NS
n6/n3	3.83	1.02	0.12	**
UFA/SFA	0.64	0.58	0.04	NS
MUFA/SFA	0.58	0.48	0.04	NS
PUFA/SFA	0.07	0.10	0.00	**
CLA/C18_1t11	0.34	0.43	0.03	*
C14:1/C14:0	0.05	0.05	0.00	NS
AI	1.55	1.96	0.15	NS

LSM – least square mean; SE : standard error; NS: not significant effect; * $P \leq 0.05$, ** $P < 0.01$.

SCFA – short chain fatty acids; MCFA – medium chain fatty acids; LCFA – long chain fatty acids; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acids; AI – atherogenic index ($C12:0 + 4 \times C14:0 + C16:0 / MUFA + PUFA \text{ n-6 and n-3}$).

In milk of PMS ewes, the portion of MCFA was higher ($P \leq 0.01$) than in PM ewes (Tab. 5). Within this group for PMS breed the C15:0 and C16:0 ($P \leq 0.01$) as well as C14:0 ($P \leq 0.05$) were higher compared to PM. The value of C15:0 for PM was similar to Tsigai ewes given by Gerchev and Michaylova (2009), whereas the portion of that acid in milk of PMS was much the same like for Serda breed (1.23–1.56 g/100 g) fed fresh forages of different Mediterranean species (Addis et al., 2005). The amount of palmitic acid in milk of PM breed is comparable to results obtained for Churra (22.04 g/100 g) and dairy breeds in Sardinia (De La Fuente et

al., 2009; Nudda et al., 2005), while the value of that acid for PMS ewes is similar to indigenous breeds in Pakistan (Talpur et al., 2009).

The differences in LCFA content in both tested breeds have been observed (Tab. 5). The portion of ($P \leq 0.01$) C18:0, C18:1 *c*7 and ($P \leq 0.05$) C18:1 *c*9 tended to be 33%, 37% and 23% respectively higher for PM breed than for PMS (Tab. 4). The obtained results are comparable to those reported for Tsigai sheep (Gerchev and Michaylova, 2009).

The milk of Polish Mountain Sheep was characterized by significantly higher amount ($P \leq 0.01$) of linolenic acid (C18:3) as well as fatty acids with 20

carbon atoms in chain like C20:4, C20:3, C20:5 (EPA) and C22:5 (DPA) (Tab. 4).

The content of C18:2, which belong to LCFA in milk of PM as well as PMS was higher compared to results obtained by Bonczar et al. (2009) and Patkowska-Sokoła et al. (2005). The similar value of linolenic acid, ranged between 1.62–2.98 g/100 g has obtained by Addis et al. (2005) for Sarda ewes. Other reported milked sheep breeds had lower amount of that acid than recorded in presented study for PMS (De La Fuente et al., 2009; Nudda et al., 2005).

Long-chain fatty acids with 16 or more carbon atoms are sourced either in the diet or because of body fat mobilization. In particular, the milk fat content of C18:0 (stearic acid) and C18:1 (oleic acid) are usually associated with the mobilization of fat depots (Chiliard et al., 2003). The high concentration of those fatty acids in PM milk could be related to the higher intensity of body fat mobilization than for Polish Mountain Sheep. Linoleic and linolenic acids are of dietary origin and are not synthesized by ruminant tissue, and their concentration in milk is dependent on the amount that flows out of the rumen. The higher content of linolenic acid (C18:3) in milk of PMS may be due to higher concentration of that acid in the diet. In spite of, that both breeds received winter diet, in case of PMS ewes were fed haylage and wheat bran, which contain higher amount of C18:3 than hay and corn silage (Tab. 2).

The larger portion of C20 fatty acids like C20:5 and C22:5 could be as a consequence of greater amount of C18:3 as a precursor to their endogenous synthesis in mammary gland (Rymer et al.,

2003). The higher concentration of those acids in PMS milk resulted, that the portion of PUFA, particularly PUFA n-3 was higher ($P \leq 0.01$) than in the milk of Polish Merino (Tab. 5). The n-6/n-3 ratio was also higher ($P \leq 0.01$) for that breed (Tab. 5).

The higher PUFA content in milk of coloured Polish Merino than in milk of studied PM ewes has been reported by Pakulski and Pakulska (2009). Whereas Patkowska et al. (2005) analyzing milk of PMS during the grazing period obtained lower portion of PUFA compared to results for this breed in presented study.

The PUFA have beneficial effect on human physiology and health. The presence of those acids in the diet reduces risk and even mortality caused by cardiovascular disease at humans (Singer et al., 1990). The essential n-3 FA are most desirable because metabolized from them eicosanoides, in contrast to eicisanoides from n-6 FA counteract many threatening diseases (Grimble, 1998). Thus, lowering n-6/n-3 ratio in human diet is very advisable.

The milk fat of PMS was characterized by 60% higher ($P \leq 0.01$) conjugated linoleic acid *c*9, *t*11 (CLA) content, than milk of Polish Merino ewes (Tab. 4). The greater proportion of vaccenic acid (2.11 vs. 1.77) was also observed for that breed, although the differences were not statistically significant. The $\Delta 9$ desaturase activity index expressed as a CLA/TVA ratio was higher ($P < 0.05$) for PMS than for PM (Tab. 5).

The CLA is produced as an intermediate during the rumen biohydrogenation of linoleic acid that leads to C18:1 *t*11 (vaccenic acid; TVA) and finally to stearic acid (C18:0). During rumen

biohydrogenation of linolenic acid, TVA but not CLA is formed (Bauman et al., 2000). The major portion of CLA (from 64 to 98%) in milk fat is produced in the mammary gland by $\Delta 9$ desaturase from TVA (Corl et al., 2001). The higher content of *c9*, *t11* CLA in milk of PMS could be related with higher activity of $\Delta 9$ desaturase enzyme in mammary gland.

The similar value for CLA *c9*, *t11* content as for PMS has been reported by Patkowska-Sokoła et al. (2005) for Polish Mountain Sheep during grazing period (0.9% of total FA) and De La Fuente et al. (2009) for Churra breed (0.89 g/100 g), but greater portion of that isomer observed by Bonczar et al. (2009) for PMS ewes (1.40%) and Gerchev and Michaylova (2009) for Tsigai sheep (1.35 g/100 g). The content of CLA in the milk of PMS was twice as much higher in comparison with typical dairy sheep breeds like Awassi (0.46%), Lacoue (0.44%), Friesland (0.35%) and Chios (0.46%) (Tsiplakou et al., 2006). With reference to PM milk higher portion of CLA has been reported by Pakulski and Pakulska (2009) for its colored strain.

The atherogenicity index (AI) characterizes the atherogenicity of dietary fat. The fat with high value of this index is assumed more detrimental to the human health. Although, the differences between studied breeds in relation to AI were not significant, the higher value (1.96 vs. 1.55) was found in milk of Polish Mountain Sheep (Tab. 5).

CONCLUSIONS

In relation to chemical composition, the milk of Polish Mountain Sheep and Polish Merino ewes was similar.

The milk fatty acid profile of the PMS was more profitable for human health and as a feed for lambs than in PM sheep, which have never been improved for milk performance. These differences could be caused by genotype and different fodder used during winter-feeding.

The milk of Polish Mountain Sheep, maintained in typical for this breed environmental conditions, was characterized by higher proportion of polyunsaturated fatty acids, particularly *n-3* and lower *n-6/n-3* ratio. The content of CLA, very important bioactive component of milk fat, was more favorable for this breed, but atherogenicity index was worse than in PM.

REFERENCES

- ADDIS M., CABIDDU H., PINNA G., DECON-DIA M., PIREDDA G., PIRISI A., MOLLE G., 2005: Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid *cis-9*, *trans-11*. *Journal of Dairy Science* 88, 3443–3454.
- AOAC (Association of Official Analytical Chem-ists) 1990: Food Composition Additives Natural Contaminants. 2. 4 Oils and Fats: 965.
- ATTI N., ROUISSI H., OTHMANE M.H., 2006: Milk production, milk fatty acid composition and conjugated linoleic acid (CLA) content in dairy ewes raised on feedlot or grazing pasture. *Livestock Science* 104, 121–127.
- BAUMAN D.E., BAUMGARD L.H., CORL B.A., GRINARI J.M., 2000: Biosynthesis of conjugated linoleic acids in ruminants. *Proceedings American Society of Animal Science Online*. Available: <http://www.asas.org/jas/symposia/proceedings/0937.pdf>.
- BONCZAR G., REGUŁA-SARDAT A., PUST-KOWICZ H., ŻEBROWSKA A., 2009: Wpływ substytucji mleka owczego mlekiem krowim na właściwości bundzu. *Zywność, Nauka, Technologia, Jakość* 5, 96–106.
- CARTA A., CASU S., USAI M.G., ADDIS M., FIORI M., FRAGHI A., MIARI S., MURA L., PIREDDA G., SCHIBLER L., SECHI T.,

- ELSEN J.M., BARILLET F., 2008: Investigating genetic component of fatty acid content in sheep milk. *Small Ruminant Research* 79, 22–28.
- CHILIARD Y., FERLAY A., ROUEL J., LAMBERET G., 2003: A review of nutritional and physiological factors affecting goat milk synthesis and lipolysis. *Journal Dairy Science* 86, 1751–1770.
- CIURYK S., MOLIK E., KACZOR U., BONCZAR G., 2004: Chemical composition of colostrum and milk of Polish Merino sheep lambing at different times. *Archives of Animal Breeding, Special Issue* 47, 129–134.
- COLLOMB M., SCHMID A., SIEBER R., WECHSLER D., RYHANEN E., 2006: Conjugated linoleic acids in milk fat: Variation and physiological effects. *International Dairy Journal* 16, 1347–1361.
- CORL B.A., BAUMGARD L.H., DWER D.A., GRINARI J.M., PHILIPS B.S., BAUMAN D.E., 2001: The role of delta (9)-desaturase in the production of *cis*-9, *trans*-11 CLA. *Journal of Nutrition Biochemistry* 12, 622–630.
- CZAUDERMA M., KOWALCZYK J., KRAJEWSKA K.A., ROZBICKA A.J., MICHALSKI J.P., 2009: Dietary selenite and conjugated linoleic acid isomers influence fatty acid concentration in the liver and femoral muscles of rats. *Journal of Animal and Feed Science* 18, 564–581.
- DE LA FUENTE L.F., BARBOSA E., CARRIEDO J.A., GONZALO C., ARENAS R., FRESNO J.M., SAN PRIMITIVO F., 2009: Factor influencing variation of fatty acid content in ovine milk. *Journal Dairy Science* 92, 3791–3799.
- GERCHEV G., MICHAYLOVA G., 2009: Variation in fatty acid composition of milk from Tsigai sheep in the region of central Balkan mountains. *Biotechnology of Animal Husbandry* 25, 945–952.
- GRIMBLE J.R.F., 1998: Modulation of inflammatory aspects of immune function by nutrients. *Nutrition Research* 18, 1297–1317.
- KONIECZNY M., 2009: Wpływa fazy laktacji na skład chemiczny i parametry fizykochemiczne mleka polskiej owcy górskiej utrzymywanej w warunkach chowu ekologicznego. *Roczniki Naukowe Zootechniki* 36 (1), 25–30.
- MARINO R., ALBENZIO M., ANNICCHIRICO G., CAROPRESE M., MUSCIO A., SANTILLO A., SEVI A., 2008: Influence of genotype and slaughtering age on meat from Altamura and Trimeticcio lambs. *Small Ruminant Research* 78, 144–151.
- MESA M.D., AQUILEVA G.M., GIL A., 2007: Efectos saludables de los lipidos n la dieta. *Alimentary Nutrition Salutory* 14, 12–26.
- MICHAYLOVA G., JAHREIS G., ODIAKOVA T., KAFEDJIEV V., 2005: Fatty acid profile of milk from sheep raised on mountain pastures. *Biotechnology of Animal Husbandry* 21, 93–96.
- MOLIK E., MURAWSKI M., BONCZAR G., WIERZCHOŚ E., 2008: Effect of genotype on yield and chemical composition of sheep milk. *Animal Science Papers and Reports* 3, 211–218.
- NUDDA A., MCGUIRE M.A., BATTACONE G., PULINA G., 2005: Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta. *Journal Dairy Science* 88, 1311–1319.
- PAKULSKI T., PAKULSKA E., 2009: Skład frakcji tłuszczowej w serach z mleka merynosów barwnych w zależności od technologii ich produkcji. *Roczniki Naukowe PTZ* 5 (2), 167–176.
- PATKOWSKA-SOKOŁA B., RAMADANI S., BODKOWSKI R., 2005: Skład chemiczny mleka polskiej owcy górskiej i owcy fryzyskiej z okresu żywienia pastwiskowego. *Roczniki Naukowe Zootechniki (suppl.)* 21, 73–75.
- PLOUMI K., BELIBASALEI S., TRIANTAPHYLIDIS G., 1998: Some factors affecting daily milk yield and composition in a flock of Chios ewes. *Small Ruminant Research* 28, 98–92.
- PULINA G., MACCIOTTO N., NUDDA A., 2005: Milk composition and feeding in the Italian dairy sheep. *Italian Journal of Animal Science* 4 (suppl.), 5–14.
- RADZIK-RANT A., 2005: Modyfikowanie zawartości kwasów tłuszczowych w tkance mięśniowej jagniąt poprzez wzbogacanie diety olejami różnego pochodzenia. *Monografie i Rozprawy*. Warszawa.
- RYMER C., GIVENS D.J., WHOLE K.W.J., 2003: Dietary strategies for increasing docosahexa-enoic acid (DHA) and eicosapentaenoic (EPA) concentration in bovine milk. <http://www.nutritionandfoodsciences.com>.

- SCERRA M., CAPARRA P., FOTI., GALO-FARO V., SINATRA M.C., SCERRA V., 2007: Influence of ewe feeding systems on fatty acid composition of suckling. *Meat Science* 76, 390–394.
- SINGER P., WIRTH M., BERGER J., 1990: A possible contribution of decrease in free fatty acids to low triglyceride levels after diets supplemented with n-6 and n-3 polyunsaturated fatty acids. *Atherosclerosis* 83, 167–175.
- TALPUR F.M., BHANGER M.J., MEMON N.N., 2009: Milk fatty acid composition of indigenous goat and ewe breeds from Sinh Pakistan. *Journal Food Composition Analysis* 22, 59–64.
- TSIPLAKOU E., MOUNTZORIS K.C., ZERVAS G., 2006: The effect of breed, stage of lactation and parity on sheep milk fat CLA content under the same feeding practices. *Livestock Science* 105, 162–167.
- WOHLT J.E., KLEYN D.H., VANDERNOOT G.W., SELFRIDGE D.J., NOVOTNEG C.A., 1981: Effect of stage of lactation, age of ewe sibling status, and sex of lamb on gross and minor constituents of Dorset ewe milk. *Journal Dairy Science* 64, 2175–2184.

Streszczenie: Skład chemiczny i profil kwasów tłuszczowych w mleku polskiej owcy górskiej i merynosa polskiego. Oznaczano skład chemiczny i profil kwasów tłuszczowych w mleku polskiej owcy górskiej (POG) i merynosa polskiego (MP). Badania prowadzono na 20 karmiących maciorach POG, pochodzących ze stada w rejonie Podhala, i 20 maciorkach MP z Polski centralnej. Próby mleka pobierano od maciorek w wieku 3–4 lat,

w czwartym tygodniu laktacji. Był to okres żywienia zimowego. Skład chemiczny mleka POG i MP kształtował się na podobnym poziomie. U obu badanych ras obserwowano różnice w profilu kwasów tłuszczowych. W mleku POG udział kwasów tłuszczowych średniołańcuchowych (MCFAs) był większy ($P \leq 0,01$) niż u maciorek MP. W grupie długołańcuchowych kwasów tłuszczowych (LCFAs), zawartość kwasów C18:0, C18:1 *c*7 ($P \leq 0,01$), C18:1 *c*9 ($P \leq 0,05$) była większa u MP, ale udział C18:3, C20:4, C20:3, C20:5 i C22:5 ($P \leq 0,01$) był większy u POG. Mleko polskiej owcy górskiej charakteryzowało się większym udziałem PUFA n-3, niższym stosunkiem n-6/n-3 ($P \leq 0,01$), jak również większą zawartością skoniugowanego kwasu linolowego *c*9, *t*11.

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Indicatory wool production of Żelaźnieńska strain of Polish Lowland Sheep maintained in semi-open shed and barn

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Abstract: *Indicatory wool production of Żelaźnieńska strain of Polish Lowland Sheep maintained in semi-open shed and barn.* The aim of this study was determination of sheep maintenance in semi-open shed on indicatory wool production. During 5 months period (September–January) one group of ewes was kept in shelter (10 animals) while the second in barn (10 animals). Wool samples, two from each ewe, were taken before shearing out of earlier prepared area (10 × 10 cm) on the left mid side. One sample from area of 4 cm² has been used to determine indicatory wool production, the second one to assay fibre length and thickness. The statistical differences in indicatory greasy and clean wool production has not been found between investigated groups. Although, the tendency to about 11% greater clean wool production has been observed in ewes kept in shelter, while these from barn produced 8% more of greasy wool. The wool from sheep kept in shelter was less than 1 μm thicker and about 8% longer and resulted in higher amount of clean wool. Higher greasy wool production in the group of ewes from barn was caused by higher, but not confirmed statistically, amount of other fleece components (sweat, fat, water, vegetable matter). The results of investigations showed tendency to higher clean wool production in ewes maintained in semi-open shed.

Key words: sheep, semi-open shed, indicatory wool production.

INTRODUCTION

The comprehensive purpose of sheep is related to their high environmental adaptability. In the past centuries most of sheep in Poland represented the dual purpose, wool and meat type; however, starting from the 1990's, meat production is the main goal in sheep husbandry in our country because of the higher profitability of this production (Radzik-Rant et al., 2000). That profitability also drives the natural demands for improving the efficiency of the sheep growing process which are often counterbalanced by the contemporary requirements of ecological conformance. Consequently, sheep growing has become a complex endeavour with its own bag of issues related to foraging, herd structuring, and housing. The new tendency in livestock production is to change management systems from intensive to semi-extensive and extensive which is connect with maintenance of animals in semi-open sheds. Nowadays, we see recently various attempts at eco-inspired diversification, e.g., utilize sheep for landscape conservation. Sheep's milk is a processed and

sold as dairy products (PDO) that protect regional cultural tradition. Sheep's also produced wool which, all in all, will never be completely phased out by synthetic fibres.

In addition to genetic and biological factors, wool production is also affected by various attributes of the sheep's environment. Obviously, the quantity and quality of forage is not without an impact: they can affect the fibre thickness – within the genetic bounds (Rayder, 1984). Many studies demonstrate a correlation of wool growth (both quantitative and qualitative) with seasonal changes like day length and temperature (Lincoln, 1990; Pearson et al., 1996; Choy et al., 1997). Changes in wool production also occur in response to thermal stress, a sudden decrease or increase of temperature for an unadoptable animals (Thwaites, 1967, Skoczylas, 1978).

The wool yield can be measured as the total fleece weight retrieved from a sheep after some specific growth interval, or, especially in research, through indicatory production taken from a pre-determined area (Schinckel, 1958).

The goal of present work was to investigate the impact of housing the sheep in semi-open shed for indicatory wool production and for the basic qualitative characteristics of the produced wool.

MATERIALS AND METHODS

The study was carried out with 20 low-land sheep of the Żelaznieńska strain selected from the herd at RZD Żelazna. The ewes under study were divided into two groups, 10 ewes each; group I was kept in a semi-open shed while group II was kept in barn. The average outdoor

temperature, taken weekly at 6 a.m. was +4.6°C. The maximum temperature, +17.3°C, was noted in September, and the minimum temperature of –5.1°C was recorded in January. Temperature in the barn was maintained according to the norm for ewes and it never dropped below +8°C (Dziennik Ustaw 167, 2003).

The goal of mothers ewe selection procedure was to bring the two groups as close as possible in terms of the relevant characteristics of their members. The selected ewes were between 2 and 4 years old going through the same series of physiological periods: preparation for mating, then mating, and the subsequent 4-month pregnancy. Taking into account the age and the ovulation rate evaluated by laparoscopy, ewes were divided into two groups, such that either group contained ewes with a similar distribution of age and the diagnosed count of ovarian follicles.

The ewes were fed according to standards for a ewe in the respective physiological stage, identically in both groups. Green forage was used as the basis in the period of preparation for mating and the subsequent mating. The exact proportions were: green forage – 4.5 kg, concentrate (triticale, barley, oat) – 0.2 kg, and carrot – 2 kg (daily, per animal); within the first three months of pregnancy: concentrate (same as above) – 0.2 kg, carrot – 2 kg, and straw – 1 kg; in the fourth month of pregnancy: concentrate rapeseed meel, triticale, barley, oat) – 0.65 kg, carrot – 2 kg, straw – 1 kg.

During the preparation for mating (in the beginning of September), all the ewes had been prepared for the test by having shaved off a 10 × 10 cm area on the left-mid side. At the end of January,

just before shearing (done one month before parturition) two wool samples were taken from that previously cleaned area. One sample, taken from a 4 cm² area, was subsequently used to assess the amount of indicator production, while the other was used to determine fibre thickness and length.

All the collected indicator samples were placed in sealed containers. They were subsequently weighed (with 10⁻⁴ g accuracy) in order to determine the amount of greasy wool. Next, the samples were dried at 105°C for 15 hours. The difference in mass before and after the conditioning was interpreted as the water content. A Soxhlet method with ethyl ether acting as the solvent was used to extract fat from the samples. The extracted fat was dried at 105°C and then weighed. Following the removal of fat as well as the mechanical separation of vegetable matters, the sample was dried again (at 105°C) until its mass ceased to drop. The final mass was assumed to represent the clean wool. The sweat content was calculated as the initial weight minus the sum of the weights of clean wool, water, fat, and the vegetable matters.

The thickness of fibres was measured using microprojection according

to Polish Standard PN-72/P04900. At least 600 fibres were measured in each sample. Staple length was determined by measuring 10 bundles of fibres per sample with the accuracy of 0.1 cm.

The obtained values have been processed statistically through a single-factor ANOVA using SPSS 2003.

RESULTS AND DISCUSSION

The greasy wool output from the indicator area (4 cm²) has been about 8% higher in the barn group than in the shed group. On the other hand, sheep in the shed group produced almost 11% more of clean wool than the animals kept in the barn. However, the differences were not statistically significant (Tab. 1).

While the greasy fleece weight depends on components like water, fat, sweat, and vegetable matters, the clean wool yield is solely the function of thickness, length, and density of the fibres per unit area (Skoczylas, 1958). The last attribute, the number of follicles and their ability to produce fibre is predetermined genetically. The thickness and length of fibres can be influenced by biological and environmental factors (Parry et al., 1995; Reis, 1992). The shed group showed a higher diameter of the

TABLE 1. The fleece components content (g) of ewes kept in semi-open shed and barn

Trait	Semi-open shed		Barn		Statistical significance
	LSM	S _E	LSM	S _E	
Greasy wool (g)	1.89	0.14	2.04	0.14	NS
Clean wool (g)	1.22	0.10	1.10	0.10	NS
Fat (g)	0.08	0.01	0.10	0.01	NS
Sweat (g)	0.40	0.04	0.60	0.04	NS
Water (g)	0.14	0.01	0.16	0.01	NS
Vegetable matters (g)	0.05	0.01	0.08	0.01	NS

NS – not significant differences

fibre (by slightly less than 1 μm) than the animals kept in the barn. This difference has not been confirmed statistically (Fig. 1). Relating the obtained thickness values to the nominal thickness according to Polish Standard PN-84/P-80053, the most of samples for the barn group was 31 μm , and for the shed group 33 μm (Fig. 2). The variability of thickness in both groups was fairly similar, as witnessed by the shape of the distribution curves and the variation coefficients (Figs 1 and 3).

The statistically significant difference in the fiber length between the two groups of ewes has not been found. Similar to the thickness, the fibers appeared to be slightly longer in the shed group: the difference in the 5th month growth was about 8% (Fig. 4). This slight tendency towards longer and thicker fibers in the shed group may be attributed to the impact of the day length, but mostly to the temperature. Numerous studies have proved that the prolactin level positively correlates with the increasing

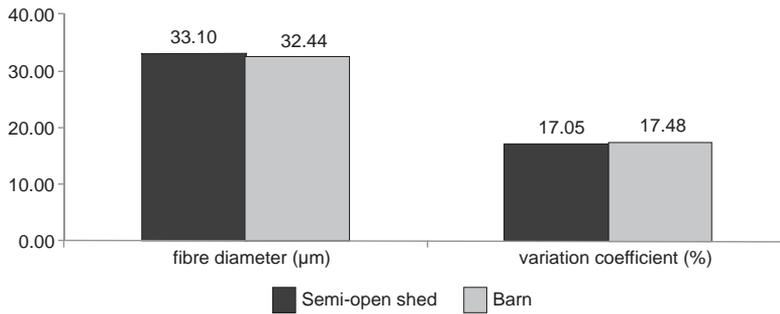


FIGURE 1. Fibre diameter (μm) of ewes maintained in semi-open shed and barn

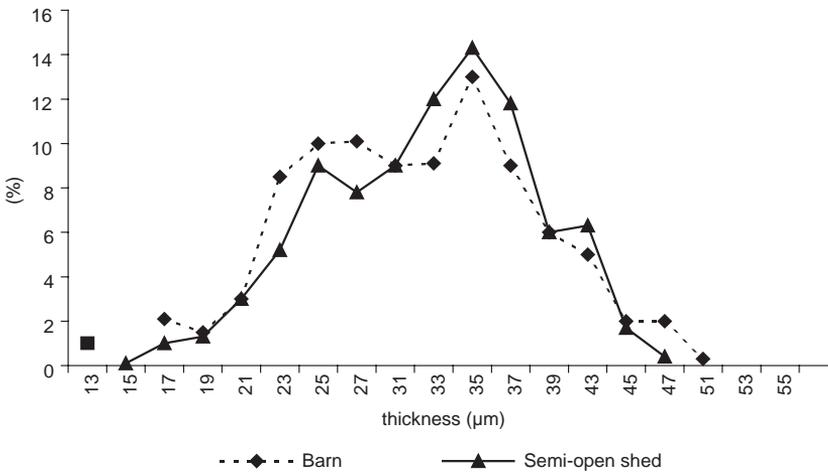


FIGURE 2. The variation of fibre thickness in ewe's wool maintained in semi-open shed and barn (nominal thickness 31 μm)

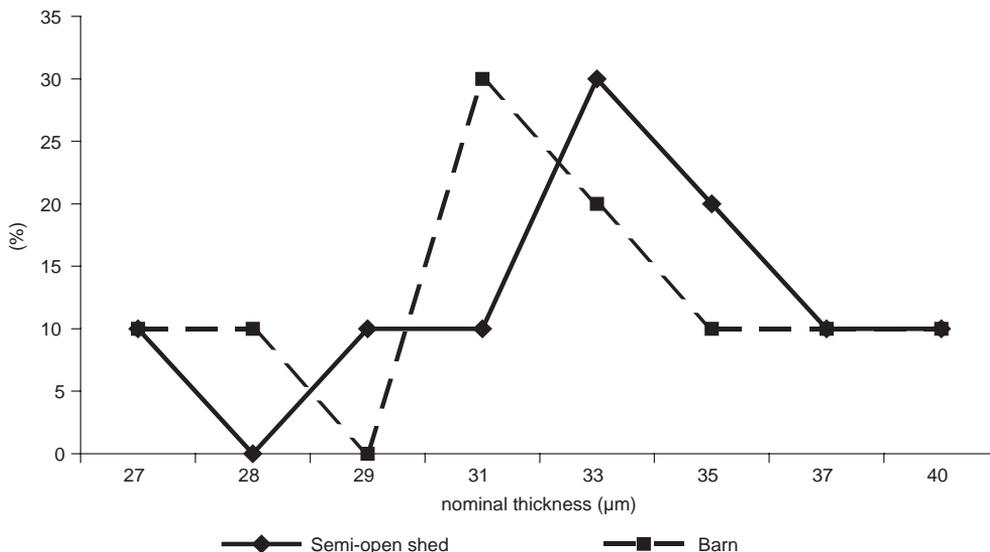


FIGURE 3. The share of nominal thickness in the wool of ewes kept in semi-open shed and barn

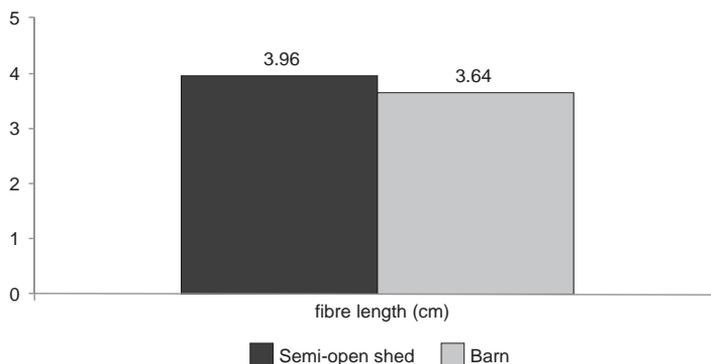


FIGURE 4. Fibre length (cm) of ewes maintained in semi-open shed and barn

day length and temperature. The prolactin level, in turn, is likely to affect the activity of follicles (Lincoln, 1990; Choy et al., 1997; Pearson et al., 1996). According to Pearson et al. (1996), the follicle activity can be stimulated by both an increase in the prolactin level, occurring mostly in the spring, and its decrease in the fall, as the day becomes shorter and the temperature drops. The changing prolactin levels mostly affect

rustic sheep in which an elevated follicle activity expresses into an increase of fibre length, wool weight, and molting, as well as the winter or summer coat growth (Santiago-Moreno et al., 2004; Lincoln, 1990). Notably, the uniform fleece is affected to a much lesser extent by the fluctuations in prolactin levels triggered by the changes in temperature and day length. Nonetheless, as shown by Lincoln (1990), some stimulation of

wool production due to decreasing temperature (and decreasing prolactin level) can be also noted among improved breeds. A study by Gebbie et al. (1999) of the impact of day length and temperature on the coat of goats suggests that the reduction of prolactin level in the fall (triggering an increase in the length and thickness of hair) is caused primarily by the temperature drop rather than the reduced day length. Thus, one can suppose that the increase in fibre length and thickness observed in shed group, and expressed into a larger indicative wool output (notwithstanding its lack of statistical significance), was caused by the lower temperature under the shed than in the barn. The larger weight of greasy wool per unit of indicative area noted among the barn ewes was due to the larger contribution of non-wool components rather than a larger yield of clean wool. The fleece of ewes from that group was found to contain a larger amount of fat, sweat, water, and vegetable matters, although the difference with respect to the other group was not statistically significant (Tab. 1). The higher production of sweat (by 50%) and fat (by 25%) among the barn ewes could be caused by the increased secretion of the sweat and fat glands resulting from their specific environmental factors. An increased content of sweat and fat in the fleece of sheep kept in barns was observed by Hapanowicz (1991). In addition to sweat and fat a higher content of water (by 14%) and vegetable matters (by 60%) have also been noted in presented study. The latter can be explained by the fact that a higher fat content in fleece facilitates retention of these matters (Skoczylas, 1978).

CONCLUSIONS

To sum up, it can be concluded that presented study showed a tendency towards a higher indicative wool production in sheep kept in semi-open shelters than in those kept in closed barns. It seems that semi-open shelters facilitate an advantageous activity of follicles.

REFERENCES

- CHOY V.J., NIXON A.J., PEARSON A.J., 1997: Distribution of prolactin receptor immunoreactivity in ovine skin and changes during the wool follicle growth cycle. *Journal of Endocrinology*, 155, 265–275.
- Dziennik Ustaw nr 167 poz. 1629 – Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 2 września 2003 r. w sprawie minimalnych warunków utrzymania poszczególnych gatunków zwierząt gospodarskich
- GEBBIE F.E., FORSYTH I.A., ARENDT J., 1999: Effects of maintaining solstice light and temperature on reproductive activity, coat growth, plasma prolactin and melatonin in goats. *Journal of Reproduction and Fertility*. 116, 25–33.
- HAPANOWICZ J., 1991: Wpływ jakości tłuszczopotu na parametry włókna wełnianego, Materiały Konferencyjne. Metlan 91, 49–54, IW, Gdynia.
- LINCOLN G.A., 1990: Correlation with changes in horns and pelage, but not reproduction, of seasonal cycles in the secretion of prolactin in rams of wild, feral and domesticated breeds of sheep. *Journal of Reproduction and Fertility*, 90, 285–296.
- Norma PN-72/P-04900: Metody badań surowców włókienniczych, wełna.
- Norma PN-84/P-80053: Surowce włókiennicze, wełna owcza potna.
- PARRY A.L., NIXON A.J., CRAVEN A.J., 1995: The microanatomy, cell replication and keratin gene expression of hair follicles during a photoperiod-induced growth cycle in sheep. *Acta Anatomica (Basel)*, 154, 283–299.
- PEARSON A.J., PARRY A.L., ASHBY M.G., CHOY V.J., WILDERMOTH J.E., CRAVEN A.J., 1996: Inhibitory effect of increased photo-

- toperiod on wool follicle growth. *Journal of Endocrinology*, 148, 157–166.
- RADZIK-RANT A., SZTYCH D., NIŻNIKOWSKI R., 2000: Produkcja, obrót i jakość wełny w Polsce. *Przegląd Hodowlany*, 3, 21–25.
- REIS P.J., 1992: Variations in the strength of wool fibres – a review. *Australian Journal of Agricultural Research*, 43, 1337–1351.
- RYDER M.L., 1984: Uzyskanie pożądaných właściwości wełny w czasie jej wzrostu. *Biuletyn Informacyjny*, 1, 8–27, IW w Gdyni.
- SANTIAGO-MORENO J., LOPEZ-SEBASTIAN A., del CAMP A., GONZALEZ-BULNES A., PICAZZO R., GOMEZ-BRUNET A., 2004: Effect of constant-release metonin implants and prolonged exposure to a long day photoperiod on prolactin secretion and hair growth in mutton (*ovis gmelini musimon*). *Domestic Animal Endocrinology*, 26, 303–314.
- SCHINCKEL P.G., 1958: Follicle density and wool production. *Australian Journal of Agricultural Research*, Vol. 8, 5, 68–76.
- SKOCZYLAS A., 1978: *Biologia owczego runa*. PWN, Warszawa.
- SPSS 2003: *User's guide 12.0*. SPSS Inc.
- THWAITES C.J., 1967: Prolonged heat stress and wool growth in sheep. *International Journal of Biometeorology*, Vol 11, 3, 297–300.
- Przez okres ok. 5 miesięcy (wrzesień–styczeń) maciorki utrzymywane były w wiacie i w owczarni, po 10 zwierząt w każdej grupie. Próby wełny, po dwie od każdej maciorki, pobrano przed strzyżą z wcześniej przygotowanego miejsca (10 × 10 cm) na lewym boku. Jedna próba z powierzchni 4 cm² posłużyła do określenia wskaźnikowej produkcji wełny, druga do oznaczenia grubości i długości. Nie wykazano statystycznie istotnych różnic odnośnie wskaźnikowej produkcji wełny potnej i czystej pomiędzy badanymi grupami owiec. Aczkolwiek zaobserwowano tendencję do większej o ok. 11% produkcji czystego włókna u maciorek utrzymywanych pod wiatą, natomiast maciorki w owczarni produkowały o 8% więcej wełny potnej. Owce w wiacie posiadały wełnę niespełna o 1 μm grubszą i o ok. 8% dłuższą, co w rezultacie dało wyższą ilość czystego włókna. Zwiększona produkcja wełny potnej w grupie maciorek z owczarni spowodowana była większym, chociaż niepotwierdzonym statystycznie, udziałem pozostałych składników runa (potu, tłuszczu, wody i zanieczyszczeń). Przeprowadzone badania wskazują na tendencję do lepszej produkcji czystego włókna u owiec utrzymywanych w pomieszczeniach półotwartych.

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Streszczenie: *Wskaźnikowa produkcja wełny u maciorek nizinnych odmiany żelaźnieńskiej utrzymywanych w różnych pomieszczeniach. Celem niniejszych badań było określenie wpływu utrzymywania owiec w pomieszczeniach półotwartych na wskaźnikową produkcję wełny.*

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SNPs polymorphisms in LGB, CSN3 and GHR genes in five goat breeds kept in Poland

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Abstract: *SNPs polymorphisms in LGB, CSN3 and GHR genes in five goat breeds kept in Poland.*

The aim of the study was to establish the frequencies of SNPs polymorphisms in β -lactoglobulin (LGB), κ -casein (CSN3) and growth hormone receptor (GHR) genes in five goat breeds kept in Poland. Blood samples from 24 farms possessing 27 goat flocks of the Alpine (A), Polish Fawn Improved (PFI), Saanen (S), Polish White Improved (PWI) and Boer (B) goat breeds were collected and used for genomic DNA extraction (219 samples in total; 32–49 samples per breed) due to the procedure proposed by the ECONOGENE consortium (2002). Researched SNPs were identified due to the KASPar® technology (KBioscience, UK 2007). Genotypes and alleles frequencies were obtained and moreover the differences between breeds were estimated by the Chi-square test in SPSS 14.0 PL software. The results expressed the highest frequencies of “g:g” homozygots and “g” allele in LGB in milk breeds (PWI, PFI, S and A). Due to the CSN3 gene the high frequencies of “a:a” homozygots and “a” allele were observed in the Saanen, Polish White Improved and Boer goats. No “g” allele was presented in the B goat in CSN3 gene. The results showed three self-distinguishing groups of goat breeds: the colored (A and PFI), the white (S and PWI) and the meat (B) goats. The Boer goat (B) presented the significant genetic difference from the other milk breeds in all examined genes.

Key words: goat, breeds, β -lactoglobulin, κ -casein, growth hormone receptor.

INTRODUCTION

Studies on polymorphisms of genes in small ruminants, including goats, have been carried out for many years all over the world (Cappuccio et al., 2006; Econogene, 2002; Strzelec, 2008). They were focused e.g. on the attempts to establish important polymorphisms in genes that might be applied as genetic markers for production traits (QTL) (Kurył and Żukowski, 1997; Ge et al., 2003), as well as being helpful to explain evolution and domestication processes of species (Luikart et al., 2001; Martin et al., 1999; Maj and Zwierzchowski, 2006; Pidancier et al., 2006). Moreover, this knowledge could help to explain the origin of breeds and relations between them, as well as their genetic similarity (Lenstra and the Econogene, 2005). The application of molecular genetics tools in the researches on the genomes of domestic animals would allow to evaluate and protect their biodiversity (Słota et al., 2007).

Goat breeding in Poland has a short tradition and the population structure of goat breeds has been established recently. Breeding interests have been focused on milk production in Poland,

therefore 4 goat milk breeds are presented in the Polish population nowadays (Strzelec and Niżnikowski, 2009). The most important milk breeds are Polish White Improved, Polish Fawn Improved, Saanen and Alpine. During the last decade, the goat meat production has been slowly developing, therefore the permanently increasing interest of breeders to possess the Boer goat has been observed recently. Total number of goats was recorded in 2009 by Central Statistical Office at the level of 119 000 heads (GUS 2011). However, due to activity of the Polish Union of Sheep-Farmers (PZO) the active population of goats dropped to the number of 152 goats registered in the herd books in 2009 (PZO 2010).

The aim of presented research was to recognize genetic potential of goat breeds in Poland and single nucleotide polymorphisms (SNP) in genes associated with milk production (β -lactoglobulin (LGB) and κ -casein (CSN3) genes) and playing an important role in metabolic paths (growth hormone receptor (GHR)) were investigated.

MATERIAL AND METHODS

Research was carried out in 2002–2003 at 24 Polish farms, housing 27 goat flocks in total, which were chosen due to the highest breeding level indicated by the Polish Union of Sheep-Farmers (PZO). Goat farms were located at the geographical coordinates of latitudes of 15.95–22.03 North and at longitudes of 49.99–54.90 East (WGS-84 system NIMA 1984) as well as at altitude between 26 and 325 meters above the sea-level.

Five breeds were examined including Alpine, Saanen, Polish Fawn Improved and Polish White Improved (milk type) as well as the Boer goat (meat type). Goats were randomly chosen from each flock by an owner due to the individual recognition of typical animals for a breed. Blood samples were taken from *v. jugularis externa* from 32–49 goats per breed avoiding the close genetic relations between individuals. Genomic DNA was obtained due to the extraction procedure proposed by the Econogene consortium (2002). The SNPs polymorphisms in the β -lactoglobulin (LGB), κ -casein (CSN3) and growth hor-

TABLE 1. Primers, SNPs positions and total number of DNA samples in β -lactoglobulin, κ -casein and growth hormone receptor genes researched in goats

Locus	Name	Primers 5'-3' (forward/reverse)	Method of genotyping	SNP position	Locali-zation	N
LGB	β -lactoglobulin	ACTCTGTGGGGTGACCTGT/ CCTCCCTGGTTCCTGAAAGT	SNP/KASPar ^a	Z33881:g.6751 A > G ^b	Exon 7	219
CSN3	κ -casein	GGTATCCTAGTTATGGACTCAAT/ GTTGAAGTAACTTGGGCTGTGT	SNP/KASPar ^a	X60763:c.471 G > A ^c	Exon 4	213
GHR	growth hormone receptor	TATGCCAGGTAAGCGACAT/ ATTGAGTACGAGGCCCTGTG	SNP/KASPar ^a	AY292282:g.77 C > T ^b	Exon 10	209

N – total number of DNA samples; ^a KBioscience technology (www.kbioscience.co.uk) 2007;

^b Caroli et al., 2001, Prinzenberg et al., 2005; ^c Cappuccio et al., 2006.

mone receptor (GHR) genes were identified due to the KASPar technology by the KBioscience Laboratory, UK (www.kbioscience.co.uk 2007). The primers, SNPs positions as well as the total number of DNA samples per gene were described in Table 1. Results were analyzed by Chi-Square for significance using Statistical Package for Social Science (SPSS) 14.0PL for Windows (2006).

RESULTS AND DISCUSSION

LGB – the β-lactoglobulin gene

Statistical differences in both cases of frequencies of genotypes (Fig. 1) as

well as frequencies of alleles (Fig. 2) in LGB gene between goat breeds were observed ($p < 0.001$). The lowest observed heterozygosity of LGB was reported in Alpine goat, and then in Polish Fawn Improved goat, and moreover only “g:g” homozygots were presented in these two breeds. Analogous heterozygosity level was observed in goat breeds of Polish White Improved and Boer. Similar pattern of both LGB genotype and allele frequencies was observed in Saanen and Polish White Improved goats. Within the LGB gene, the observed heterozygosity occurred to be higher in Saanen, Polish White Improved and Boer goats compar-

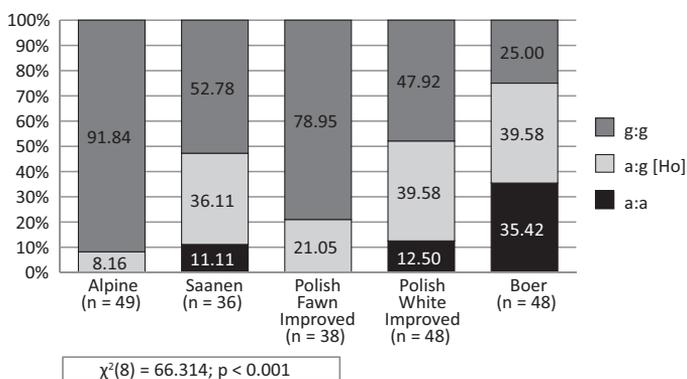


FIGURE 1. Frequencies of β-lactoglobulin (LGB) genotypes in five Polish goat breeds

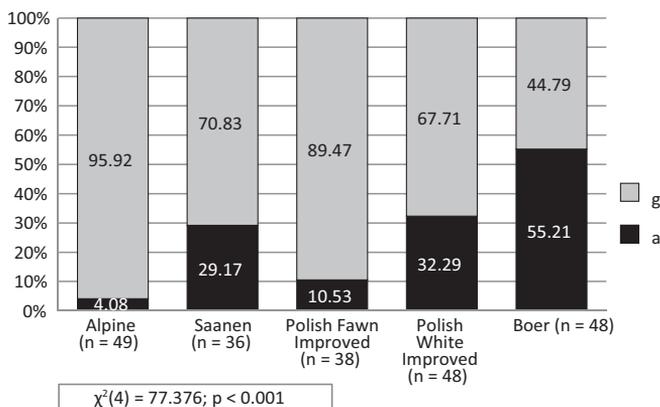


FIGURE 2. Frequencies of β-lactoglobulin (LGB) alleles in five Polish goat breeds

ing to the corresponding values (25.10%) reported by Cappuccio et al. (2006) in this gene in goats in general. The highest frequency of “g” allele were presented by goats of Alpine and Polish Fawn Improved, that may be explained by the using of the Alpine and German Noble Fawn goats in breeding schemes of Polish Fawn Improved goat and improving of its milk productivity in the past. Generally, the observed heterozigosity in LGB gene in whole examined goat breeds did not exceeded 40%, and the lowest genetic

variability was observed in goats of Alpine and Polish Fawn Improved.

CSN3 – the κ-casein gene

Statistically approved differences in both frequencies of genotypes (Fig. 3) as well as frequencies of alleles (Fig. 4) between goat breeds were observed ($p < 0.001$). The total lack of heterozigosity in Boer goats was presented and the “a:a” homozygots were only reported. The highest heterozigosity was observed in goats of Alpine and Polish Fawn Improved,

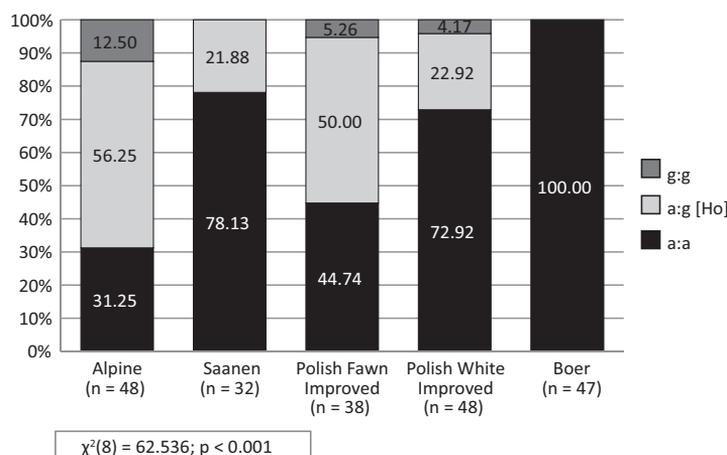


FIGURE 3. Frequencies of κ-casein (CSN3) genotypes in five Polish goat breeds

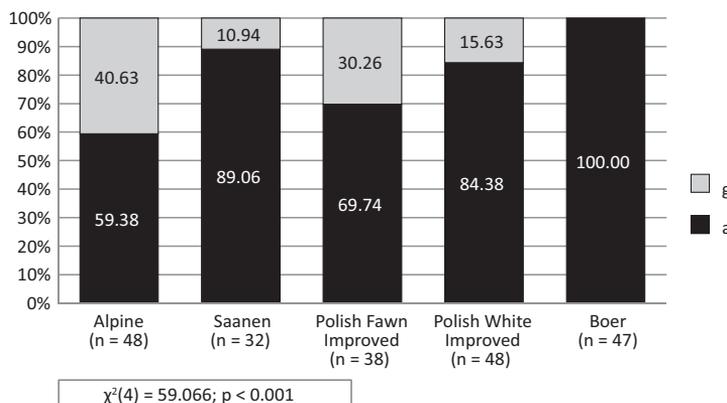


FIGURE 4. Frequencies of κ-casein (CSN3) alleles in five Polish goat breeds

which indicated the high variability of CSN3 genotypes in these two breeds. Similar tendency was observed in case of frequency of CSN3 alleles in these breeds as well. Saanen and Polish White Improved goats demonstrated high frequencies of “a:a” homozygots as well as the highest frequencies of “a” allele. In study of Cappuccio et al. (2006), the observed heterosigosity of CSN3 gene was reported at 34.60% in goats in general. The examined “a” allele corresponds to the B variant of CSN3 in goats and seemed to play an important role in milk production, because this variant was recognized to be responsible for higher milk yield as well as the higher content of proteins and caseins in milk what is very important for the cheese yield (Angulo et al., 1994; Chiatti et al., 2007). Similar tendencies were observed in the milk cattle (McLean et al., 1984; Ng-Kwai-Hang et al., 1984; Barłowska et al., 2007). Obtained results showed differences in SNP polymorphism in the CSN3 gene between the white coated goats (even the Boer goat) and the colored

coated goats. The 100% frequency of “a” allele in the Boer goat may indicate the good maternal abilities to maintain healthy and vitality offspring, probably due to the high protein and caseins content in its milk. This hypothesis would be worth to check in broader studies.

GHR – the growth hormone receptor gene

Statistical differences in both cases of frequencies of genotypes (Fig. 5) as well as frequencies of alleles (Fig. 6) in GHR gene were observed between goat breeds ($p < 0.01$). The highest heterozygosity in GHR gene was reported in Alpine goat and moreover this breed demonstrated the highest genetic variability among all examined goat breeds. The highest frequencies of “t:t” homozygots and “t” allele in Boer goats were reported. In study of Cappuccio et al. (2006), the observed heterosigosity of GHR gene was reported at 43.30% in goats in general. The polymorphism in the GHR seemed to be connected with some metabolic processes in liver (Jiang et al., 1999), but

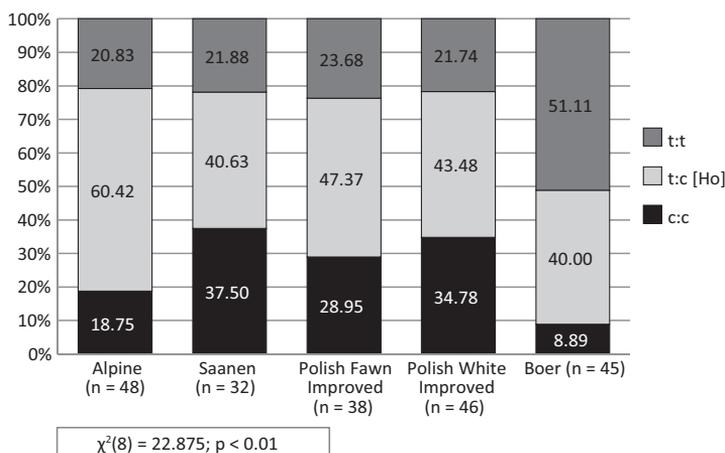


FIGURE 5. Frequencies of growth hormone receptor (GHR) genotypes in five Polish goat breeds

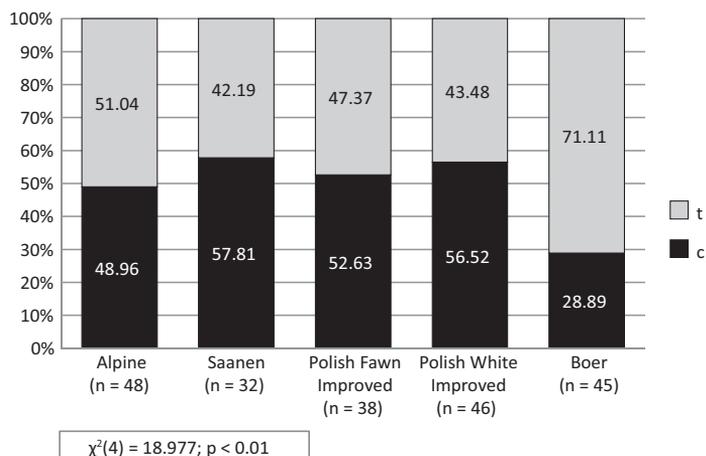


FIGURE 6. Frequencies of growth hormone receptor (GHR) alleles in five Polish goat breeds

its effect on the animal growth should be in question with other proteins involved in the growth hormone path (Cogan and Phillips 1998; Franco et al., 2005). The genetic studies carried out on polymorphisms of different regions of GHR gene in goats (Maj and Zwierzchowski, 2005; Hradecká et al., 2006) did not indicate statistically important connections with production traits, e.g. growth, milk yield or chemical composition of milk.

CONCLUSIONS

The highest frequencies of “g” allele and “g:g” homozygots in the LGB gene were observed in milk goats, especially in Alpine and Polish Fawn Improved goats, which also presented the absence of “a:a” homozygots. The high frequency of “a” allele and “a:a” homozygots in CSN3 gene were observed in Saanen, Polish White Improved and Boer goats. No “g” allele was observed in the Boer breed. The Alpine and Polish Fawn Improved goats presented higher frequency of “a” allele than “g” allele, giving the lower

frequency of “a:a” and “a:g” genotypes in CSN3 gene. In case of GHR gene, similar frequencies of both alleles and “t:t” homozygots were observed in the milk goats. The highest frequency of GHR heterozygots was observed in Alpine goats. The Boer goat expressed the remarkable genetic difference from the milk breeds within GHR gene. Considering the frequencies of alleles and genotypes of genes involved in milk production (LGB, CSN3), the three groups of goat breeds were distinguished: the colored goats (Alpine and Polish Fawn Improved), the white goats (Saanen and Polish White Improved) and finally, the meat goat (Boer goat). Obtained results in all examined genes (LGB, CSN3 and GHR) allowed to conclude that the Boer goat presents the remarkable genetic difference from the other goat breeds.

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REFERENCES

- ANGULO C., DIAZ CARRILLO E., MUNOZ A., ALONSO A., JIMENEZ I., SERRADILLA J.M., 1994: Effect of electrophoretic goat's κ -casein polymorphism on milk yield and main components yield. V. World Congress on Genetics Applied to Livestock Production, Guelph, 19, 333–336.
- BARŁOWSKA J., LITWIŃCZUK Z., KĘDZIERSKA-MATYSEK M., LITWIŃCZUK A., 2007: Polymorphism of caprine milk alphas1-casein to performance of four goat breeds. *Pol. J. Vet. Sci.* 10 (3), 159–164.
- CAPPUCCIO I., PARISET L., AJMONE-MAR-SAN P., DUNNER S., CORTES O., ERHARDT G., LÜHKEN G., GUTSCHER K., JOOST S., NIJMAN I.J., LENSTRA J.A., ENGLAND P.R., ZUNDEL S., OBEXER-RUFF G., BEJA-PEREIRA A., VALENTINI A., THE ECONOGENE CONSORTIUM, 2006: Allele frequencies and diversity parameters of 27 single nucleotide polymorphisms within and cross goat breeds. *Mol. Ecol. Notes* 6, 992–997.
- CAROLIA., JANN O., BUDELLI E., BOLLA P., JAGER S., ERHARDT G., 2001: Genetic polymorphism of goat [kappa]-casein (CSN3) in different breeds and characterisation at DNA level. *Anim. Genet.* 32 (4), 226–230.
- CHIATTI F., CHESSA S., BOLLA P., CIGNALINO G., CAROLI A., PAGNACCO G., 2007: Effect of κ -casein polymorphism on milk composition in the orobica goat. *J. Dairy Sci.*, 90 (4), 1962–1966.
- COGAN J.D., PHILLIPS J.A., 1998: Growth disorders caused by genetic defects in the growth hormone pathway. *Adv. Pediatr.* 45, 337–361.
- ECONOGENE, 2002, from www.econogene.eu
- FRANCO M.M., ANTUNES R.C., SILVA H.D., GOULARDT L.R., 2005: Association of PIT1, GH and GHRH polymorphisms with performance and carcass traits in Landrace pigs. *J. Appl. Genet.* 46 (2), 195–200.
- GE W., DAVIS M.E., HINES H.C., IRVIN K.M., SIMMEN R.C.M., 2003: Association of single nucleotide polymorphisms in the growth hormone and growth hormone receptor genes with blood serum insulin-like growth factor I concentration and growth traits in Angus cattle. *J. Anim. Sci.* 81, 641–648.
- GUS, 2011. Bank danych regionalnych, www.stat.gov.pl
- HRADECKÁ E., ŘEHOUT V., ČITEK J., 2006: The polymorphism of growth hormone receptor gene in Holstein and Czech pied bulls. *Acta fytotechnica at zootechnica – Mimoradne číslo*, 224–227.
- JIANG H., OKAMURA C.S., LUCY M.C., 1999: Isolation and characterisation of a novel promoter for the bovine growth hormone receptor gene. *J. Biol. Chem.* 274 (12), 7893–7900.
- KASPar® TECHNOLOGY KBioscience, 2007: from www.kbioscience.co.uk.
- KURYŁ J., ŻUKOWSKI M., 1997: Genetic markers in domestic farm animals (Polish) In *Animals biotechnology (in Polish)* (eds. Zwierchowski L.), PWN, Warsaw.
- LENSTRA J.A., ECONOGENE CONSORTIUM, 2005: Evolutionary and demographic history of sheep and goats suggested by nuclear, mtDNA and Y-chromosome markers. International workshop, 5–7 March 2005, Turin, Italy: „The role of biotechnology for the characterisation and conservation of crop, forestry, animals and fishery genetic resources”, Villa Gualino, Turin, Italy, 97–100.
- LUIKART G., GIELLY L., EXCOFFIER L., VIGNE J.D., BOUVET J., TABERLET P., 2001: Multiple maternal origins and weak phylogenetic structure in domestic goats. *PNAS.* 98 (10), 5927–5932.
- MAJ A., ZWIERZCHOWSKI L., 2005: A LINE-1 element insertion in the 5'-noncoding region of caprine growth hormone receptor gene. *Biochem. Genet.* 43, 465–470.
- MAJ A., ZWIERZCHOWSKI L., 2006: Molecular evolution of coding and non-coding sequences of the growth hormone receptor (GHR) gene in the family Bovidae. *Folia biologica (Kraków)*, vol. 54, no 1–2, 31–36.
- MARTIN P., OLLIVIER-BOUSQUET M., GROSCLAUDE F., 1999: Genetic polymorphism of caseins: a tool to investigate casein micelle organization. *Int. Dairy J.* 9, 163–171.
- McLEAN D.M., GRAHAM E.R.B., PONZONI R.W., MCKENZIE H.A., 1984: Effects of milk protein genetic variants on milk field and composition. *J. Dairy Res.* 51, 531–546.
- NG-KWAI-HANG K.F., HAYES J.F., MOXLEY J.E., MONARDES H.G., 1984: Association of genetic variants of casein and milk serum pro-

- teins with milk, fat and protein production by dairy cattle. *J. Dairy Sci.* 67, 835–840.
- NIMA TECHNICAL REPORT TR8350.2 (1984) Department of Defence World Geodetic System 1984, Its Definition and Relationships With Local Geodetic Systems, Third Edition, National Geospatial-Intelligence Agency.
- PIDANCIER N., JORDAN S., LUIKART G., TABERLET P., 2006: Evolutionary history of the genus *Capra* (Mammalia, Artiodactyla): Discordance between mitochondria DNA and Y-chromosome phylogenies. *Mol. Phylogenet. and Evol.* 40, 739–749.
- PRINZENBERG E.M., GUTCHER K., CHESSA S., CAROLI A., ERHARDT G., 2005: Caprine κ -casein (CSN3) polymorphism: new development in molecular knowledge. *J. Dairy Sci.* 88, 1490–1498.
- PZO, 2010. Hodowla owiec i kóz w Polsce w 2009 roku. Warszawa, 63–70.
- SŁOTA E., REJDUCH B., BUGNO M., RYCHLIK T., ZĄBEK T., 2007: Characterization of animal genetic resources using molecular genetics methods. *An. Animal Sci., Suppl.* 1, 33–42.
- SPSS 14.0PL for Windows, v. 14.0.1 (2006).
- STRZELEC E., 2008: Polimorfizm wybranych genów u ras kóz utrzymywanych w różnych warunkach środowiskowych. Rozprawa doktorska. SGGW w Warszawie, (maszynopis).
- STRZELEC E., NIŻNIKOWSKI R., 2009. Pochodzenie, znaczenie hodowlane oraz charakterystyka populacji kóz na świecie i w Polsce. *Przeg. Hod.*, 4, 7–12.
- w posiadaniu łącznie 27 stad pobrano próby krwi od kóz ras: alpejskiej (A), polskiej barwnej uszlachetnionej (PFI), saaneńskiej (S), polskiej białej uszlachetnionej (PWI) oraz od burskiej (B). Łącznie pobrano 219 prób krwi po 32–49 prób w rasie i następnie wyekstrahowano DNA genomowe wg procedury ekstrakcji zaproponowanej przez konsorcju ECONOGENE (2002). Poszczególne polimorfizmy SNP zostały określone za pomocą technologii KASPar® (KBioscience UK, 2007). Uzyskane wyniki posłużyły do określenia frekwencji genotypów i alleli w obrębie badanych genów. Wyniki zostały poddane analizie statystycznej za pomocą testu chi-kwadrat w programie SPSS v.14 (2006). Otrzymane wyniki wskazały najwyższą frekwencję homozygot „g:g” oraz allelu „g” w genie LGB u mlecznych ras kóz (A, PFI, S oraz PWI). W przypadku genu CSN3 najwyższą frekwencją homozygot „a:a”, a także allelu „a” odznaczały się kozy ras saaneńskiej, polskiej białej uszlachetnionej oraz burskiej. W genie CSN3 nie odnotowano wystąpienia allelu „g” u kóz rasy burskiej. Podsumowując, uzyskane wyniki pozwoliły wskazać trzy samowydzielające się grupy rasowe kóz: barwne kozy mleczne (A i PFI), białe kozy mleczne (S i PWI) oraz kozę mięsną (B). Ponadto kozy rasy burskiej wykazały znaczącą odrębność genetyczną w stosunku do pozostałych ras kóz w obrębie badanych genów.

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Streszczenie: *Polimorfizm SNP w genach LGB, CSN3 i GHR u pięciu ras kóz utrzymywanych w Polsce**. Celem badań było ustalenie frekwencji polimorfizmów SNP w genach β -laktoglobuliny (LGB), κ -kazeiny (CSN3) oraz receptora hormonu wzrostu (GHR) u pięciu ras kóz utrzymywanych w Polsce. Z 24 gospodarstw mających

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Effect of a herbal preparation Prisma Jet on intestinal microflora composition and meat quality of broiler chickens

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Abstract: *Effect of a herbal preparation Prisma Jet on intestinal microflora composition and meat quality of broiler chickens.* The experiment was conducted with 38,000 ROSS 308 chickens kept in two buildings of a poultry production farm. The chickens from the experimental group were receiving and addition of a herbal preparation Prisma Jet to a feed mixture. On day 42, the chickens were slaughtered and samples of their intestines were collected for microflora composition analyses and these of meat for qualitative analyses. Determinations were carried out for the counts of lactic acid bacilli *Lactobacillus* spp., bacteria of the family *Enterobacteriaceae* sp., mesophilic heterotrophs as well as yeast and filamentous fungi in digesta of the small intestine and blind gut (cecum) of the chickens. In turn, breast and leg muscles were determined for the chemical composition, physicochemical properties and fatty acid profile in the intramuscular and abdominal fat. The study demonstrated that the Prisma Jet preparation evoked a decrease in the total bacterial count in the small intestine and an increase in the count of beneficial intestinal microflora in the cecum. The preparation was also shown to improve meat quality, which was indicated by more favorable physicochemical parameters and fatty acid profile.

Key words: broiler chickens, phytobiotics, intestinal microflora, meat quality.

INTRODUCTION

The intensification of poultry production involves a higher stock density per area unit, more intensive feeding and the shortening of the rearing period of chickens. Such procedures may, however, evoke difficulties in maintaining appropriate animal husbandry conditions or in keeping a good health status of the flock. A frequent cause of those problems are disorders of the gastrointestinal system. What is more, poor zoohygienic conditions, including among others a high concentration of such toxic gases as ammonia or hydrogen sulfide, may contribute to the deterioration of the end product, which is poultry meat.

In the case of animals the gastrointestinal tract is a site linking directly the external environment with the body. Its objective is on the one hand to isolate the body from pathogenic microorganisms and on the other hand to be the site for the absorption of indispensable nutrients. The proper functioning of the gastrointestinal tract (GIT) is possible owing to specific microflora – bacteria, fungi, moulds and

protozoa, that constitute a specific biofilm. Bacteria of the genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus*, naturally occurring in the GIT, belong to the most desirable ones and serve an important function in providing the appropriate conditions (low pH, nutrients) for the growth of other positive microorganisms as well as prevent the colonization of pathogenic microflora – *Clostridium perfringens*, *Salmonella*, *Staphylococcus* or *Escherichia coli* bacteria (Gajewska et al., 2002). In order to assure the growth of positive microflora of the digestive tract many feed producing companies offer specific preparations the goal of which is to model or enhance the colonization of positive bacteria. The most commonly applied preparations of this type include: probiotics, prebiotics, acidifying and plant preparations. The latter, used mainly in the form of extracts or essential oils, prove successful in poultry production and, importantly, are characterized by the highest relative efficacy to antibiotic growth stimulants (Grela and Semeniuk, 2006). In addition, herbal plants are naturally a rich source of, among others, polyunsaturated fatty acids (including linolenic acid), whose presence in animal feeding enables simple and inexpensive modification of fatty acid profile of the intramuscular fat, which in turn improves the health-promoting properties of meat. Plant preparations, by affecting the gastrointestinal tract of birds, additionally improve digestion and thus feed conversion. As a result, smaller quantities of detrimental compounds are excreted from their bodies, which greatly improves zoohygienic conditions in a poultry house, and physicochemical characteristics of the end product, i.e. poultry meat.

The reported study was aimed at determining the effect of a herbal preparation Prisma Jet on the composition of gastrointestinal microflora and meat quality of broiler chickens.

MATERIAL AND METHODS

The experiment was conducted at a commercial farm of slaughter poultry located in the Mazowieckie Province. Numbers of chickens in flocks accounted for 18 000 and 20 000 in building I and II, respectively. The experimental material were ROSS 308 type broiler chickens kept on litter under standard conditions, following standard recommendations for poultry rearing. A factor differentiating those two groups was the addition of a Prisma Jet preparation (stimulating appetite and growth) to starter, grower and finisher type feed mixtures. Chickens from the control group (I) were fed a mixture without the addition of Prisma Jet preparation, whereas those from the experimental group (II) were administered Prisma Jet preparation with a feed mixture, at a dose of 1 kg/t of starter and grower, and 2 kg/t of finisher mixture. The Prisma Jet preparation contains natural active substances originating from plants of the poppy family (*Papaveraceae* Juss.), including: tetterwort (*Chelidonium majus* (majus) L.), bloodroot (*Sanguinaria canadensis* L.), plume poppy (*Macleaya cordata* L.), and Kelway's coral plume (*Macleaya microcarpa* L.). Its major active substances are alkaloids (3%) and derivatives of benzophenanthridine: chelidonine, sanguinarine, chelerythrine (constituting 90% of the active substances).

The chickens were fed starter (0–14 day), grower (15–35 day) and finisher (35–42 day) type feed mixtures according to the feeding program for broilers elaborated by the poultry production farm. The composition of feed mixtures administered to the chickens over the rearing period was presented in Table 1.

cooled at a temperature of 4°C, then 48 h after the slaughter, breast and thigh muscles were determined for: pH – acc. to Polish Standard PN-77/A-82058, water absorbability – acc. to Polish Standard PN-73/A-82110, free drip (free drip of juice from muscle tissue was computed from the difference between meat mass

TABLE 1. Basic composition of mixtures from analysis (%)

	Dry matter	Crude ash	Crude protein	Crude fat	Crude fiber
Starter	86.04	4.91	20.96	5.00	3.22
Grower	87.61	4.97	18.49	7.33	4.28
Finisher	87.61	4.97	18.49	7.33	4.28

Once rearing had been completed (day 42), 6 cocks and 6 hens with body weight approximating the mean body weight in the group were selected from each group for slaughter. Their intestines were sampled for quantitative and qualitative microbiological analyses of small intestine and cecum digesta. Determinations were carried out for counts of the following microorganisms: lactic acid bacilli *Lactobacillus* spp., bacteria of the *Enterobacteriaceae* sp. family (obligatorily harmful bacteria – *Salmonella* sp. and *Shigella* sp., and potentially harmful bacteria – *Escherichia coli*, *Proteus* sp. and *Klebsiella pneumoniae*), groups of mesophilic heterotrophs (*Pseudomonas* (*P. aureofaciens*, *P. fluorescens*, *P. putida*, *P. stutzeri*, *P. paucimobilis*), *Achromobacter*, *Acinetobacter* (*A. junii*, *A. lwoffii*), *Bacillus*, *Cellulomonas*, *Commomonas* (*C. acidovorans*), *Clostridium*, *Flavobacterium* (*F. multivorum*), *Staphylococcus*), as well as yeast and filamentous fungi. The analyses were conducted at the Department of Agricultural Microbiology, Warsaw University of Life Sciences. Chicken carcasses were

on the 24th and 48th hour of storage in foil bags at a temperature of 4°C, and for proximate chemical composition (dry matter and ash) – acc. to Polish Standard PN-73/A-82110. In addition, intramuscular fat and abdominal fat of the chickens were assayed for fatty acid profile acc. to Polish Standard PN-En ISO 5509, 2001. Results obtained were elaborated statistically with the analysis of variance counted with the least squares method using a statistical software SPSS 14.0 PL for Windows.

RESULTS AND DISCUSSION

Neither yeast nor filamentous fungi were detected in digesta of the small intestine and cecum in both groups. A negative result was also reached in the test for the presence of the *Salmonella* sp. genus bacteria. In the control group, despite no addition of the stimulant, counts of all analyzed microorganisms in the small intestine digesta were higher when compared with the experimental group. The group of control birds was characterized by a significantly ($P \leq 0.01$) higher count

of *Lactobacillus* sp. bacteria, those of the genus *Enterobacteriaceae* sp. and mesophilic heterotrophs as compared to the group of experimental chickens (Tab. 2).

In the case of cecum, the numbers of *Lactobacillus* sp. bacteria and mesophilic heterotrophs did not differ significantly between the control and experimental group. Only the number of *Enterobacteriaceae* sp. was significantly higher in the control than in the experimental group administered the Prisma Jet feed additive (Tab. 3).

The substantially lower count of *Enterobacteriaceae* bacteria in the cecum of experimental birds, compared to the control birds, indicates a beneficial, systematic effect of Prisma Jet over the entire rearing period. This is confirmed by the coli/lacto index indicating the number of the *Enterobacteriaceae* genus bacteria and lactic acid bacilli in the small intestine, which in this study appeared to be more beneficial in the control group. In contrast, in the case of cecum, a more positive index was recorded in the experimental group of birds receiving Prisma

Jet preparation. A study by Gajewska et al. (2009) demonstrates that the efficacy of herbal preparations is diversified and depends on several factors. Michalczyk et al. (2008) confirm the advisability of applying plant preparations and report on their positive effect on intestinal microflora.

Investigations by Ouwehand et al. (2010) conducted under *in vitro* conditions demonstrate that such substances as: carvacrol, cinnamic aldehyde, citral, limonene and thymol, suppress the colonization of *Clostridium perfringens* bacilli and *Salmonella* sp. bacteria only at a high concentration of essential oils (500 mg/l). In turn, *E. coli* bacteria and some bacilli of the genus *Lactobacillus* (*L. reuteri*, *L. fermentum*) and *Bifidobacterium* (*B. longum*, *B. breve*, *B. animalis*) were susceptible to the effects of low concentrations of essential oils – barely 5–50 mg/l. In addition, Mitsch et al. (2004) claim the reason of varied efficacy to be the use of plant preparations and type of the properties of the major active substances. In turn, Arschad et al. (2008) demonstrate

TABLE 2. Number jtk bacteria/g contents of the small intestine

Group	<i>Lactobacillus</i>	<i>Enterobacteriaceae</i>	<i>Mesophilic heterotrophs</i>
Control	$4.08 \cdot 10^5$ ^A	$1.71 \cdot 10^5$	$5.00 \cdot 10^4$
Experimental	$2.14 \cdot 10^3$ ^B	$1.14 \cdot 10^3$	$6.00 \cdot 10^3$

^{A, B} – $P \leq 0.01$

TABLE 3. Number jtk bacteria/g contents of the cecal

Group	<i>Lactobacillus</i>	<i>Enterobacteriaceae</i>	<i>Mesophilic heterotrophs</i>
Control	$8.00 \cdot 10^6$	$3.58 \cdot 10^6$ ^A	$7.23 \cdot 10^6$
Experimental	$4.32 \cdot 10^6$	$0.89 \cdot 10^6$ ^B	$4.41 \cdot 10^6$

^{A, B} – $P \leq 0.01$

that side effects are likely to occur as a result of prolonged administration of the active substance.

Results of the analyses of chemical properties of meat in cocks did not demonstrate any significant differences in the composition of their breast muscles. In contrast, a significant difference was recorded in dry matter of leg muscles (Tab. 4). In the case of hens, no significant differences were found in the chemical composition of breast nor leg muscles (Tab. 4).

The chemical composition of breast and leg muscles is a very stable characteristics, and its modification is difficult. Our previous investigations as well as those by Gornowicz (2008), and Czaja and Gornowicz (2004) point to the marginal significance of plant preparations

in modifying the composition of mainly breast muscles.

Determinations conducted in the reported experiment included: active acidity of meat (pH), free drip and water absorbability of breast and thigh muscles.

The physicochemical properties of breast muscles of cocks did not demonstrate any statistically significant differences in particular feeding groups. In the case of thigh muscles, a statistically significant difference was noted in pH value (Tab. 5).

Amongst the physicochemical properties of the breast muscles of hens a significant increase was observed in the pH value and water absorbability in the birds administered a feed mixture with the addition of a herbal preparation Prisma

TABLE 4. Chemical parameters (dry matter and crude ash) of breast and leg muscles (%)

Male	Group	DRY MATTER		CRUDE ASH	
		Breast muscles	Leg muscles	Breast muscles	Leg muscles
LSM	I	26.38	24.96 ^B	1.18	0.83
LSM	II	26.77	28.55 ^A	1.20	1.05
SE		0.91	0.576	0.02	0.09
Female					
LSM	I	28.32	26.41	1.17	0.96
LSM	II	26.84	28.62	1.19	0.99
SE		2.33	0.78	0.01	0.01

^{A, B} – $P \leq 0.01$

TABLE 5. Physical parameters of breast and leg muscles – male

		Group	pH	Free loss (%)	WHC
Breast muscles	LSM	I	5.98	1.84	81.48
	LSM	II	6.04	1.75	87.16
	SE		0.07	0.32	2.28
Leg muscles	LSM	I	6.17 ^b	1.21	87.22
	LSM	II	6.48 ^a	1.19	88.69
	SE		0.08	0.11	1.17

^{a, b} – $P \leq 0.05$

TABLE 6. Physical parameters of breast and leg muscles – female

	Group		pH	Free loss (%)	WHC
	LSM	I			
Breast muscles	LSM	I	5.87 ^b	1.82	73.32 ^A
	LSM	II	6.08 ^a	1.71	87.34 ^B
	SE		0.06	0.15	2.15
Leg muscles	LSM	I	6.29	1.44	89.60
	LSM	II	6.54	1.41	89.14
	SE		0.08	0.25	2.11

^{A, B} – $P < 0.01$; ^{a, b} – $P \leq 0.05$

Jet. In contrast, no significant differences were found in respective properties of the thigh muscles (Tab. 6).

Pietrzak et al. (2009) as well as Fiłonik and Niemiec (2002) emphasize that leg muscles are characterized by a lower pH value than the breast muscles. In addition, Pietrzak et al. (2009) demonstrated a high stability of the discussed parameter in both breast and leg muscles. However, investigations by Kim et al. (2009) and Gornowicz (2008) do not confirm that tendency, for those authors reported a decreased pH value in both breast and leg muscles after herbal additives administration to birds.

A parameter correlated with the pH value is water absorbability. Its value is higher in the muscles with a reported higher value of pH (Połtowicz, 2000). Higher water absorbability may, thus, be expected in leg muscles. This was confirmed by results of this experiment as well as by findings of Pietrzak et al. (2009) and Gornowicz (2008).

Contemporarily, investigations addressing the quality of broiler chickens meat tend to, among other things, achieve an improvement in the nutritive value through decreasing the total content of fat and cholesterol and through increasing the contribution of polyunsaturated fatty acids coupled with reach-

ing the most beneficial ratio of n-3 to n-6 fatty acids in poultry meat.

Those characteristics may be improved most of all through the modification of animal feeding and through the application of feed additives affecting lipid composition in meat (Pikul, 2009).

The herbal preparation administered to the chickens from group II affected, to a great extent, modifications of the fatty acid profile in both intramuscular and abdominal fat (Tabs 7 and 8).

Analyses demonstrated a decrease in the content of saturated fatty acids (SFA) in the intramuscular fat of breast muscles and in the abdominal fat which was additionally characterized by a higher contribution of polyunsaturated fatty acids. Both groups of the birds were also shown to be characterized by an increased contribution of n-3 PUFA acids and by the improved n-6: n-3 ratio. All those differences turned out statistically significant.

The Prisma Jet preparation evoked a significant effect on the fatty acid profile assayed in the intramuscular and abdominal fat of hens. Analyses demonstrated a lower contribution of saturated fatty acids in the intramuscular fat of their breast muscles, a higher contribution of monounsaturated fatty acids in their abdominal fat, as well as

TABLE 7. Fatty acid composition (% of total fat) of breast, leg muscles and abdominal fat – male

		Group		SFA	MUFA	PUFA	n-6	n-3	n-6: n-3
Breast muscles	LSM	I	25.018 ^A	36.230	36.061	32.093	32.093	3.968 ^b	8.097 ^a
	LSM	II	22.956 ^B	36.201	36.984	32.730	32.730	4.254 ^a	7.695 ^b
	SE		0.381	0.474	0.698	0.647	0.647	0.091	0.094
Leg muscles	LSM	I	23.594	35.824	37.233	33.511	33.511	3.722 ^B	9.007 ^A
	LSM	II	22.581	35.949	36.735	32.419	32.419	4.316 ^A	7.521 ^B
	SE		0.476	0.717	0.867	0.790	0.790	0.110	0.076
Abdominal fat	LSM	I	24.753 ^A	37.196	34.495 ^B	30.987 ^b	30.987 ^b	3.508 ^B	8.844 ^A
	LSM	II	22.690 ^B	36.756	36.680 ^A	32.462 ^a	32.462 ^a	4.218 ^A	7.701 ^B
	SE		0.270	0.371	0.466	0.409	0.409	0.063	0.113

^{A, B} – $P < 0.01$; ^{a, b} – $P < 0.05$

TABLE 8. Fatty acid composition (% of total fat) of breast, leg muscles and abdominal fat – female

		Group		SFA	MUFA	PUFA	n-6	n-3	n-6: n-3
Breast muscles	LSM	I	22,906 ^a	34,769	38,612	34,498 ^a	34,498 ^a	4,114	8,396 ^a
	LSM	II	21,936 ^b	36,458	37,455	33,288 ^b	33,288 ^b	4,167	7,990 ^b
	SE		0,243	0,365	0,458	0,363	0,363	0,051	0,119
Leg muscles	LSM	I	22,127	36,529	36,664	32,890	32,890	3,774 ^a	8,716 ^A
	LSM	II	21,804	36,622	36,770	32,654	32,654	4,116 ^b	7,935 ^B
	SE		0,435	0,407	0,702	0,639	0,639	0,082	0,041
Abdominal fat	LSM	I	21,939	36,549 ^B	37,715 ^a	33,848 ^A	33,848 ^A	3,867 ^B	8,632 ^A
	LSM	II	21,867	37,778 ^A	36,249 ^b	32,214 ^B	32,214 ^B	4,035 ^A	7,862 ^B
	SE		0,300	0,134	0,360	0,330	0,330	0,038	0,055

^{A, B} – $P < 0.01$; ^{a, b} – $P < 0.05$

an increase in the level of n-6 PUFA in the intramuscular fat of breast muscles and in the abdominal fat, and that of n-3 PUFA in the intramuscular fat of thigh muscles and in abdominal fat. Both groups of the birds were characterized by a lower n-6: n-3 ratio. All those differences were statistically significant.

Maślanko and Pisarski (2009) investigated the effect of particular medicinal plants (hop, lime, dropsy plant, mint and nettle) on the fatty acid profile of the abdominal fat in chickens. The greatest effect on the reduction of the n-6: n-3 ratio was found by those authors in the case of dried dropsy plant addition to the feed mixture.

In turn, Zuidhof et al. (2009) observed an improvement in the fatty acid profile (an increase in PUFA and a decrease in SFA level, and a lower n-6: n-3 ratio) after the addition of 17% of linseed to a standard feed mixture for poultry. The administration of a lower dose (10%) affected the fatty acid profile only to a negligible extent.

The greatest impact on the improvement of the investigated parameters was noted by Lopez-Ferrer et al. (2001), who were applying fish oil as a feed additive. Results achieved in their study demonstrate a significant increase in the content of polyunsaturated fatty acids in fat of thigh muscles and a decrease in the

n-6: n-3 ratio. Simultaneously, many authors (Bou et al., 2004; Lopez-Ferrer et al., 2001) emphasize the negative impact of a too high addition of fish oil to feed mixtures for broiler chickens on the sensory attributes of their meat (taste and aroma).

Results of this study enable stating the positive effect of plant preparations on the composition of intestinal microflora and meat quality of broiler chickens.

REFERENCES

- ARSCHAD N., NEUBAUER C., HASNAIN S., HESS M., 2008: Peganum harmala can minimize *Escherichia coli* infection in poultry, but long-term feeding may induce side effects. *Poultry Science*, 87 (2): 240–249.
- BOU R., GUARDIOLA F., TRES A., BARROETA A.C., CODONY R., 2004: Effect of Dietary fish oil, α -tocopheryl acetate and zinc supplementation on the composition and consumer acceptability of chicken meat. *Poult. Sci.*, 82, 282–292.
- CZAJA L., GORNOWICZ E., 2004: Wpływ udziału mieszanki ziół w paszy kurcząt brojlerów na wodochłonność i skład chemiczny mięśni. *Rocz. Nauk. Zoot.* 31, 77–86.
- FIŁONIK A., NIEMIEC J., 2002: Wpływ zastosowanych stymulatorów wzrostu na wyniki produkcyjne, poziom lipidów i właściwości fizykochemiczne mięsa. *Rocz. Nauk. Zoot., Supl.*, 16, 305–310.
- GAJEWSKA J., BUCKA J., ŻABIK A., RIEDEL J., MICHALCZUK M., 2009: Wpływ naturalnych preparatów roślinnych na stan mikroflory jelitowej brojlerów kurzych. *Ochrona Środowiska i Zasobów Naturalnych*, 41: 302–309.
- GAJEWSKA J., NIEMIEC J., ROKOSZ-BURLAGA H., 2002: Effect of addition of „Greenline” preparations to feed mixtures for broilers on the composition of their intestinal microflora. *Acta Microbiologica Polonica*, 51, 1: 71–78.
- GORNOWICZ E., 2008: Wpływ wybranych czynników odchowu kurcząt brojlerów na kształtowanie się cech jakościowych tuszek i mięsa. *Rocz. Nauk. Zoot. Monografie i Rozprawy*, Kraków 6–13; 26–33.
- GRELA E.R., SEMENIUK W., 2006: Konsekwencje wycofania antybiotykowych stymulatorów wzrostu z żywienia zwierząt. *Med. Wet.*, 62 (5), 502–507.
- KIM Y.J., JIN S.K., YANG H.S., 2009: Effect of dietary garlic bulb and husk on the physicochemical properties of chicken meat. *Poult. Sci.*, 88, 398–405.
- LOPEZ-FERRER S., BAUCELLS M.D., BARROETA A.C., GRASHORN M.A., 2001: n-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. *Poult. Sci.*, 80, 741–752.
- MAŚLANKO W., PISARSKI R.K., 2009: The effect of herbs on the share of abdominal fat and its fatty acid profile in broiler chickens. *Annales Uniwersytetu Marii Curie-Skłodowskiej*, 27 (3), 28–34.
- MICHALCZUK M., GAJEWSKA J., GÓRSKA A., NIEMIEC J., ROKOSZ-BURLAGA H., 2008: Efektywność preparatu roślinnego i kwasu 3-hydroksy-3-metyloasmałowego w żywieniu kurcząt brojlerów. *Roczniki naukowe Polskiego Towarzystwa Zootechnicznego*, t. 4, 2: 65–72.
- MITSCH P., ZITTERL-EGLESEER K., KOHLER B., GABLER C., LOSA R., ZIMPERNIK I., 2004: The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. *Poultry Science*, 83 (4): 669–675.
- OUWEHAND A.C., TIIHONEN K., KETTUNEN H., PEURANEN S., SCHULZE H., RAUTONEN N., 2010: In vitro effects of essential oils on potential pathogens and beneficial members of the normal microbiota. *Veterinarni Medicina*, 55 (2): 71–78.
- PIETRZAK D., MROCZEK J., GARBACZEWSKA A., FLOROWSKI T., RIEDEL J., 2009: Wpływ wybranych dodatków do paszy o działaniu przeciwbakteryjnym na jakość mięsa i tłuszczu kurcząt. *Med. Wet.* 65 (4), 268–271.
- PIKUL J., 2009: Lipidy mięsa drobiowego [w:] *Przetwórstwo mięsa drobiu – podstawy biologiczne i technologiczne. Praca zbiorowa pod redakcją Smolińskiej T., Kopcia W.*, Wydawnictwo Uniwersytetu Przyrodniczego we Wrocławiu 153–159.

POŁTOWICZ K., 2000: Wpływ początkowego poziomu pH mięśni piersiowych na wybrane wskaźniki jakości mięsa kurcząt brojlerów należących do trzech genotypów. *Rocz. Nauk. Zoot., Supl.* 98, 161–165.

SPSS 2006: SPSS, 14.0 for Windows user's guide, 2006, by SPSS Ins. USA.

ZUIDHOF M.J., BETTI M., KORVER D.R., HERNANDEZ F.I.L., SCHNEIDER B.L., CARNEY V.L., RENEMA R.A., 2009: Omega-3-enriched broiler meat: 1. Optimization of a production system. *Poult. Sci.*, 88, 1108–1120.

Streszczenie: *Wpływ ziołowego preparatu Prisma Jet na skład mikroflory jelit i jakość mięsa kurcząt brojlerów.* Badania przeprowadzono na 38 000 kurczętach ROSS 308 utrzymywanych w dwóch budynkach na fermie produkcyjnej. Kurczęta z grupy doświadczalnej otrzymywały w paszy dodatek ziołowego preparatu Prisma Jet w paszy. W 42 dniu kurczęta ubito i pobrano próby jelit do zbadania składu mikroflory oraz mięsa do analiz jakościowych. Oznaczono liczebność pałeczek kwasu mlekowego *Lactobacillus* spp., bakterii z rodziny *Enterobacteriaceae* sp., grupy mezofilnych heterotroficznych bakterii oraz drożdży i grzybów strzępkowych w treści jelita cienikiego i ślepego kurcząt. W mięśniach piersiowych i nóg określono skład chemiczny, właściwości fizykochemiczne oraz profil kwasów tłuszczowych w tłuszczu śródmięśniowym i sadelkowym.

Stwierdzono, że preparat Prisma Jet spowodował spadek ogólnej liczebności bakterii w jelicie cieniowym i wzrost liczebności pożytecznych bakterii jelitowych w jelicie ślepy. Wykazano również wpływ preparatu na poprawę jakości mięsa – korzystniejsze wskaźniki fizykochemiczne i profil kwasów tłuszczowych.

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Carcass efficiency and fatty acid content of farmed pheasants (*Phasianus colchicus*) meat

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Abstract: *Carcass efficiency and fatty acid content of farmed pheasants meat (Phasianus colchicus) meat.* 30 (15 ♂ and 15 ♀) carcasses of 16-week-old farmed pheasants were examined for meat properties and the content of fatty acids. All carcasses were dissected and 70g samples of meat tissue were taken from breast and thigh muscles. The thighs of males were significantly greater than those in females, but the perirenal fat depots were larger in females. There was a significant difference in the mass of the heart between sexes. The content of fatty acids differed significantly in male and female muscles. The muscle tissues of males contained more mono- and polyunsaturated but not the saturated fatty acids, than those of female pheasants.

Key words: pheasant, meat efficiency, fatty acids.

INTRODUCTION

The meat of pheasant (*Phasianus colchicus*) is one of the most valued by consumers. There were several introductions of varying pheasant species in Poland, therefore the availability of its meat is rather high. There are two main sources of pheasant's meat: hunting and farming (Tucak et al., 2008; Hofbauer et al., 2010). Scientific reports confirm, that releases of pheasants for hunting

purposes has a positive outcome for vegetation structure and bird communities (Draycott et al., 2008).

Markets demand pheasant's meat due to its high nutritive value, the content of high-quality protein and low fatness, considering the growing interest in healthy animal products (Kuźniacka et al., 2007). Recently, pet food producers showed an interest for new ingredients of high quality, including meats of quail, ostrich and pheasant (Rojas et al., 2011).

Kuźniacka et al. (2010) reported that there were no significant differences in meat properties from two most popular varieties of pheasants: Mongolian and common. Many authors compare the biological value of meat obtained from hunted pheasants and those reared in captivity, indicating that the natural nutrition pattern is more favored for high protein and low fat content (Tucak et al., 2004; 2008; Hofbauer et al., 2010). Nuernberg et al. (2011) noted that both, hunted and farmed pheasants accumulate more intramuscular fat in thigh than in breast muscles, however their general fatness is still lower than in black coot and wild duck.

Subsequently, the dietary effects of farming on the fatty acids content in pheasants meat are even more pronounced. Commonly accepted, as an indicator of health promoting values of the meat, n-6/n-3 polyunsaturated fatty acids (PUFA) ratio was twofold higher for farmed than that for hunted birds, and its value in thigh muscles reached 29 (vs. 10.1). This clearly shows the importance of proper nutrition regime for the meat properties of game animals reared in captivity.

Therefore the aim of presented study was to evaluate the meat effectiveness, including edible viscera content in carcasses and fatty acids content in breast and thigh muscles of farmed male and female pheasants.

MATERIAL AND METHODS

30 carcasses of 16-weeks-old farmed pheasants (15 ♂ and 15 ♀) were examined for meat properties and the content of fatty acids. From 9th to 16th weeks of life animals were fed complete dry feed (Tab. 1), and slaughtered in an abattoir on the farm.

After 24 h of cooling in 4°C carcasses were dissected and samples of meat tissue (approx. 70 g) were taken from breast and thigh muscles of the left half of the carcass. The yield of carcass and breast and leg muscles ratios were calculated on the basis of the right half of the carcass. Moreover, perirenal fat and edible viscera shares in carcasses were assessed.

The fatty acid content in intramuscular fat was according to PN-EN ISO 5509 norm – October 2001. The results were statistically developed by variance analysis, calculated by the least square method in statistical programme SPSS 14.0 PL for Windows (SPSS 2006).

RESULTS AND DISCUSSION

Table 2 presents the meat efficiency of pheasant males and females. The physiological differences in growth of both sexes demand their separate analysis.

Higher live weight (LW) was described for males and was similar to those obtained in other studies on pheasant farms (Tucak et al., 2008; Kuźniacka et al., 2010). Average LW difference

TABLE 1. The content of complete feed for pheasants

DM [%]	Crude ash [%]	Crude protein [%]	Crude fat [%]	Crude fiber [%]	Gross ME [MJ/kg]
87.13	4.66	16.86	2.38	3.68	15.47

TABLE 2. Meat efficiency of pheasants

Sex	Live weight [g]	Carcass weight [g]	Carcass yield [%]	Muscles [%]		Perirenal fat [%]
				breast	thigh	
♂	1226.20	882.30	71.90	31.20	21.60 ^A	0.13 ^B
♀	1124.90	829.50	73.50	30.70	17.90 ^B	3.80 ^A
SE	46.07	42.31	0.87	1.33	0.87	0.71

^{A, B} – significant at $P \leq 0.01$.

between male and female pheasant was 101 g (Tab. 2).

The carcass yield (CY) was slightly higher in females than that in males which is similar to results of the study on chicken (Szalkowska and Meller, 1998). But compared to other studied on pheasants, the dressing efficiency is similar which may indicate that 16 weeks of age is an optimal moment for slaughter (Adamski and Kuźniacka, 2006).

There were no major differences in the share of breast muscles in carcasses of both sexes in our study. Tucak et al. (2004) reported lower values for farmed pheasants, but due to lacking nutritional value of feed in that paper, it is hard to determine if it was the effect of feeding. In the recent study, Kuźniacka et al. (2010) report significantly higher share of breast muscles in female carcass.

Interestingly, we noted significantly lower share of thigh muscles in females than that in males (Tab. 2). The content of perirenal fat in females was also significantly higher than that in male carcasses ($P < 0.01$). This may indicate that female carcasses are less favored in terms of consumer demands for healthy meat (Nuernberg et al., 2011).

Concerning the share of edible viscera in carcasses, there were significant difference ($P \leq 0.01$) in the heart weight between male and female pheasants (Tab. 3).

TABLE 3. Edible viscera share in carcasses of pheasants [%]

Sex	Stomach	Heart	Liver
♂	2.74	0.81 ^A	2.25
♀	2.42	0.52 ^B	1.90
SE	0.15	0.04	0.16

^{A, B} – significant at $P \leq 0.01$.

The content of fatty acids in meat and fat tissues of poultry corresponds mainly to the species and may vary according to diet composition (Hargis and Van Elswyk, 1993; Pikul, 1996).

Current recommendations for increased consumption of PUFA and monounsaturated fatty acids (MUFA) base on the results of long term clinical studies (Massaro et al., 2010; Gillingham et al. 2011). Therefore, the meat markets demand high quality animal products with valued fatty acids profile.

The content of saturated fatty acids (SFA) was similar in both muscle samples. Nuernberg et al. (2011) report three-fold higher SFA content in thigh muscles of 28-weeks-old farmed pheasants. This may be the consequence of the increased *de novo* synthesis and deposition of SFA in thigh intramuscular fat of older birds and confirm the notion that the optimal slaughter time for farmed pheasants is between 16th to 20th weeks of age.

The monoenoic fatty acids level was higher in females than that in males, but the deposition of polyenoic fatty acids was greater in males, regardless of the muscle location (Tab. 4). Similar results were reported for chicken (Pietrzak et al., 2006). Poultry lipids have more valued content than that of other animal fats. It contains 60–70% of PUFA. MUFA (approx. 45–50%), like oleic acid, are desired for dietetic reasons. According to Pikul (1996), the fatty acid profile of poultry intramuscular fat depends on various reasons, with the emphasis on the content of offered feed. Birds can synthesize saturated and monoenoic fatty acids from non-lipid feeds, which are mainly palmitic with stearic and palmitoleic with oleic acids respectively. However

TABLE 4. Fatty acid content in the meat of pheasants (g/100 g)

Sex	SFA	MUFA	PUFA	PUFA n-3	PUFA n-6	PUFA n-6/n-3
breast						
♂	32.44	29.27 ^B	26.01 ^A	6.18 ^A	25.45 ^A	4.12 ^B
♀	33.51	37.22 ^A	23.73 ^B	4.06 ^B	23.25 ^B	5.72 ^A
SE	0.49	0.49	0.32	0.13	0.31	0.24
thigh						
♂	32.41 ^a	36.40 ^B	26.26 ^A	4.07 ^A	26.05 ^A	6.42 ^B
♀	30.72 ^b	40.72 ^A	22.98 ^B	2.54 ^B	22.81 ^B	9.04 ^A
SE	0.49	0.49	0.32	0.13	0.31	0.24

^{a, b} – significant at $P \leq 0.05$; ^{A, B} – significant at $P \leq 0.01$.

polyenoic acids, like linoleic (n-6) and linolenic (n-3), are not synthesized by animals and have to be supplied in diets.

In our study the PUFA content was significantly higher in males than that in females. The PUFA n-3 were preferentially deposited in male breast muscles. The difference was significant at $P < 0.01$.

The n-6/n-3 PUFA ratio values, obtained in present study were generally lower than those reported by Nuernberg et al. (2011) for farmed pheasants. With comparable tendency for higher n-6/n-3 ratio in females (5.72 and 9.04 vs 10.5 and 29.0 respectively in breast and thigh muscles) regardless of sex, it seems probable that there were other environmental factors responsible for such major disadvantageous differences. Further studies are needed to clarify these observations.

CONCLUSION

Farming of pheasants is considered diminishing regarding the nutritional values of meat (Nuernberg et al., 2011). The deliberate selection of components in complete feed production process seems advisable. However, low volume

of current consumers demands in most of east european countries may be the most limiting factor for the improvement of the nutritional value of pheasants meat.

REFERENCES

- ADAMSKI M., KUŹNIACKA J., 2006: The effect of age and sex on slaughter traits of pheasants (*Phasianus colchicus* L.). Anim. Sci. Pap. Rep. 24, 11–18.
- DRAYCOTT R.A.H., HOODLESS A.H., SAGE R.B., 2008: Effects of pheasant management on vegetation and birds in lowland woodlands. J. Appl. Ecol. 45, 334–341.
- GILLINGHAM L.G., HARRIS-JANZ S., JONES P.J.H., 2011: Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids 46, 209–228.
- HARGIS P.S., VAN ELSWYK M., 1993: Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer. World's Poult. Sci. J. 49, 251–264.
- HOFBAUER P., SMULDERS F.J.M., VODNANSKY M., PAULSEN P., EL-GHAREEB W.R., 2010: A note on meat quality traits of pheasants (*Phasianus colchicus*). Eur. J. Wildl. Res. 56, 809–813.
- KUŹNIACKA J., ADAMSKI M., BERNACKI Z., 2007: Effect of age and sex of pheasants (*Phasianus colchicus* L.) on selected physical properties and chemical composition of meat. Ann. Anim. Sci. 7(1), 45–53.

- KUŹNIACKA J., ADAMSKI M., JAROSZEWSKA A., 2010: Meat traits of pheasants from different origin and at different age. *Fleischwirtschaft Int.* 6, 75–77.
- MASSARO M., SCODITTI E., CARLUCCIO M.A., DE CATERINA R., 2008: Basic mechanisms behind the effects of n-3 fatty acids on cardiovascular disease. *Prostagl. Leukotr. & Ess. Fatty Acids* 79, 109–115.
- NUERNBERG K., SLAMECKA J., MOJTO J., GASPARIK J., NUERNBERG G., 2011: Muscle fat composition of pheasants (*Phasianus colchicus*), wild ducks (*Anas platyrhynchos*) and black coots (*Fulica atra*). *Eur. J. Wildl. Res.*, 57, 795–803.
- PIETRZAK D., MROCZEK J., ADAMCZAK L., WOJDA Ł., NIEMIEC J., 2006: The effect of an increased supplement B-group vitamins on the composition and properties of chicken meat and fat. *Animal Science, Supplement* (1), 156–157.
- PIKUL J., 1996: Lipidy mięsa drobiu. *Gosp. Mięsna* 48(7), 8–34.
- POLISH STANDARD PN-ISO 5509 (2001). Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids (in Polish).
- ROJAS M., GONZÁLEZ I., DE LA CRUZ S., HERNÁNDEZ P.E., GARCÍA T., MARTÍN R., 2011: Application of species-specific polymerase chain reaction assays to verify the labeling of quail (*Coturnix coturnix*), pheasant (*Phasianus colchicus*) and ostrich (*Struthio camelus*) in pet foods. *Anim. Feed Sci. Technol.* 169, 128–133.
- SPSS (2006) SPSS 14.0 for Windows user's guide by SPSS Ins USA.
- SZAŁKOWSKA H., MELLER Z., 1998: Wpływ wieku, genotypu i płci na wartość rzeźną kurcząt brojlerów. *Materiały konferencji naukowej: Agrobiznes w Regionie południowo-wschodniej Polski. Produkcja zwierzęca i przetwórstwo*, 53.
- TUCAK Z., ŠKRIVANKO M., POSAVČEVIĆ Š., PERIŠKIĆ M., BOŠKOVIĆ I., JUMIĆ V., 2008: The influence of keeping pheasants in captivity vs. nature on the biological value of meat and its use in human nutrition. *Coll. Antropol.* 32, 959–962.
- TUCAK Z., ŠKRIVANKO M., KRZNAŘIĆ M., M., POSAVČEVIĆ Š., 2004: Indicators of biological value of the pheasant meat originated from natural and controlled breeding. *Acta Agric. Slov., suppl.* 1, 87–91.

Streszczenie: Parametry rzeźne i zawartość kwasów tłuszczowych w mięsie bażantów (*Phasianus colchicus*) pochodzących z hodowli zamkniętej. 30 tuszek pozyskanych od 16-tygodniowych bażantów (15 ♂ i 15 ♀) poddano ocenie pod kątem paramentów rzeźnych oraz oznaczono zawartość kwasów tłuszczowych w tłuszczu śródmięśniowym. Dokonano dysekcji wszystkich tuszek oraz pobrano próbki (około 70 g) mięśni piersiowych i udowych. Masa mięśni udowych była istotnie wyższa u samców niż u samic, odwrotnie niż masa tłuszczu sadełkowego. Zaznaczyły się istotne różnice w masie serca między płciami. Zawartość kwasów tłuszczowych była istotnie różna u samców niż u samic. Stwierdzono obecność większego stężenia jedno- i wielonienasyconych kwasów tłuszczowych u samców, natomiast więcej nasyconych kwasów tłuszczowych zawierały tkanki samic bażantów.

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