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Effect of dietary arginine supplementation on body weight changes and productivity of sows

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Abstract: *Effect of dietary arginine supplementation on body weight changes and productivity of sows*. The objective of this study was to determine whether provision of additional arginine to pregnant and lactating sows could influence body weight changes in females during reproductive cycle as well as reproductive and rearing performance of the piglets. The study included 36 F1 crossbred sows (Polish Landrace × Polish Large White), including 12 primiparous (P) and 24 multiparous (M) sows, which after insemination were randomly allocated to experimental group E (6 P, 12 M) and control group C (6 P, 12 M). Sows from both groups received complete diets, which were fed to meet requirements. Sows from group E were supplemented with amino acid (L-arginine 98%) at 0.3 kg/t for pregnant sows (from 4 weeks after insemination) and at 0.5 kg/t for lactating sows. Sows from group C were not supplemented. Feeding group (C, E) had a significant effect on sow body weight at weaning ($P \leq 0.01$), while lactation (P, M) had a significant effect on sow body weight at mating and weaning, and also on body weight change during the weaning-to-mating period ($P \leq 0.01$). The number of piglets born to primiparous sows from group E versus C was higher by 1.3 piglets (11.11%) ($P > 0.05$). Weaned piglets at age 28 days, reared by primiparous sows, were significantly heavier than the progeny of multiparous sows ($P \leq 0.01$), by 0.76 kg/animal (10.08%) in group C and by 0.97 kg/animal (12.63%) in group E. Piglets from group E versus C were heavier at weaning by 0.35 kg (4.22%), when born to primiparous sows, and by 0.14 kg (1.86%), when born to multiparous sows. No interaction between variables group and lactation has been shown for any of the parameters studied. It seems appropriate to supplement pregnant and nursing primiparous sows with dietary arginine.

Key words: sows, feeding, arginine, productivity

INTRODUCTION

The increased reproductive potential and excellent reproductive performance of present-day sows, resulting from breeding progress and heterosis effect, justify the need to modify the diets to meet the growing production requirement of the females for energy and nutrients (Ji 2004, Rekiel et al. 2015, Rekiel et al. 2016). Diets have an effect on reproductive performance and reproductive longevity of the sow. Quantitative and qualitative dietary changes are introduced in successive stages of the reproductive cycle, namely gestation, lactation, and pre-estrus period. Dietary changes are also dictated by the need to maintain optimum protein and fat reserves in the body of sows during their productive life. Diets can and should be modified by supplementing, for example, synthetic amino acids (Ramanau et al. 2004, Rehfeldt et al. 2012, Wu et al. 2013, Rekiel

et al. 2014, Rutkowski et al. 2014). These supplements enhance sow productivity expressed as the number and weight of piglets born and weaned, the quantity and quality of colostrum, and body condition (Bojcuková and Kratký 2006, Beyer et al. 2007, Heo et al. 2008, Yang et al. 2009).

Rat and pig experiments attempted to determine the role of arginine in the body's life processes. Arginine was found to regulate angiogenesis and the development of blood vessels, and to have a beneficial effect on placenta and its function (Gao et al. 2012, Liu et al. 2012). Arginine is an essential substrate for nitric oxide (NO) synthesis, which points to its role in the dilation of blood vessels, thus enhancing delivery of nutrients and oxygen to the conceptus (Zhu et al. 2017). Arginine is involved in muscle metabolism and in maintenance of normal nitrogen balance, and helps to increase muscle mass and reduce adipose tissue. According to Wu et al. (2004), because arginine deficiency, due to reduced intestinal synthesis, is an important metabolic problem from the aspect of growth, increasing arginine reserves may promote neonatal growth.

Literature studies reveal that opinions on the role of arginine in reproductive processes are divided. Zeng et al. (2008) showed the dietary arginine supplementation of rats to increase embryo survival. However, Li et al. (2010) reported that 0.8% L-arginine supplemented to the diets of gilts between 1st and 25th day of gestation reduces litter size. In turn, Li et al. (2014) demonstrated that arginine added to the diet of sows between 14th and 28th day of gestation increased embryo survival.

A review of the available literature indicates that arginine was supplemented to the diets of sows at different gestation stages and during lactation (Li et al. 2010, 2014, Bass 2012, Liu et al. 2012, Quesnel et al. 2014, Zhu et al. 2017). Analysis was made of its effect on the organism, including reproductive performance – embryonic and fetal survival as well as embryonic and fetal growth and development (Bass et al. 2017), weight of piglets born (Che et al. 2013, Quesnel et al. 2014, Fonseca DeSilva 2016), colostrum and milk production and quality (Dallanora et al. 2016, Krogh et al. 2016, Zhu et al. 2017). Studies were also conducted on the effect of arginine on the body's immune response (Che et al. 2013).

Palencia et al. (2017) evaluated the effectiveness of supplementing sow diets with arginine on fetal development. After analysing the results of more than 10 experiments, the authors concluded that 53% of studies observed a positive effect of feeding arginine to the sows, as expressed by increased embryo survival and better fetal growth and development. The number and weight of piglets born increased as a result of improved placental development. Bass et al. (2017) stated that L-arginine supplementation in late gestation had no effect on the number and weight of piglets born and on rearing performance. Other studies confirmed a beneficial effect of arginine supplementation on fertility in gilts (Mateo et al. 2007, Che et al. 2013), neonatal weight in piglets (Che et al. 2013, Fonseca DeSilva 2016), chemical composition of colostrum (Krogh et al. 2016), and milk fat yield and content (Zhu et al. 2017). The application of arginine

was also reported to offer metabolic and immunological advantages (Che et al. 2013).

The aim of the study was to determine the effect of dietary supplementation of arginine to pregnant and lactating sows on their body weight changes during reproductive cycle as well as reproductive and rearing performance of the piglets.

MATERIAL AND METHODS

The study involved 36 F1 crossbred sows (Polish Landrace × Polish Large White), which were monitored for body weight changes (mating to weaning), number of piglets born and reared and their body weight, as well as the weaning-to-mating interval.

Primiparous (P) and multiparous (M) sows were divided into experimental group E (18 sows, including 6 P and 12 M) and control group C (6 P and 12 M sows).

Sows from groups C and E received complete diets prepared from the same feed materials, which were fed to pregnant and lactating sows in different proportions. The parameters characterizing the diets with regard to energy and nutrient content per kg are presented in Table 1. The diets fed to the sows from the experimental group were supplemented with amino acid (L-arginine 98%) at 0.3 kg/t for pregnant sows and at 0.5 kg/t for lactating sows. Supplemental arginine (Arg) diets were fed to the experimental sows from 4 weeks after insemination to farrowing, and then from the beginning of lactation until the termination of rearing on day 28. No supplemental Arg was fed to the control sows. Daily rations of the sows were changed according to their physiological condition (gestation, lactation). The diet was 2.2 kg/sow/day up to day 30 after insemination, 2.7 kg/sow/day from 31st to 75th day, and 3.2 kg/sow/day from 76th to 110th day of gestation. The ration was decreased to 2.2 kg/day for 4 days before farrowing, and gradually increased after farrowing for 4–5 days. Daily requirement was set at 2.2 kg for lactating sows (maintenance requirement) and at 0.5 kg for each piglet (production requirement). From day 5 after birth, piglets were supplemented with a complete loose-mix

TABLE 1. Level of energy and nutritive value of the feed mixtures for pregnant and lactating sows

Energy and nutrients	Mixture	
	pregnant sows	lactating sows
Metabolizable energy (MJ)	12.2	13.1
Crude protein (g)	145	175
Calcium (g)	7.5	5.0
Crude fibre (%)	6.5	4.2
Lysine (g)	6.5	9.2
Methionine + Cysteine (g)	4.0	5.2
Threonine (g)	4.2	5.5
Arginine (g)	0.9	0.9

diet prepared on the farm. It was made from the following feed materials: extruded barley, low-gluten winter wheat, skimmed milk powder, dried whey, feed hemoglobin, fish meal (72% CP), soybean meal HP300, and 3 supplemental acidifiers.

Sows were weighed at mating and weaning (day 28). Number of piglets born alive and reared to 28th day, litter weight at birth, and individual body weight of piglets on weaning day were recorded.

The results were statistically analysed using two-way variance analysis (IBM SPSS Statistics 24), which included the effects of feeding group (C, E), next lactation (P, M), and the interaction of both by following model:

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

where

Y – trait measured;
μ – overall mean;
a_i – effect of feeding group (i = 1, 2);
b_j – effect of lactation (j = 1, 2);
ab_{ij} – interaction (feeding group × lactation);
e_{ijk} – random error.

RESULTS AND DISCUSSION

Dietary intake per sow was around 66 kg during early gestation, 119 kg during mid-gestation, 109 kg in late gestation, and 9 kg prior to farrowing (303 kg in total). Lactating sows consumed from 145 to 165 kg of the diet depending on small differences in the appetite of the females and the number of piglets they reared.

The difference in body weight at the mating of P and M sows was significant ($P \leq 0.01$) – Table 2. No significant differences for this trait were confirmed between groups C and E. The weaning weight of the sows differed between groups C and E ($P \leq 0.01$) and between groups P and M ($P \leq 0.01$). Body weight change in the sows from weaning to mating differed significantly ($P \leq 0.01$) between primiparous and multiparous sows. Primiparous sows increased their body weight in the analysed period by 11.24% (group C) and 6.45% (group E). For multiparous sows, body weight decreased by 5.72% (group C) and 4.93% (group E).

The number of piglets born to primiparous sows from group E was higher by 1.3 piglets (11.11%) compared to group C and this result is worth noting despite the lack of significant differences between the groups. The number of piglets weaned from litter was higher by only 0.5 piglet ($P > 0.05$) in experimental compared to control primiparous sows, which resulted from the losses during four weeks of rearing. They were twice as high in group E compared to group C (difference of 0.84 piglet).

The number of piglets born alive, the weight of one-day-old piglet, and the number of 28-day-old piglets did not differ between groups E and C and between groups P and M. However, differences were observed in body weight of 28-day-old piglets between primiparous and multiparous sows. After 4 weeks of rearing, piglets from young sows were heavier than the progeny of multiparous sows ($P \leq 0.01$), by 0.76 kg/animal (10.08%) in group C and by 0.97 kg/animal (12.63%) in group E.

TABLE 2. Production results

Item	Groups of sows (G)								Significance *P*-value		
	control (C)				experimental (E)						
	primiparous (P)		multiparous (M)		primiparous (P)		multiparous (M)		group	lactation	group × lactation
	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*			
Weight of sow at mating (kg)	151.3	1.98	194.1	4.21	144.2	2.54	190.5	3.89	NS	0.001	NS
Weight of sow at weaning (kg)	168.3	0.14	183.0	2.43	153.5	2.93	181.1	2.27	0.004	0.001	NS
Body weight change of sow during the weaning-to-mating period (kg)	17.0	0.50	–11.1	3.99	9.3	2.01	–9.4	2.73	NS	0.008	NS
Number of piglets born alive on day 1 of age (head)	11.7	0.72	13.8	0.57	13.0	0.58	13.8	0.71	NS	NS	NS
Weight of piglet on day 1 of age (kg)	1.26	0.05	1.23	0.02	1.25	0.05	1.22	0.02	NS	NS	NS
Number of piglets on day 28 of age (head)	10.8	0.54	12.3	0.49	11.3	0.33	12.0	0.58	NS	NS	NS
Weight of piglet on day 28 of age (kg)	8.30	0.31	7.54	0.18	8.65	0.13	7.68	0.24	NS	0.001	NS
Rearing losses of piglets (head)	0.83	0.31	1.50	0.36	1.67	0.56	1.75	0.31	NS	NS	NS
Weaning-to-estrus period (days)	12.2	0.48	11.9	0.99	12.5	0.72	13.8	0.86	NS	NS	NS

NS – not significant ($P > 0.05$).

Compared to control piglets, experimental piglets from primiparous sows were heavier at weaning by 0.35 kg (4.22%), and those from multiparous sows by 0.14 kg (1.86%).

Piglet losses during rearing were comparable between the groups ($P > 0.05$). Pre-estrus period did not differ significantly for the compared groups E and C, and P and M (Table 2).

The differences noted in the weight of mated primiparous and multiparous sows are natural and expected. They result from the several year long somatic development of the sows. The non-significant body weight differences of the sows during reproductive cycle, which were observed between the groups, fall within normal range. Rather than being caused by the experimental factor, they result from individual variation and random assignment of animals for groups. Other than Zhu et al. (2017), there is no other study in the available literature to analyse the effect of supplemental arginine on body weight changes and fat reserves of the sows during their productive life.

Production results were often improved when supplementing higher amounts of some amino acids to the diets of pregnant and lactating sows (Ramanau et al. 2004, Heo et al. 2008, Rehfeldt et al. 2012, Kim et al. 2013, Wu et al. 2013, Rutkowski et al. 2014). In our study, young experimental (primiparous) sows produced larger litters. The findings of Yang et al. (2009) and Wu et al. (2013) show the benefits of feeding supplemental arginine to sows immediately after mating, as expressed by improved fertility. In our experiment, the diets with supplemental arginine were given to the sows three weeks after their insemination. The obtained improvement in fertility corresponds with the results of experiments by Mateo et al. (2007), Che et al. (2013) and Li et al. (2014). As reported by Quesnel et al. (2014), dietary arginine supplementation of late gestation sows had a slight effect on reducing within-litter birth variation. In the study by Che et al. (2013), arginine supplementation of sows from 30th to 114th day of gestation increased the number of piglets born alive as well as litter weight compared to sows from the control group. Arginine supplementation was also observed to offer metabolic and immunological benefits such as reduced urea levels, and elevated levels of immunoglobulins and PRRS-specific antibodies. Fonseca DeSilva (2016) supplemented L-arginine in sow diets from 30th to 60th, and from 80th to 114th day of gestation to determine the effect of amino acid supplementation on reproductive and productive capacity of the sows and their progeny. The supplementation had a positive effect on the mean birth weight of the piglets. The proportion of piglets born with body weight above 1.81 kg was higher in the sows supplemented with arginine compared to the unsupplemented control sows.

Dallanora et al. (2016), who determined the impact of supplementing lactating sows with arginine on rearing performance, body weight, and survival of the piglets, found the experimental factor to have no significant effect on piglet body weight, litter weight, and mean daily weight gains of the piglets up to 10th and 21st day of lactation.

It should be noted that primiparous sows are subjected during their productive lives to many different environmental factors, which may reduce the effect

of using dietary synthetic amino acids. This may be the reason for no improvement in fertility in multiparous sows from group E compared to group C in our experiment. The observed changes in the body weight of reared piglets, more beneficial for the progeny of young females as well as the experimental sows, may be indicative of the beneficial effect of additional arginine supplementation on their postnatal growth and development, which is known to largely depend on the sow's milk production and the quality of colostrum and milk. Our experiment did not monitor milk production and food quality. The results of few studies confirm the beneficial effect of supplementing arginine during lactation on the composition of sow colostrum (Krogh et al. 2016). In the above experiment, supplemental arginine reduced lactose content and increased dry matter content in colostrum ($P \leq 0.05$). However, the authors did not observe arginine's effect on the colostrum and milk fat content, on the production efficiency of colostrum and milk, and on the weight of piglets and their daily gains. On the other hand, they found the concentration of protein and IGF-I to increase in sows supplemented with arginine. In summing up the results, Krogh et al. (2016) concluded that supplementation of pregnant and lactating sows with arginine influences macro-chemical composition of colostrum, but has no effect on colostrum yield and on milk yield and composition. In our study, feeding supplemental arginine to late-pregnant sows (beginning of synthesis of colostrum components) and lactating sows could have a beneficial effect on rearing performance of the piglets. According to Bass et al. (2017), late supplementation of L-arginine (L-Arg) during gestation has no effect on lactational performance. It appears that additional supplementation of this amino acid (as a follow-up at 3 weeks after insemination) between 40th and 80th–90th day of gestation in our study had no considerable effect on fetal development, as confirmed by uniform mean neonatal body weight in the groups, which is supported by Bass et al. (2017). The study by Zhu et al. (2017) analysed the effect of dietary arginine supplementation on the yield of sows, litter quality, plasma metabolite and hormone concentration, and milk yield and composition in the sows. Arginine-supplemented feeds were provided during lactation. The mean daily weight gains of piglets from sows supplemented with 0.5 or 1.0% L-Arg-HCl from 3rd to 14th day of lactation (experimental groups) were higher than in the control group ($P \leq 0.05$). There was no significant effect of the supplementation on mean daily consumption of feed, body weight loss, and loss of backfat thickness in lactating sows. The supplementation of 0.5 or 1.0% L-Arg-HCl contributed to an increase in milk yield ($P \leq 0.05$) and milk fat content. Milk protein and lactose remained unchanged. It was also observed that supplementing the diet with 1.0% L-Arg-HCl increased plasma prolactin and insulin concentrations in sows between 14th and 21st day of lactation, as well as the concentrations of NEFA, insulin-like growth factor-1 (IGF-1) and nitric oxide (NO) on day 21 compared to the control group ($P \leq 0.05$). Dallanora et al. (2016) reported that supplemental arginine for lactating sows had no effect on litter size and survival, nor on the

content of amino acids and the arginine and lysine ratio in milk.

CONCLUSION

Based on the results obtained, it seems appropriate to supplement the diets of primiparous sows with arginine and to conduct further research with a greater number of animals.

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Streszczenie: *Wpływ dodatku argininy w żywieniu loch na zmiany ich masy ciała i produkcyjność***.** Celem badań było wykazanie, czy dodatkowa podaż argininy w żywieniu loch prośnych i karmiących ma wpływ na zmiany masy ciała samic w cyklu reprodukcyjnym oraz wyniki rozrodu i odchowu prosiąt. Badaniami objęto łącznie 36 loch mieszańców F1 (pbz × wbp), w tym 12 pierwiastek (P) i 24 wieloródki (M), które po inseminacji podzielono losowo na grupy: doświadczalną – E (6 P i 12 M) i kontrolną – C (6 P i 12 M). Lochy z obu grup żywiono mieszankami pełnoporcjowymi, których podaż była zgodna z zapotrzebowaniem. Dla loch z grupy E zastosowano dodatek aminokwasu (L-arginina 98%), w ilości odpowiednio: 0.3 kg/t dla loch prośnych (od 4. tygodnia po inseminacji) i 0.5 kg/t dla loch karmiących. Lochy C nie otrzymywały tego dodatku. Stwierdzono istotny wpływ grupy żywieniowej (C, E) na masę loch przy odsadzeniu ($P \leq 0{,}01$) oraz laktacji (P, M) na masę loch przy kryciu, odsadzeniu i zmianę masy w okresie odsadzenie – krycie ($P \leq 0{,}01$). Liczba prosiąt urodzonych przez pierwiastki

z grupy E w porównaniu z grupą C była większa o 1,3 prosięcia (11,11%) ($P > 0,05$). Odsadzone prosięta w wieku 28 dni, odchowywane przez lochy pierwiastki były istotnie cięższe od potomstwa loch wieloródek ($P \leq 0,01$), w grupie C o 0,76 kg/szt. (10,08%), a w grupie E o 0,97 kg/szt. (12,63%). Prosięta z grupy E w porównaniu z grupą C pochodzące od pierwiastek były cięższe przy odsadzeniu odpowiednio o 0,35 kg (4,22%), a od wieloródek o 0,14 kg (1,86%). Nie wykazano interakcji między zmienną grupa a zmienną laktacja dla wszystkich badanych wskaźników. Stosowanie dodatku argininy do paszy wydaje się być uzasadnione dla prośnych i odchowujących prosięta loch pierwiastek.

Słowa kluczowe: lochy, żywienie, arginina, produkcyjność

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Variability of cows' milk functional fatty acids level in grazing period

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Abstract: *Variability of cows' milk functional fatty acids level in grazing period.* The aim of this study was to determine variation in the content of functional fatty acids (FFA) and to examine the value of the atherogenic (AI) and thrombogenic (TI) indexes in cows' milk during the grazing season. The study was carried out in a low input farm located in Mazovia province. Milk samples were collected from 8 lactating cows, which were in a similar phase of lactation (100 ±30 day) during the 5 months grazing period (from June to October). Analysis of health-promoting compounds in examined milk lipid fraction was performed using capillary gas chromatography method. The highest concentrations of functional fatty acids of cows milk fat have been reported in September (32.91 g/100 g of fat), substantially the lowest one at the beginning and at the end of grazing period (July and October). The most desirable (the lowest) values of AI and TI indexes were achieved in September and July, respectively. The most favourable stearoyl-CoA desaturase (SCD) activity and the levels of n-6 to n-3 ratio have been presented in July. We concluded that the content of functional fatty acids in cows' milk varies significantly in particular months of grazing period, influencing its health promoting quality.

Key words: AI, cows' milk, functional fatty acids, grazing, TI, trans-vaccenic acid, ∑CLA – sum of selected isomers of conjugated dienes of linoleic acid

INTRODUCTION

Present scientific research focuses on the naturally occurring health-promoting properties of animal products and factors affecting these properties i.e. feeding (Ellis et al. 2006, Collomb et al. 2008, Kuczyńska et al. 2011a, Kuczyńska et al. 2015). Functional fatty acids are compounds of bovine milk fat of high biological value and present beneficial impact on human health. Recent studies have shown that FFA's provide anti-carcinogenic, anti-inflammatory and anti-diabetic properties and may prevent against cardiovascular diseases due to their anti-atherosclerotic properties (Taylor and Zahradka 2004, Field et al. 2009, Miciński et al. 2012).

Functional fatty acids are a group of health-promoting compounds of milk fat that include following acids: butyric acid (C4:0 – BA) inhibits DNA synthesis, exhibits anti-carcinogenic and anti-oxidative effect (Canani et al. 2012). Vaccenic acid (C18:1 t11 – TVA) inhibits proliferation of cancer/tumour cells in a tissue culture and plays significant role in maintenance of cells' membrane lipid

structure (Lim et al. 2004). Oleic acid (C18:1 c9 – OA) arises hypolipidemic, hypocholesterolemic and anti-atherosclerotic properties. Recent studies in rodent models revealed that OA reduces type 2 diabetes and alleviates symptoms of multiple sclerosis by synthesis of myelin (Natali et al. 2007, Vassilious et al. 2009).

Linoleic acid (C18:2 c9, c12 – LA) and α-linoleic acid (C18:3 c9, c12, c15 – LNA) are main essential fatty acids (EFA) – as they need to be exogenously provided to human organism. Apart from being a precursor of arachidonic acid (C20:4 – ARA), linoleic acid enhances liquidity of cell membrane. Linoleic acid is a precursor of n-3 fatty acids and is a predominating fatty acid found in grasses about hypolipidemic effect (Abedi and Sahari 2014).

The term conjugated diens of linoleic acid (CLA) refers to positional (carbon 6, 8 to 12, 14) and geometric [*cis(c)-cis*, *cis-trans(t)*, *trans-cis* and *trans-trans*] isomers of linoleic acid. Studies conducted over several years show that CLA presents range of beneficial effects on human health including anti-carcinogenic effect and modulation of immune system in animal model and allergic and to as well as inflammatory responses in humans (Collomb et al. 2008). Among the isomers of CLA – C18:2 c9, t11 – CLA9 is a predominating in respect of quantity and above mentioned health-promoting properties.

Among FFA there are important representatives of n-3 and n-6 essential fatty acids (EFA) such as: arachidonic acid (C20:4 – ARA), eicosapentaenoic acid (C20:5 – EPA), docosapentaenoic acid (C22:5 – DPA) and docosahexaenoic acid (C22:6 – DHA). Several studies indicate significance not only of the content of particular acids, but what is more important the ratio of n-6 fatty acids to n-3 fatty acids in foodstuff (Simopoulos 2002). The high fatty acids ratio of n-6 to n-3 presents hypocholesterolemic effect. Most recent studies suggest the n-6 fatty acids to are important factors in depression treatment (Deacon et al. 2017).

Recently, the role of milk and dairy products in human nutrition is a subject of discourse among scientists (Mills et al. 2011). Several studies confirmed that milk obtained from grazing cows was characterized by more preferable composition in the terms of nutritional value, regarding the level of functional fatty acids (Butler et al. 2011, Kuczyńska et al. 2011b, Rutkowska et al. 2015). This is caused by the feeding regime at the traditional farms and higher biological value of pasture e.g. higher proportion of herbs and clovers compared to non-organic ones (Collomb et al. 2008). The content of FFA in cows' milk including CLA and polyunsaturated fatty acids (PUFA) are mostly influenced by the dietary factor e.g. feeding regime and botanic composition of pasture, that differs throughout grazing season in Poland (Puppel et al. 2017).

Stearoyl-CoA desaturase (SCD) is an endogenous enzyme catalyzing the introduction of the first *cis*-double bond in the Δ-9 position (between carbons 9 and 10) in several fatty acyl-CoA substrates, including the process of synthesis of C18:2 c9, t11 (CLA9) from VA (Kuczyńska et al. 2015).

Atherogenic index (AI) indicates the ratio of selected saturated fatty acids (C14:0, C16:0 and C18:0) to selected

unsaturated fatty acids, which contribute to the adhesion of lipid cells and aggregation of plaque in blood vessels there-by induce or prevent against cardiovascular diseases. Thrombogenic index (TI) refers to the ratio of the sum of main saturated fatty acids and selected unsaturated fatty acids. It exhibits the tendency to produce clots in blood vessels. The level of aforementioned acid as well as AI and TI indices value are indicators of anti-atherosclerotic value of cows' milk fat (Ulbricht and Southgate 1991, Garaffo et al. 2011, Abedi et al. 2014).

The aim of this study was to determine variation in the content of functional fatty acids (FFA) and to examine the value of atherogenic (AI) and trombogenic (TI) indexes in cows' milk during the grazing season. The secondary objective was to present changes of AI and TI values and the activity of SCD enzyme during grazing period.

MATERIAL AND METHODS

Animals

Forty milk samples were collected from 8 Polish Holstein-Friesian cows, similar in days in milk, stage of lactation (100 ±30 days) kept at low input farm located in Mazovian province. The experiment was conducted during the grazing period with *ad libitum* access to green forage. Individual milk samples were collected during pasture feeding season 5 fold at a monthly intervals (from June to October). The cows were milked daily at evening milking at 16:30. Milk samples were transferred to the sterile bottles, preserved with Mlekostat CC, and immediately submitted to the Cattle Breeding Division Milk Testing Laboratory of Warsaw University of Life Science – SGGW for the fatty acids content analysis.

Chemical analysis

To examine the milk fatty acid composition, fat was extracted according to the Röse–Gottlieb procedure (AOAC 1995), at room temperature. Amount of 60 mg of crude fat were subjected to fatty acid methylation according to the transesterification method by EN ISO 5509:2000. Analysis of fatty acid methyl esters (FAME) was carried out with a gas chromatograph (GC instrument type 6890A Agilent) with a flame-ionization detector, using a Varian CP-SIL 88 fused-silica capillary column (100 m in length, 0.25 mm in diameter and 0.20 μm film thickness). Modified capillary gas chromatography was used to determine the profile of milk's 40 fatty acids – from butyric acid (C4:0) to docosahexaenoic acid (C22:6), according to Kuczyńska et al. (2015) methods. The separation of 1 μl of each sample was performed at pre-programmed temperature: column temperature was kept at 130°C for 1 min, then increased to 175°C at rate of 5.5°C/ /min (kept at 175°C for 8 min), and then to 215°C at rate of 2.75°C/min, and was kept at 215°C for 10 min. Subsequently, the temperature increased to 230°C at rate of 20°C/min and was kept at 230°C for 10 min. Total run time was 52.477 min. Purified helium was used as a carrier gas, with a head pressure of 49.6 kPa and a constant column flow of 1.5 ml/min. The flow rate of the carrier gas was average velocity to 23.535 cm/s. The injection system (Agilent Technologies type G 4513A) used a split ratio of 1 : 100

and an injector temperature 180°C. The detection has been performed by detector (FID), and the temperature was set at 250°C. Peaks of individual fatty acids were identified by using time retention of pure fatty acids standards: PUFA 1 from marine, composed of 18 fatty acid standards from C14:0 to C24:1 and with individual C18:1 t11 and CLA standards (Sigma-Aldrich). Additionally, selected saturated and monounsaturated fatty acids were analyzed in order to formulate index of activity of stearoyl-CoA desaturase enzyme as an indicator of endogenous precursors of unsaturated fatty acids in milk. Activity of SCD enzyme was assessed and calculated as proposed by Kuczyńska et al. (2015), using the following formula: (C16:1 + C18:1) / / (C14:0 + C16:0 + C18:0 + C16:1 + + C18:1).

From among examined fatty acids following families has been detected:

SFA = (C4:0 + C6:0 + C8:0 + C10:0 + + C12:0 + C14:0 + C15:0 + C16:0 + + C17:0 + C18:0 + C20:0 + C24:0)

MUFA = (C10:1 c9 + C12:1 c9 + C14:1 c9 + C15:1 + C16:1 c9 + C17:1 c9 + + C18:1 c9 + C18:1 t9 + C18:1 c11 + + C18:1 t11 + C18:1 c12 + C18:1 c15)

PUFA = (C18:2 c9,t11 + C18:2 t10, c12 + C18:2 c9, t13 + C18:2 t16, c14 + C18:2 t9, t12 + C18:3 + C20:3 + C20:4 + C20:5 + + C22:5 + C22:6)

n-3 = (C18:3 + C20:3 + C20:5 + C22:5 + + C22:6)

n-6 = (C18:2 + C18:3 + C20:4)

The formula used to establish AI (according to Ulbricht and Southgate 1991):

AI = [C12:0 + (4 × C14:0) + C16:0] / / [ΣMUFA + Σn-6 + Σn-3]

The formula used to establish TI:

TI = (C14:0 + C16:0 + C18:0) / [(0.5 × × MUFA) + (0.5 × n-6) + (3 × n-3) + + (n-3 / n-6)]

Statistical analysis

The data obtained were analyzed statistically using analysis of variance (least squares) by using the SPSS 23.0 packet software.

The statistical model:

$$Y_{ijk} = \mu + A_i + e_{ij}$$

where:
Y_{ijk} – dependent variable;
μ – overall mean;
A_i – month effect (June, July, August, September, October);
e_{ij} – residual error.

RESULTS AND DISCUSSION

The nutritional requirements of the animals and the nutritive value of their diet were calculated following the nutrition standards set out in the INRATION 4.0 software. Table 1 summarizes the feeding characteristics during grazing period, from June to October. In the scheme of the experiment included a percentage of legume seeds: white clover (*Trifolium repens*), red and bastard clover in the pasture was 30%; however, grasses

TABLE 1. Chemical composition and nutritional value of pastures (% of DM)

Trait	Grazing period				
	June	July	August	September	October
Ash	8.33	8.81	8.89	8.98	8.21
Crude protein	18.52	18.82	19.23	18.85	14.20
Ether extract	3.89	4.13	3.56	3.54	2.40
Crude fiber	26.62	28.62	29.01	29.85	27.82
Unit of milk production (balance %)	0.84	0.91	0.89	0.88	0.85
Protein digested in the small intestine when rumen-fermentable nitrogen is limiting	115.23	115.75	102.56	101.90	88.96
Protein digested in the small intestine when rumen-fermentable energy is limiting	96.53	95.62	93.24	93.20	84.92
Neutral detergent fiber	48.56	52.84	45.63	49.58	42.30
Acid detergent fiber	31.26	40.30	28.96	28.95	30.40

dominated: *Lolium perenne* (40%), *Festuca rubra* L. (20%) and *Dactyllis glomerata* L. (10%).

The basic content and percentage of fatty acids in milk have been the subject of many studies, but the literature directly related to our research is limited (Capuano et al. 2014). Table 2 presents the effect of the month on the content of functional fatty acids (FFA), which varied significantly through the grazing period, with the highest concentration (32.91 g/100 g of fat) in September and the lowest in October (25.68 g/100 g of fat). Predominating in the quantity of all FFA was oleic acid (C18:1 c9), which the highest amounts have been reported in September (26.47 g/100 g of fat). The rapid decrease of its level was observed in October (19.65 g/100 g of fat) – Table 2. Two-year study of Stergiadis et al. (2015) comparing 3 pasture-based systems of milk production also showed the lowest OA level in October milk samples (21.4 g/100 g of fat). While the highest amount was observed in March (23.4 g/100 g of fat). Kuczyńska et al. (2011a) stated no significant differences in OA content in summer feeding season (June – July). Additionally, the highest level of butyric acid (C4:0 – BA) was achieved in June (2.22 g/100 g of fat), while in September samples presented the lowest concentration (1.67 g/100 g of fat; $P \leq 0.05$). Trans-vaccenic acid (C18:1 t11 – TVA) concentration was characterized by the highest value in August milk (1.83 g/100 g of fat), and achieved the lowest level in October samples (0.71 g/100 g of fat). A similar trend was also observed by Stergiadis et al. (2015) with TVA values of 0.30 and 0.23 g/100 g of fat noted August and October, respectively.

Studies conducted over several years, regarding biological value and quality of milk obtained from different management systems, show that milk from organic and

TABLE 2. Changes in the concentration of functional fatty acids in cow's milk during grazing period (g/100 g of fat)

Functional fatty acid	Grazing period									
	June		July		August		September		October	
	LSM	*SEM*	*LSM*	*SEM*	*LSM*	*SEM*	*LSM*	*SEM*	*LSM*	*SEM*
C4:0 (BA)	2.22^{a}	0.154	1.96	0.164	2.15	0.164	1.67^{a}	0.195	1.75	0.178
C18:1 t11 (TVA)	1.40^{aB}	0.142	1.33^{cd}	0.152	1.83^{acEF}	0.152	1.11^{E}	0.180	0.71^{BdF}	0.164
C18:1 c9 (OA)	21.52^{ab}	1.129	25.65^{aC}	1.207	24.16^{dE}	1.207	26.47^{b}	1.429	19.65^{CdE}	1.304
C18:2 c9, c12 (LA)	1.27	0.088	1.24	0.094	1.22	0.094	1.21	0.112	1.24	0.102
C18:3 c9, c12, c15 (LNA)	0.87^{A}	0.046	0.82^{B}	0.049	0.83^{C}	0.049	0.81^{D}	0.058	0.57^{ABCD}	0.053
C20:4 (ARA)	0.10^{a}	0.046	0.10^{b}	0.049	0.10^{c}	0.049	0.12	0.058	0.14^{abc}	0.053
C20:5 (EPA)	0.04^{A}	0.006	0.05^{B}	0.006	0.05^{C}	0.006	0.05^{D}	0.007	0.09^{ABCD}	0.007
C22:5 (DPA)	0.07^{aB}	0.005	0.07^{Cd}	0.005	0.07^{E}	0.005	0.09^{aC}	0.006	0.10^{BDE}	0.006
C22:6 (DHA)	0.07^{AB}	0.010	0.08^{Cd}	0.011	0.08^{EF}	0.011	0.01^{ACE}	0.013	0.13^{BdF}	0.012
∑CLA	1.25^{ABCd}	0.013	1.34^{Ae}	0.014	1.40^{Bef}	0.014	1.37^{Cg}	0.017	1.30^{dfg}	0.015
∑FFA	28.81^{A}	1.261	32.64^{B}	1.348	31.79^{C}	1.348	32.91^{D}	1.595	25.68^{ABCD}	1.456

Means (*LSM*) in the rows marked with the same letter differ significantly $^{A\text{-}F}P \leq 0.01$; $^{a\text{-}f}P \leq 0.05$.

BA – butyric acid; OA – oleic acid; TVA – trans-vaccenic acid; LA – linoleic acid; LNA – α-linolenic acid; ARA – arachidonic acid; EPA – eicosapentaenoic acid; DPA – docosapentaenoic acid; DHA – docosahexaenoic acid; ∑CLA – sum of selected isomers of conjugated dienes of linoleic acid; ∑FFA – sum of BA, OA, TVA, LA, LNA, ARA, EPA, DPA, DHA.

low input dairy farms presented higher concentration of TVA compared with conventional ones. For example, Butler et al. (2008) showed variable levels of TVA in low input non-certified organic dairy farms (4.19 g/100 g of fat), certified organic (3.55 g/100 g of fat) and in high input production system (2.25 g/100 g of fat). Tunick et al. (2015), during spring – summer periods in organic and conventional farms, determined values of TVA – 3.21 and 2.49 g/100 g of fat, respectively. The similar results were obtained by Ellis et al. (2006) in organic milk and conventional milk TVA level 2.06 and 1.75 g/100 g of fat respectively (in average values of 12-month collecting). However, Kuczyńska et al. (2011) reported in the summer feeding season, TVA levels fluctuating between 6.90 and 2.31 g/100 g of fat for different treatment.

In present study, only the levels of LA did not differ significantly during grazing period. However significant differences were observed in the α-linoleic acid (LNA) content, with the lowest value reported in October (0.57 g/100 g of fat) and the highest in June, July and August (0.87, 0.82 and 0.83 g/100 g of fat, respectively, at $P \leq 0.05$). On the other hand, Tunick et al. (2015) showed different LA and LNA content in milk samples obtained during spring – summer season from conventional (LA – 3.66 g/100 g, LNA – 0.63 g/100 g of fat) and organic farms (LA – 2.71 g/100 g, LNA – 0.79 g/100 g of fat). Furthermore, Kuczyńska et al. (2011) demonstrated different values for LA (1.48 g/100 g of fat) and LNA (0.914 g/100 g of fat).

The obtained data showed that the content of CLA isomers exhibited sinusoidal trend with significantly the highest level in August (1.40 g/100 g of fat) and the lowest one in June (1.25 g/100 g of fat). The similar result has been obtained by Lipiński et al. (2012) in August samples containing 1.68 g/100 g of CLA (C18:2 c9, t11; rumenic acid). Butler et al. (2011) reported significant ($P < 0.01$) influence management (organic vs. conventional) and the season (summer vs. winter) on the concentration of CLA. They found in organic farms higher level of rumenic acid – 0.81 g/100 g of fat in comparison with conventional ones – 0.47 g/100 g of fat during summer feeding season.

In Butler et al. (2011) study the content of CLA10 has been not influenced by the management (0.05 g/100 g of fat), but has been differed through the seasons ($P \leq 0.01$) obtaining values in summer and winter feeding season of 0.04 and 0.06 g/100 g of fat, respectively. The similar trend has been noted in the case of arachidonic acid (C20:4 – ARA) with the level of 0.12 g/100 g of fat both in organic and conventional system of production and values of 0.14 and 0.11 g/100 g of fat in summer and winter season.

In current study, gradual increase in the content of ARA through grazing period has been observed, with significantly the highest level in October (0.139 g/100 g of fat; $P \leq 0.01$). Highly significant variation in the concentration of eicosapentaenoic acid (C20:5 – EPA) and docosapentaenoic acid (C22:5 – DPA) has been reported, with the highest values obtained in October (0.087 and 0.099 g/100 g of fat, respectively; $P \leq 0.01$). While the lowest levels were found in milk derived in June (0.042 and 0.069 g/100 g of fat, respectively). In

study Butler et al. (2011), highest concentration – 0.162 g/100 g of fat – of docosahexaenoic acid (C22:6 – DHA) was found in September and significantly ($P \leq 0.01$); lower content was observed in June (0.074 g/100 g of fat). In the study mentioned before, DHA value remained on the same level regardless of the management and season of feeding.

The total content of n-3 and n-6 fatty acids did not differ significantly throughout the pasture season, however, the ratio of n-6 to n-3 exhibited significant ($P \leq 0.01$); variability with the highest value obtained in June samples (2.26 g/100 g of fat) and the lowest (1.92 g/100 g of fat) in July (Table 2). Stergiadis et al. (2015) also showed no significant differences in the group of n-3 acids, although the n-6 ($P \leq 0.01$) acids and ratio of n-6 to n-3 ($P \leq 0.01$) differed across the pasture season. The highest levels of both components were observed in March (1.7 and 2.4 g/100 g of fat, respectively) and in October (1.5 and 2.3 g/100 g of fat, respectively) while the lowest level was noted in August (1.25 and 1.7 g/100 g of fat, respectively).

The activity of SCD varied through grazing season with the highest values reported in July, slightly lower in September and the lowest in October. Similar trend was observed in the concentration of α-linoleic acid

TABLE 3. Changes in the concentration of selected families of fatty acids and in the values of stearoyl-CoA desaturase during grazing period

Selected families of fatty acids	Grazing period									
	June		July		August		September		October	
	LSM	*SEM*	*LSM*	*SEM*	*LSM*	*SEM*	*LSM*	*SEM*	*LSM*	*SEM*
∑SFA	66.54	1.576	62.60^{a}	1.685	65.44	1.685	63.96	1.993	69.45^{a}	1.819
∑UFA	30.83^{ab}	1.365	34.99^{ac}	1.459	34.16^{d}	1.459	35.48^{be}	1.726	29.17^{cde}	1.576
∑MUFA	28.34^{a}	1.261	32.29^{aB}	1.348	31.74^{C}	1.348	32.28^{D}	1.595	25.42^{BCD}	1.456
∑PUFA	4.50^{ABC}	0.207	4.64^{dE}	0.221	4.77^{Adf}	0.221	5.00^{BEf}	0.261	4.87^{C}	0.239
∑n-6	2.48	0.084	2.38	0.089	2.57	0.089	2.63	0.106	2.58	0.097
∑n-3	1.10	0.055	1.24	0.059	1.15	0.059	1.24	0.069	1.27	0.063
n-6/n-3	2.26^{ABC}	0.069	1.92^{AD}	0.073	2.23^{DeF}	0.073	2.12^{Be}	0.087	2.03^{CF}	0.079
SCD	0.298	0.015	0.341^{A}	0.016	0.323^{b}	0.016	0.337^{c}	0.019	0.272^{Abc}	0.017

Means (*LSM*) in the rows marked with the same letter differ significantly $^{A-F}P \leq 0.01$; $^{a-f}P \leq 0.05$.
SFA – saturated fatty acid, UFA – unsaturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; SCD – stearoyl-CoA desaturase.

TABLE 4. Atherogenic and thrombogenic properties of milk during grazing period

Trait	Grazing period									
	June		July		August		September		October	
	LSM	*SEM*	*LSM*	*SEM*	*LSM*	*SEM*	*LSM*	*SEM*	*LSM*	*SEM*
AI	2.71^{ab}	0.091	2.25^{aC}	0.097	2.37^{D}	0.097	2.24^{bE}	0.115	3.18^{CDE}	0.105
TI	1.77^{a}	0.098	1.51^{abcD}	0.105	1.63^{b}	0.105	1.63^{c}	0.125	2.06^{D}	0.114
C12:0	3.27^{AB}	0.187	2.30^{AC}	0.200	2.73^{de}	0.200	2.09^{BdF}	0.236	3.35^{CeF}	0.216
C14:0	11.06^{aB}	0.428	9.55^{aC}	0.458	10.19^{d}	0.458	8.87^{BE}	0.541	12.06^{CdE}	0.494
C16:0	30.14^{a}	1.148	29.28^{B}	1.227	30.25^{e}	1.227	27.84^{D}	1.451	34.65^{aBcD}	1.325
C18:0	12.25^{A}	0.759	13.48^{Bc}	0.811	13.19^{De}	0.811	17.80^{ABDF}	0.960	10.23^{ceF}	0.876

Means (*LSM*) in the rows marked with the same letter differ significantly $^{A-F}P \leq 0.01$; $^{a-f}P \leq 0.05$.
AI – atherogenic index; TI – thrombogenic index.

(LNA), with the lowest concentration recorded in October. The highest content of monounstaurated fatty acids (MUFA) was noted in July (32.29 g/100 g of fat), slightly lower in August (32.29 g/100 g of fat) and the lowest in October (25.42 g/100 g of fat). Differences in the concentration of polyunsaturated fatty acids (PUFA) were statistically significant ($P \leq 0.01$). Similar results for MUFA has been reported Stergiadis et al. (2015), with the lowest level (26.5 g/100 g of fat) observed in October.

The lowest value of both AI and TI indexes was observed in September (2.24) and in July (1.51). While the statistically significant the highest levels of these indices were found in October (3.18 and 2.06, respectively) along with the highest content of ARA, EPA, DPA, DHA – Table 2. In the study of Stergiadis et al. (2015) the lowest values of AI (2.42) has been reached from March to October.

CONCLUSION

Fatty acid composition of cows' milk has been varied through grazing period. The most favorable profile of cows' milk lipid fraction has been obtained in July samples, including the highest content of FFA and ΣCLA. Values of AI and TI indices achieved the lowest level in September and in July, respectively, improving nutritional value of milk. We concluded that the content of functional fatty acids in cows' milk varies significantly in particular months of grazing season, influencing its' health promoting quality.

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Streszczenie: *Zmienność koncentracji funkcjonalnych kwasów tłuszczowych w mleku w okresie żywienia pastwiskowego krów.* Celem pracy było określenie zmienności zawartości funkcjonalnych kwasów tłuszczowych (FFA) oraz zbadanie wartości wskaźnika miażdżycowego (AI) i wskaźnika trombogennego (TI) w mleku krowim w poszczególnych miesiącach sezonu pastwiskowego. Badanie zostało przeprowadzone w gospodarstwie niskonakładowym, zlokalizowanym na terenie województwa mazowieckiego. Próbki mleka pobierano od 8 krów będących w zbliżonej fazie laktacji (100 ±30 dni) w ciągu 5 miesięcy sezonu pastwiskowego (od czerwca do października). Najwyższe stężenie funkcjonalnych kwasów tłuszczowych w tłuszczu mlekowym wykazano we wrześniu (32,91 g/100 g tłuszczu), znacznie niższe na początku i pod koniec sezonu wypasania (czerwiec i październik). Podobne tendencje odnotowano w poziomie ΣCLA (1,40 g/100 g tłuszczu). Najbardziej pożądane (najmniejsze) wartości AI i TI uzyskano w lipcu. Dodatkowo stosunek kwasów n-6 do n-3 osiągnął w lipcu najkorzystniejsze wartości. Konkludując, monitorowanie profilu kwasów tłuszczowych w trakcie żywienia pastwiskowego potwierdza wysoką jakość prozdrowotną mleka pozyskanego przy zastosowaniu tradycyjnego modelu żywienia w okresie letnim.

Słowa kluczowe: AI, mleko krowie, funkcjonalne kwasy tłuszczowe TI, kwas trans-wekanowy, ΣCLA – suma wybranych izomerów skoniugowanego kwasu linolowego

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Polish crossbred pigs' blood haematological parameters depending on their age and physiological state

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Abstract: *Polish crossbred pigs' blood haematological parameters depending on their age and physiological state*. Based on the assumption that factors such as age or physiological status may have a significant effect on the haematological parameters of pigs' blood, the aim of this paper was to present how the values of basic haematological parameters of the blood of crossbred Polish Landrace × Polish Large White pigs differ depending on age or physiological state (pregnancy or lactation). We estimated red blood cell parameters, i.e. Ht, Hb, RBC, MCHC, MCH and MCV, and white blood cell indices (WBC and the percentage of each white blood cell type in the total count) in the blood of piglets (n = 335), weaners (n = 563), fatteners (n = 446), gilts (n = 120) and sows (n = 203) during gestation and lactation. The blood parameters in the growing pigs were characterized by a linear increase with the age of the pigs: RBC (5.79, 6.33, 6.81 and 7.14 10^{12} l^{-1}), Hb (5.71, 6.52, 7.55 and 8.21 mmol·l^{-1}) and Ht (0.31, 0.33, 0.35 and 0.39 l·l^{-1}) in piglets, weaners, 'Grower' fatteners and 'Finisher' fatteners, respectively. WBC was lower ($P \leq 0.05$) in piglets than in weaners and both groups of fatteners (13.36, 17.14, 21.06 and 18.03 · 10^9 l^{-1}, respectively), with linearly increasing LYM and a decreasing proportion of NEU in the leukocyte population. In sows, red blood cell parameters showed only slight fluctuations during gestation and lactation, whereas WBC was significantly higher, with lower LYM and higher NEU counts in the blood of sows in early pregnancy and in postpartum sows in comparison with sows in advanced gestation and lactating sows. The results confirmed that haematological parameters are substantially affected by the animals' age and physiological state. Knowledge of these relationships and the presented data will facilitate correct interpretation of the results of analyses of pigs' blood.

Key words: pig, blood, haematological parameters, technological groups

INTRODUCTION

Blood components participate in a wide range of biochemical and physiological activity in the body, and therefore their analysis is one of the most efficient and common means of determining the functionality of the body and to detect and/or predict possible disorders (Friendship and Henry 1996, Klem et al. 2010).

Many factors may influence these parameters in a given species – genotype, age, sex, technological group, and environmental conditions, especially nutrition (von Borell et al. 2007, Adenkola et al. 2009, Czech et al. 2009, Rekiel et al. 2011, Mayengbam and Tolenkhomba 2015). The physiological state of the animal is also associated with a number

of significant changes in blood parameters (Faustini et al. 2000, Czech and Grela 2002). Reference ranges for pigs in the available literature rarely take into account technological groups, including age, physiological state and breed. This makes it more difficult to interpret results and to draw correct conclusions regarding the health or body condition of pigs.

Based on the assumption that factors such as age or physiological status have a significant effect on the haematological parameters of pigs' blood, the aim of this paper was to present how basic haematological parameters of the blood of Polish Landrace × Polish Large White (PL × PLW) crossbred pigs differ depending on their age or physiological state (pregnancy or lactation).

MATERIAL AND METHODS

The data constituting the material for this study were obtained in experiments approved by the Local Ethics Committee (33/2006 of 04.07.2006; 4/2009 of 20.01.2009; 7/2009 of 20.01.2009; 31/2010 of 16.02.2010; 35/2011 of 07.06.2011; 6/2012 of 17.01.2012).

Location and experimental design

All results used in this publication originate from trials carried out at the Institute of Animal Nutrition and Bromatology and the Department of Biochemistry and Toxicology of the University of Life Sciences in Lublin in 2005–2014.

The studies were performed on Polish Landrace × Polish Large White (PL × × PLW) crossbred pigs. These were growing pigs, including 335 piglets, 563 weaners and 446 fatteners, as well as 120 gilts and 203 sows during gestation and lactation. Piglets from each sow were weighed and underwent tooth clipping, tail docking, and castration. After the 28-day rearing period, they were housed in pens, 25–30 each, until the end of the fattening period. The gilts and sows were penned in groups of 10 in early pregnancy, in pairs in late pregnancy, and individually during farrowing and lactation.

Nine experimental groups were formed: piglets – P; weaners – W; fatteners in the growing period – G; fatteners in the finishing period – F; gilts – GL; sows in early pregnancy – SEP; sows in late pregnancy – SLP; postpartum sows – PS; and lactating sows – LS. The piglets were given Pre-starter feed for 2–3 weeks before weaning and for 2 weeks after weaning, and Starter feed until they reached a body weight (BW) of 28–30 kg. The fatteners were fed Grower (31–60 kg BW) and Finisher feed (61–110 kg BW). The sow groups comprised both gilts and adult sows. The nutritive value of feeds for all groups was in compliance with the nutrient requirements of pigs (NRC 1998). All animals except pregnant sows were fed *ad libitum* and all had free access to water. The animals were housed on grates.

The conditions in the pig unit were in accordance with welfare standards (Marchant-Forde 2009). The animals were under continual veterinary supervision.

Collection of blood samples for analyses

Blood was collected for analysis on the following days: piglets (P) – 21st day of age, about 6–7 kg BW; weaners (W) – at about 25–30 kg BW; fatteners (G) – at

about 55–60 kg BW; fatteners (F) – at about 95–100 kg BW; gilts (GL) – directly before first mating (about 130 kg); sows in early pregnancy (SEP) – 56th day of gestation; sows in late pregnancy (SLP) – 98th day of gestation; postpartum sows (PS) – day 2–3 post-partum; lactating sows (LS) – 21st day of lactation.

For 12 h prior to blood collection the animals did not have access to feed, except for the piglets. Blood was collected from the jugular vein in the early morning. Each blood sample for determination of haematological parameters was immediately poured into a test tube containing disodium salt of ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant, at a rate of 2 $mg \cdot ml^{-1}$ of blood (Oyewale 1992). The blood was refrigerated (4°C), and transported to the laboratory (about 1 h). The analyses were performed immediately after the material was delivered.

Laboratory analyses

After thorough mixing (about 10 min) on a UMH 5 roller mixer, the blood samples were stored at room temperature. EDTA samples were assayed using an ABACUS-Vet haematology system with a species-specific setting for pigs in Multi Species System Software (Siemens Medical Solutions Diagnostics Inc. Tarrytown, NY, USA) (Klem et al. 2010).

Blood was tested to determine the following haematological parameters: haematocrit (Ht), haemoglobin concentration (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood corpuscular count (WBC), and leukocyte profile (leucogram): proportions of lymphocytes (LYM), neutrophil granulocytes – neutrophils (NEU), acidophilic granulocytes – eosinophils (EOS), basophilic granulocytes – basophils (BASO) and monocytes (MONO). The percentages of white cells (differential white cell counts) were determined on May-Grünwald-Giemsa-stained slides under a microscope at 100× magnification by counting 100 white cells manually on an electronic digital counter (Counter Electronics, USA). The analyses were performed in two replications.

Statistical methods

Statistical analysis of the results was performed by one-way analysis of variance using Statistica software ver. 10 (StatSoft, Inc., Dell, USA). Significance of differences between means was determined by Tukey's test.

RESULTS

The data are shown as minimum and maximum values, means and standard deviations. Tables 1 and 2 present Ht, Hb content, RBC count, MCV, MCH, and MCHC in the blood of the growing pigs and sows. Tables 3 and 4 present white blood cell parameters: WBC count and percentage of each white blood cell type (NEU, LYM, MONO, EOS and BASO).

DISCUSSION

The most important factors determining the values of blood parameters in animals include their age and physiological state (Friendship and Henry 1996, Žvorc et al. 2006, Rekiel et al. 2008, Joksimovic-Todorovic et al. 2010).

TABLE 1. Red blood cell parameters of growing pigs

Variable		Experimental group				$\bar{x}$	*SEM*
		P	W	G	F		
		n = 335	n = 563	n = 446	n = 446		
Ht ($l \cdot l^{-1}$)	$\bar{x}$	0.31[a]	0.33[a]	0.35[a]	0.39[a]	0.35 ±0.099	0.10
	min	0.18	0.17	0.25	0.35		
	max	0.43	0.48	0.45	0.45		
Hb ($mmol \cdot l^{-1}$)	$\bar{x}$	5.71[a]	6.52[a]	7.55[a]	8.21[a]	6.99 ±3.09	2.19
	min	3.33	3.11	4.90	5.00		
	max	8.09	9.93	10.20	11.42		
RBC ($10^{12}\ l^{-1}$)	$\bar{x}$	5.79[a]	6.33[a]	6.81[a]	7.14[a]	6.52 ±2.22	1.66
	min	4.11	3.85	4.04	5.00		
	max	7.47	8.81	9.58	9.28		
MCV (fl)	$\bar{x}$	51.4[a]	46.1[abc]	49.3[b]	51.2[c]	49.50 ±15.51	3.97
	min	28.66	28.69	37.93	39.67		
	max	74.14	63.51	60.67	62.73		
MCH (pg)	$\bar{x}$	15.95[a]	15.61[bc]	16.11[b]	17.10[ac]	16.19 ±7.85	2.09
	min	8.36	8.99	5.65	9.83		
	max	23.54	22.23	26.57	24.37		
MCHC ($g \cdot l^{-1}$)	$\bar{x}$	314.2[ab]	338.2[bc]	322.1[cd]	337.9[ad]	328.1 ±53.41	39.17
	min	285.3	290.5	276.3	245.1		
	max	343.1	385.8	368.0	430.7		

P – piglets; W – weaners; G – fatteners in growing period; F – fatteners in finishing period.
Means in the same row with the same letters differ significantly between experimental groups, $P \leq 0.05$.

Red blood cell parameters

The haematocrit, haemoglobin content and red blood cell count in the blood of the piglets were significantly lower than in the other groups of growing pigs. The low levels of these indices in the neonatal piglets may be due to the growth, development and maturation of their organs and doubling of body mass (Kabalin et al. 2008, Petrovič et al. 2009). Research by Friendship and Henry (1996) on weaned piglets (P) revealed lower RBC counts and other erythropoietic indices (Ht and Hb) than in older animals, which is consistent with physiological processes taking place in the body. The red blood cell parameters (Hb, Ht, and RBC) of the piglet blood obtained in our study were at a similar level to those reported by other authors (Kabalin et al. 2008, Czech et al. 2009). They also come close to the lower limit of the reference range for pigs without regard to age (Winnicka 2011).

Changes in the haematological parameters of weaners are rarely addressed in

TABLE 2. Red blood cell parameters of sows

Variable		Experimental group					$\bar{x}$	*SEM*
		GL	EPS	LPS	PS	LS		
		n = 120	n = 203	n = 203	n = 203	n = 203		
Ht ($l \cdot l^{-1}$)	$\bar{x}$	0.37	0.34	0.37	0.36	0.35	0.35 ±0.089	0.13
	min	0.25	0.25	0.24	0.28	0.25		
	max	0.47	0.43	0.43	0.44	0.45		
Hb ($mmol \cdot l^{-1}$)	$\bar{x}$	7.63^{ac}	6.33^{ab}	6.96	6.42^{c}	7.63^{b}	7.00 ±2.38	1.73
	min	4.50	4.30	5.13	3.83	5.02		
	max	10.76	8.36	8.79	9.01	10.24		
RBC ($10^{12}\ l^{-1}$)	$\bar{x}$	5.89	5.28	5.94	5.55	5.93	5.72 ±2.09	1.90
	min	4.00	3.11	4.03	3.81	3.03		
	max	7.78	7.45	7.85	7.29	8.83		
MCV (fl)	$\bar{x}$	60.2^{abc}	58.1	57.2^{a}	57.0^{b}	57.1^{c}	57.92 ±10.22	5.08
	min	47.70	47.84	45.91	45,33	47.93		
	max	72.70	68.36	68.49	68.67	66.27		
MCH (pg)	$\bar{x}$	20.17	18.79	19.52	20.29	19.47	19.64 ±4.18	2.48
	min	16.21	16.89	12.2	14.19	16.51		
	max	23.01	20.69	26.84	26.39	22.43		
MCHC ($g \cdot l^{-1}$)	$\bar{x}$	338.1^{a}	339.9^{b}	340.3	355.9^{abc}	334.0^{c}	341.60 ±40.79	43.56
	min	328.8	319.6	275.1	269.9	307.5		
	max	347.4	360.2	405.4	441.9	360.5		

GL – gilts; SEP – sows in early pregnancy; SLP – sows in late pregnancy; PS – postpartum sows; L – lactating sows.
Means in the same row with the same letters differ significantly between experimental groups, $P \leq 0.05$.

research studies. The range of red blood cell indices obtained in the present study was similar to the reference values for this group of animals given by Friendship and Henry (1996). However, this range is lower than the commonly accepted reference values for pigs reported by Winnicka (2011).

Analysis of the all red blood cell parameters, except for MCV, in the growing pigs (P, W, G and F group) shows a linear increase in their values with the increase in body weight. The differences between these groups were statistically significant ($P < 0.05$). No significant differences were noted for the Ht values or RBC between gilts at mating (GL) and sows, either during gestation or lactation. Determination of reference values for haematological parameters of fatteners' blood was the research objective of such authors as Friendship and Henry (1996), Chmielowiec-Korzeniowska et al. (2008) and Klem et al. (2010). In the study by Chmielowiec-Korzeniowska et al. (2008), the haematocrit level and

TABLE 3. White blood cell parameters of growing pigs

Variable		Experimental group				$\bar{x}$	*SEM*
		P	W	G	F		
		n = 335	n = 563	n = 446	n = 446		
WBC (10^9 l^{-1})	$\bar{x}$	13.36^{ab}	17.14^{a}	21.06^{ac}	18.03^{bc}	17.34 ±9.55	1.37
	min	6.49	8.89	5.92	9.02		
	max	20.23	25.39	36.2	27.04		
NEU (%)	$\bar{x}$	59.82^{ab}	47.56^{ac}	40.56^{a}	37.95^{bc}	46.47 ±25.49	4.38
	min	37.24	21.32	13.41	12.60		
	max	82.4	73.82	67.72	63.30		
LYM (%)	$\bar{x}$	36.54^{ab}	49.55^{ac}	53.99^{a}	58.96^{bc}	49.76 ±31.09	5.72
	min	7.78	19.30	25.60	20.30		
	max	65.30	79.80	82.38	97.62		
MONO (%)	$\bar{x}$	1.99	2.28	3.89	2.31	2.62 ±2.33	0.33
	min	0.18	0.10	0.59	0.20		
	max	3.80	4.46	7.19	4.42		
EOS (%)	$\bar{x}$	1.58	0.43	1.20	0.72	0.98 ±1.09	0.14
	min	0.00	0.00	0.00	0.00		
	max	3.82	2.45	2.40	2.00		
BASO (%)	$\bar{x}$	0.07	0.18	0.36	0.06	0.17 ±0.26	0.10
	min	0.00	0.00	0.10	0.20		
	max	0.66	0.36	0.62	1.00		

Legend as in Table 1.

RBC count of fatteners were found to increase with age, as confirmed in the present study. An increase in haemoglobin content and erythrocyte counts in pigs during the first months of life was also reported by Petrovic et al. (2009). These authors attributed the increases in Hb and RBC to rapid organ development, which is responsible for erythrocyte formation and maturation, and to the extension of the erythrocyte life span (over 120 days). This is usually accompanied by a decrease in MCH and MCHC values, although this was not confirmed in our study. Throughout the fattening period, the range of MCH and MCHC values was within the limits given by Friendship and Henry (1996) and by Winnicka (2011), but was closer to their lower values. It should be noted that due to the many factors affecting the MCH value, a current trend has been to exclude this parameter in diagnosis of disorders of the red blood cell system, whereas MCHC is thought to be the most accurate red blood cell parameter (Žvorc et al. 2006). Chmielowiec-Korzeniowska (2008) observed a decrease in this parameter throughout the fattening period. Value of MCHC recorded in the piglet group in

TABLE 4. White blood cell parameters of sows

Variable		Experimental group					$\bar{x}$	*SEM*
		GL	EPS	LPS	PS	LS		
		n = 120	n = 203	n = 203	n = 203	n = 203		
WBC (10^9 l^{-1})	$\bar{x}$	14.07^{ab}	15.09^{acd}	13.44^{ce}	15.26^{bef}	14.11^{df}	14.39 ±7.44	1.24
	min	9.20	7.18	3.50	7.52	6.92		
	max	18.94	23.0	23.38	23.0	21.3		
NEU (%)	$\bar{x}$	41.26^{ab}	60.85^{ac}	48.35^{cd}	67.72^{ad}	58.85^{bd}	55.41 ±16.81	4.50
	min	20.10	41.40	31.40	56.84	42.30		
	max	62.42	80.30	65.30	78.60	75.40		
LYM (%)	$\bar{x}$	53.11^{ab}	36.06^{ac}	48.59^{cd}	29.55^{bd}	37.55	40.97 ±17.82	5.03
	min	35.60	19.42	31.28	17.50	12.20		
	max	70.62	52.70	65.90	41.60	62.90		
MONO (%)	$\bar{x}$	1.28	3.09	2.59	2.73	2.79	2.50 ±2.23	0.30
	min	0.20	0.38	0.18	0.06	0.68		
	max	2.36	5.80	5.00	5.40	4.90		
EOS (%)	$\bar{x}$	4.15	0.00	0.43	0.00	0.72	1.06 ±1.22	0.19
	min	0.00	0.00	0.00	0.00	0.00		
	max	9.10	0.00	1.10	0.00	2.21		
BASO (%)	$\bar{x}$	0.20	0.00	0.04	0.00	0.09	0.07 ±0.27	0.09
	min	0.00	0.20	0.00	0.00	0.00		
	max	0.80	0.00	0.90	0.00	1.60		

Legend as in Table 2.

the present study was comparable to that obtained for piglets by Egeli et al. (1998) and Kabalin et al. (2008). The range of MCHC values obtained in our study for weaners and fatteners appeared to be substantially wider than that reported by Friendship and Henry (1996), while similar to that presented for fatteners by Klem et al. (2010).

The periods of the reproduction cycle are an important factor affecting red blood cell indices in sows. According to Žvorc et al. (2006), haemoglobin decreases during the first half of gestation in sows, with the lowest level observed in the second month. Their observations correspond with our results, where the value of this parameter at that time was the lowest in comparison with other physiological periods. More than 2 weeks before parturition (on average the 98th day of gestation), both Hb and RBC values were considerably higher (although the differences were not confirmed statistically). Values of RBC often increase during late pregnancy owing to enhanced production of erythropoietin promoted in erythropoiesis (Douglas and Weiss 2010). In the present study, these parameters fell 2–3 days after

parturition and increased again during lactation. Values of Ht, Hb, RBC, MCV and MCH in the sows at all reproductive stages were within the ranges reported by Winnicka (2011) and Friendship and Henry (1996). Levels of MCV and MCH in the sows during early pregnancy were lower than those reported by Žvorc et al. (2006) for sows in early gestation. Value of MCV in sows in advanced pregnancy and in lactating sows was lower than in a study by Rekiel et al. (2008). Values of MCHC in the sows were within the limits given by Winnicka (2011), except for the sows immediately postpartum, in which it surpassed the upper limit of these reference values. It is worth noting that Winnicka (2011) gives only the mean values for a given species without taking into account age or physiological state. Comparison with the reference values determined specifically for sows by Friendship and Henry (1996) revealed that only the MCHC value for sows directly after birth was within that range, while the other red blood cell parameters of sows in early or advanced pregnancy and of lactating sows were below the lower limits. The values of Ht and Hb recorded in the sows in early pregnancy were also found to be lower than the values for sows in early gestation reported by Žvorc et al. (2006).

Analysis of our results indicates that haematological parameters are perceptibly influenced by physiological state. The levels of blood components reflect the pathological and physiological changes taking place in the body throughout life. The gestation and lactation period is a time of marked fluctuations in many haematological parameters, which reflect the changes occurring in the body due to increased blood plasma volume (Žvorc et al. 2006, Rekiel et al. 2008), changes in the content of some steroid hormones (Joksimović-Todorović et al. 2010), or increased erythropoiesis in the final stage of pregnancy. These changes are visible in the blood profile, and therefore values given for a species as a whole without distinguishing physiological periods is not sufficiently precise.

White blood cell parameters

White blood cells have a primary role in the body's defence mechanisms. Total leukocyte counts in swine fall within a wide range of normal values (Friendship and Henry 1996, Klem et al. 2010). An increase in WBC concentration, apart from pathological states, can also be observed in animals after strenuous exercise or feeding, which is termed physiologic leukocytosis. It occurs among sows in the final stage of gestation and directly after farrowing, as well as in suckling piglets. The leukocyte count in group P was close to values reported by Egeli et al. (1998), while in groups W, G and F it was within the reference values for weaners and fatteners presented by Friendship and Henry (1996), and the value for fatteners during the first fattening stage (G) was within the range reported by Klem et al. (2010).

The leukocyte count in groups P, W, F was in the range of reference values reported by Winnicka (2011), while in group G it exceeded the upper limit by over $20 \cdot 10^9$ l^{-1}. Similarly, Chmielowiec-Korzeniowska (2008) showed that mean leukocyte counts during the fattening period clearly surpassed the upper limits of reference values presented by Winnicka (2011). The number of

leukocytes in piglet blood was lower than in the older groups of growing pigs ($P < 0.05$), with the significantly highest WBC count noted in the fatteners in the first fattening (G) stage. Among the sows, a significantly higher leukocyte count was noted in the sows in early pregnancy (SEP) and sows just after parturition (PS).

The percentages of each WBC type in all groups of growing pigs were within the reference limits determined by Winnicka (2011). A great deal of literature, including this study, indicates that piglets have a higher percentage of neutrophils than lymphocytes but that this proportion is quickly reversed (Friendship and Henry 1996, Klem et al. 2010). In addition, the percentages of all leukocyte types in the weaners (W) and fatteners (G and F) were within the reference limits given by Friendship and Henry (1996) for these groups.

Analysis of the results obtained for the white blood cell profile revealed that the percentage of neutrophils (NEU) was significantly lower in the fatteners in both fattening stages than in the other groups. The value of this parameter in the gilts was also quite low. An inverse relationship was observed for the percentage of lymphocytes, which was higher in these groups ($P < 0.05$). In the blood of adult sows, the highest leukocyte count was noted immediately after parturition, and was significantly higher than in the final period of gestation and during lactation. The percentage of neutrophils was also highest at this time, while the percentage of lymphocytes was significantly ($P < 0.05$) the lowest. There were no statistically significant differences between the groups in the percentages of the other white blood cell types, i.e. monocytes, basophils and eosinophils. The leukocyte count in sows in early pregnancy was slightly higher than the value reported by Žvorc et al. (2006). In sows in advanced pregnancy, the WBC was higher than that reported by Rekiel et al. (2008), while in lactating sows it was lower than the values obtained by Joksimović-Todorović et al. (2010).

Parturition in mammals induces stress and thereby causes a number of changes in the blood profile, including postpartum leukocytosis with lymphopenia. Lymphocytosis occurs when the elevated postpartum body temperature declines. All changes return to baseline values within five days after farrowing. Our study and a paper by Joksimović-Todorović et al. (2010) confirmed that the leukocyte count differs between physiological periods in sows. In our study, the highest leukocyte count, detected directly after parturition, was significantly ($P < 0.05$) higher than the counts recorded during late gestation and lactation. The neutrophil counts were also highest in the first few days after farrowing. Unlike the total leukocyte count and the proportion of neutrophils in it, the mean percentage of lymphocytes in the leukocytes was lowest on the first few days of lactation. The share of monocytes and basophils did not change significantly during the lactation period. The percentages of eosinophils and basophils were generally low, and they were not found in the blood of sows during early pregnancy and postpartum.

The percentages of each white blood cell type in the total WBC count of all five sow groups were within the reference limits provided by Friendship and Henry

(1996) and Winnicka (2011) for gilts and sows, excluding the percentage of neutrophils in the SEP and PS groups, which exceeded the upper limits recommended by these authors, and the percentage in the LS group, which surpassed the upper limit given by Winnicka (2011). Furthermore, the percentage of eosinophils in all the sow groups was below the lower limit of reference values for sows given by Friendship and Henry (1996).

CONCLUSIONS

The results of the study suggest that haematological parameters are substantially affected by the age and physiological state of pigs. Piglets are characterized by lower red blood cell indices (Ht, Hb, and RBC) and white blood cell indices (primarily WBC count, percentage of LYM) as compared to fatteners.

The gestation and lactation periods are marked by fluctuations in haematological parameters – the RBC count increases in the final stage of gestation, while in postpartum sows the number of WBC, including neutrophils, rises noticeably.

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Streszczenie: *Parametry hematologiczne krwi polskich mieszańców świń w różnym wieku i stanie fizjologicznym.* Celem pracy było zaprezentowanie jak zmieniają się wartości podstawowych parametrów hematologicznych krwi u mieszańców świń wbp × pbz w różnym wieku i stanie fizjologicznym (ciąża, laktacja). W pracy przedstawiono parametry czerwonokrwinkowe (Ht, Hb, RBC, MCHC, MCH, MCV) oraz wartości parametrów układu białokrwinkowego (WBC, procentowy udział poszczególnych rodzajów krwinek białych w ogólnej ich liczbie) we krwi 21-dniowych prosiąt (n = 335), warchlaków (n = 563), tuczników (n = 446) oraz loszek (n = 120) i loch (n = 203) w okresie ciąży i laktacji. U rosnących świń oznaczane czerwonokrwinkowe parametry krwi charakteryzowały się liniowym wzrostem wraz z wiekiem: RBC (5,79; 6,33; 6,81 i 7,14 · 10^{12} l^{-1}), Hb (5,71; 6,52; 7,55 i 8,21 mmol·l^{-1}) oraz Ht (0,31; 0,33; 0,35 i 0,39 $l \cdot l^{-1}$) odpowiednio u prosiąt, warchlaków oraz tuczników „Grower" i „Finiszer". U prosiąt liczba WBC była mniejsza ($P \leq 0,05$) niż u warchlaków i obu grup tuczników (odpowiednio: 13,36; 17,14; 21,06; 18,03 · 10^{9} l^{-1}), z liniowo wzrastającym udziałem LIM, a malejącym NEU w populacji leukocytów. Krew loch podczas ciąży i laktacji charakteryzowała się niewielką zmiennością parametrów czerwonokrwinkowych, wartość WBC była zaś istotnie większa, z mniejszym udziałem LIM, ale większym NEU u loch niskoprośnych oraz w okresie poporodowym w porównaniu z wysokoprośnymi i karmiącymi bądź będącymi w laktacji. Uzyskane wyniki potwierdziły, że wartości parametrów hematologicznych krwi są zależne od wieku i stanu fizjologicznego zwierząt.

Słowa kluczowe: świnie, krew, wskaźniki hematologiczne, grupa produkcyjna

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Trichinellosis – history, current status, new threats

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Abstract: *Trichinellosis – history, current status, new threats*. The paper presents the epizootic situation of trichinellosis in historical terms, the present state, as well as emerging new threats. At the turn of the last few years, new species of trichinella species have emerged, as well as the spectrum of species of wild and domestic animals, which are a potential source of parasite transmission, has been widespread. Quite a significant threat is the problem of increasing occurrence of trichinella at herbivorous animals, which until recently were mostly treated *ex officio* as trichinella-free. The changing epizootiological image of trichinella makes it seems necessary now to intensify the preventive measures in the prevention of meat of all species susceptible to trichinella by means of etching method. Such activities should guarantee the safety of marketed animal origin food and thus effectively reduce the epidemiological risk.

Key words: trichinellosis, wild boar, epizootic risk, food safety

INTRODUCTION

Diverse environmental conditions (temperature, precipitation, humidity, feeding base), in which wild animals are present, affect the incidence of many diseases with a complex etiological basis. Periodic migrations, and also concentration of significant numbers of animals in livestock herds, often in relatively small areas, directly and indirectly affect the spread of individual disease units. It is not unimportant that wild animals are vectors of many diseases, so-called zoonoses, which can be transmitted to humans. The infection may occur by various transmission means, either by direct contact with animals or by indirect means through contact with feces, secretions, fur, feathers, and by ingestion of meat or its products containing the pathogen. People are significantly more at risk of zoonoses transmitted by mammals than birds. This is due to the higher degree of similarity between the organisms and intracellular environments of human to mammals (Hugh-Jones et al. 1995, Śmigielska 2010).

One of the most dangerous zoonoses for humans is trichinellosis. This disease is an example of a typical zoonotic disease with a fairly complex epizootiological process. Despite a number of preventive measures in many countries, mainly in the form of multidimensional constraints and restrictions, as well as

mandatory diagnostic tests to remove trichinella from the food chain, these parasites are unable to stop by any barriers, either geographically or administratively. Thus, it was and continues to be a very serious epidemiological problem, both in Poland and in many other countries in the world. Economic epizootic risk in terms of animal production, mainly pigs and food safety, is also important. There are currently 250 mammal and bird species being the trichinellosis reservoir (Britov 1997, Murrell and Pozio 2000, Gołąb and Sadkowska-Todys 2003, Gottstein et al. 2009, Hurniková and Dubinský 2009, Bilska-Zając et al. 2011, Flis 2011c, Holzbauer et al. 2014, Gliński 2016).

DEVELOPMENT OF TRICHINELLOSIS AT THE TURN OF THE CENTURY

The first mention of a disease that resembled trichinellosis was from 422 BC. According to reports, the mass outbreaks of the Carthaginians during the Sicilian War were supposed to determine the outcome of the conflict. Nevertheless, the trichinosis has occurred much earlier. Its presence was confirmed in ancient Egypt by the detection of trichinella larvae in the intercostal muscles of the dead mummified around 1200 BC. Probably in the Middle East, trichinosis could be quite common, as reflected in the Mosaic Law, in the form of a ban on the consumption of pork. In subsequent historical epochs, the source texts contain descriptions of diseases resembling trichinellosis (Gould 1970, Gołąb and Sadkowska-Todys 2006).

It is commonly reported that as a disease unit, it was first described in 1863, which was the sound of a German order to perform microscopic examination of a pig meat intended for consumption to verify the presence of trichinella. Actually, the trichinosis was described and named in 1835 in London by a first-year medicine student at St. Bartholomew hospital. During the autopsy of the deceased corpses, he found the occurrence of muscle larvae. He described these larvae as a new species of nematode and called them *Trichina spiralis* (Gołąb and Sadkowska-Todys 2006).

In our country, the earliest mention of trichinellosis comes from 1879, when it was ordered to investigate the meat of all pigs and boars intended for human consumption for the presence of trichinosis, in the Prussian partition. However, in the area of present Wielkopolska, the pork meat has been examined since 1868. In the Kingdom of Poland, there were no legal guidelines for mandatory meat testing for trichinellosis, and in the Austrian partition, in spite of the Imperial and Royal guidelines for meat testing, there was no mandatory examination of pork for trichinosis. In the interwar period under the Regulation of the President of the Republic of Poland of 2 March 1928, the obligation of official pig testing for the presence of trichinosis, except for animals intended for consumption on their own farms, was introduced. Despite this, in 1919–1937, 1,753 cases of trichinellosis in humans were reported, of which 54 cases were fatal. In the postwar period, there was an increase in the number of cases and the appearance of further epidemiological outbreaks. This led to the situation that mandatory obligation to test the meat of wild boars and pigs for slaughter at their own farm, was

introduced in 1953 (Gołąb and Sadkowska-Todys 2006, Różycki et al. 2016).

At the turn of the last decades, the epizootic and epidemiological picture of trichinosis has changed, and various preventive measures have been undertaken. The occurrence of trichinella in the wild is one of the basic factors that affect the onset of this disease in domestic animals, mainly pigs. Thus, the basic preventive measures are the observance of the zoohygienic principles in all pig farms, especially for rat extermination. However, the most important preventive action is obligatory testing of commercial meat for the presence of trichinella. Farm animals are subjected to official testing of pig and nutria meat, and for wild animals, the wild boars meat testing is mandatory. Due to the emergence of new of trichinella as well as their detection, the most effective treatment in previously untreated species, the most effective method of testing is the use of etching of muscle samples from animals, for which the meat is intended for human consumption (Gottstein et al. 2009, Pozio and Zarlenga 2013, Flis 2016b, Gliński 2016).

ETIOLOGY AND DEVELOPMENT OF *TRICHINELLA*

Trichinellosis is caused by internal polyxenic parasites belonging to the trichinella family Trichinellidae. They are capable of living and developing in a fairly wide range of hosts. In general, they are referred to as viviparous parasites of carnivorous and omnivorous mammals. Nevertheless, in the period of the last few decades, the epizootic and epidemiological picture of this disease has changed significantly. New species of *Trichinella* pathogenic to humans have been identified. In addition, the area of the parasite occurrence has increased, as well as the most recent sources of infection have been subject to specific modifications. Due to the possibility of trichinosis occurrence both in the forest and synanthropic environment, the reservoir of trichinella can be wild and domestic animals, and thus the possibilities of trichinella appearance is widespread. There are currently nine species and twelve genotypes of *Trichinella* classified. In temperate climate of Europe and Asia, two species of trichinella are the most common: *Trichinella spiralis* and *T. britovi*. In addition, there are also *T. nativa*, *T. pseudospiralis*, *T. murelli*. Other species and genotypes occur on other continents. In our country, trichinellosis is most often induced by *T. spiralis* and *T. britovi*. In addition, at wild animals, there are also found *T. nativa* and *T. pseudospiralis*, which may also occur in birds. Due to the fact that some of these species (*T. nativa*) are resistant to freezing, therefore precautions that have been taken so far (deep freezing of meat before consumption) should not be used, because they do not provide safety and may lead to an increased epidemiological risk. In addition, the two *Trichinella* species, *T. pseudospiralis* and *T. papua*, do not stimulate the muscle cells to produce a collagenic envelope around the larvae, which make them undetectable by means of traditional compressor method (Gołąb and Sadkowska-Todys 2003, Oivanen 2005, Gawor 2011, Pozio and Zarlenga 2013, Gliński 2016).

Trichinella occurs in food as a result of the consumption of meat or its products containing encapsulated or non-encap-

sulated trichinella larvae. Assuming that the consumption of 1 g of meat, in which more than two *T. spiralis* larvae is present, may cause clinical symptoms. After getting into the host, the incubation period lasts up to a few days and consists of three stages: intestinal, muscular, and recovery (sometimes death). The first stage is related to the dissolution of the connective tissue sac surrounding the parasite larva by the gastric juice. Once the larvae has reached a puberty, which occurs within 4–5 days of the release and subsequent female fertilization, they penetrate the mucous membranes of the intestinal wall, where each of them produces from about 200 to 1,000 live larvae of not more than 1 mm in size. Larvae get into the bloodstream and are spread throughout the whole organism, and consequently they are located in skeletal transversely striated muscles, where they tend to twist and ooze (muscular stage), with the exception of *T. pseudospiralis* and *T. papua*. Over time, the capsules are calcified and the larvae do not lose their vitality for many years. Considering that each female can be fertilized several times, significantly more larvae get to the host's muscles than was consumed along with the infected meat, hence the level of invasion can be very high (Cabaj 2006, Gawor 2011, Gliński 2016).

RESERVOIR OF TRICHINELLOSIS

Forest environment

The main reservoir of the parasite is wild mammals, mainly from the order of Carnivora, therefore the most important way to transmit the parasite and the possibility of infection at humans, is considered the so-called forest cycle. At predatory animals, trichinella is found most often in foxes, wolves, raccoons, martens, polecats, badgers, and bears. It is also found in small rodents. The wild predators way of eating through cannibalism and scavenging influences on the maintenance of trichinosis in the environment. However, because the meat of predatory animals is not consumed, the main source of the parasite's invasion to humans are omnivorous wild boars, which, through the involvement of carrion in their diet, are infected by the trichinella and form its natural reservoir (Gołąb and Sadkowska-Todys 2003, Cabaj 2006, Gawor 2011, Osten-Sacken and Solarczyk 2016). According to various authors, the prevalence of trichinella, especially *T. spiralis* and *T. britovi* in foxes in our country ranges from a few to a dozen percent in the samples tested. In other predatory species, the turnout is generally lower, but this may be due to the lower frequency of this type of studies and smaller number of animals in a sample (Cabaj 2006, Gawor 2011, Chmurzyńska et al. 2013, Gawor 2016). In wild boars, in our country, *T. spiralis* is most often found, whose share in the examined samples is about three times higher than that of *T. britovi* (Bilska-Zając et al. 2013). In 2016 in West Pomerania, *T. pseudospiralis* for the first time was found in the wild boar; it is one of the two species that do not encapsulate within the muscle cell. The fact of the first finding of this species in wild boar, in connection with the fact that it is not detected by the compressor test, leads to an increased possibility of its transmission to humans (Bilska-Zając et al. 2016).

The frequency of trichinellosis in wild boars varies considerably in different years. At the end of the 1980s, the percentage of trichinosis was about 0.30%. In the mid-1990s, the percentage of infected wild boars was 0.25% and in 2000 it was 0.18%, while in 2002 – 0.28% (Lis 1991, Gołąb and Sadkowska-Todys 2003). In 2003–2009, the prevalence of trichinellosis in wild boars ranged from 0.28 to 1.09% (Flis 2011a). In 2010, the prevalence of trichinosis in wild boars was 0.34%, and in 2011 – 0.32% with significant variation in particular regions of the country (Flis 2011c, Flis 2012), whereas in subsequent year, the share of infected wild boars amounted to 0.30% (Szkucik et al. 2012a). On the other hand, the sanitary and veterinary assessment of wild game carcasses in 2000–2011 shows that during veterinary-sanitary assessment, 31.21% of the disqualified animal carcasses were found to be useless for consumption due to trichinellosis (Szkucik et al. 2012a).

Over the past decade, the number of diagnosed cases of trichinosis in wild boars has increased almost 2.5-fold (Fig. 1). Despite significant differences in the occurrence of trichinellosis in individual regions of the country in 2015, 752 cases were identified. The highest number of *Trichinella* was found in the western and north-western provinces (Fig. 2). This condition can be directly related to the annually increasing number of this species in Poland, which entails the intensification of pressure to hunt wild boar (Fig. 3). During the last 5 hunting seasons, despite the fluctuations in individual seasons, it remained at an average level of about 246 thousand individuals. In turn, the size of hunting acquirement in the same period increased by about 75%, and the rate of hunting population exploitation representing the real hunting pressure increased from 72.6 up to 128.7% in the same period. Due to the fact that the rates of densities in wild boars populations in western and northwestern Poland are significantly higher for many years, the level of culling is significantly higher in these regions, which in turn entails the

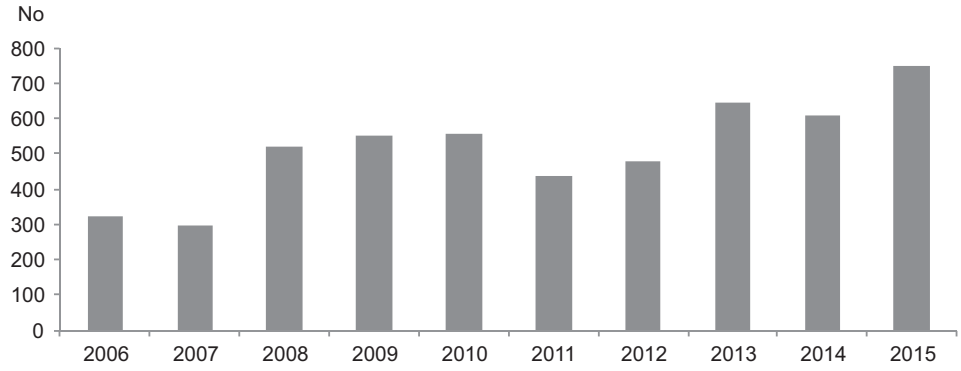


FIGURE 1. The confirmed cases of trichinosis in wild boar in Poland in 2006–2015

Source: Own elaboration on the basis of the reports of RRW-6, Chief Inspectorate of Veterinary from the results of the official study of meat, poultry, game, rabbit and aquaculture animals for the years 2006–2015.

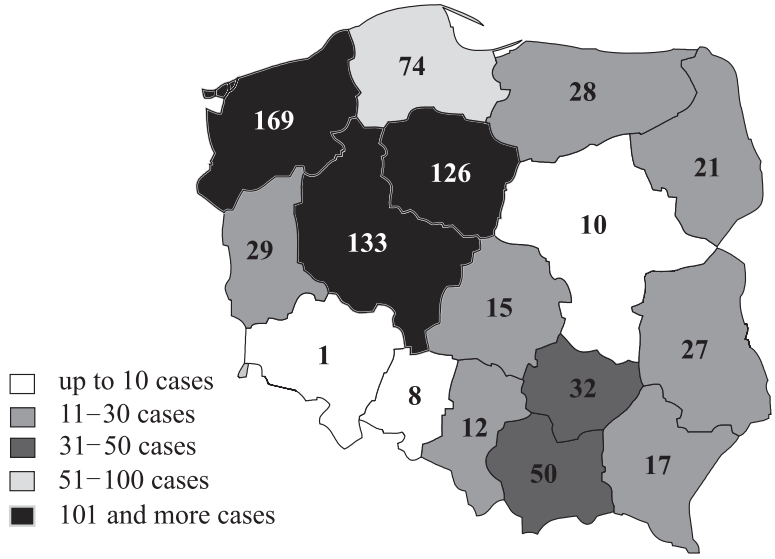
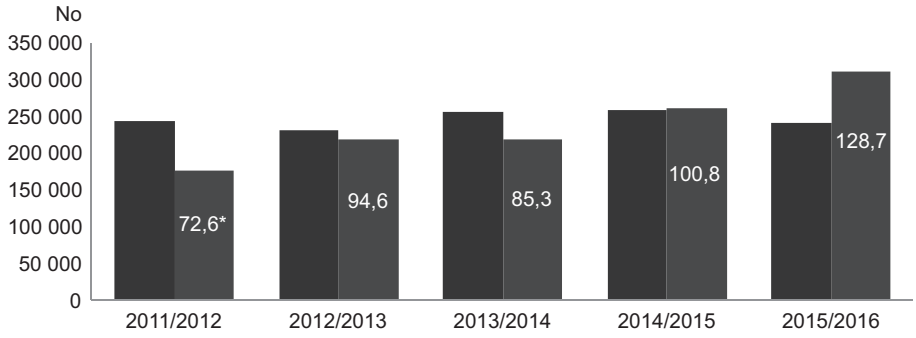


FIGURE 2. The number of ascertain cases of *Trichinella* in wild boars in Poland in 2015



* the rate of hunting and exploitation of the population

FIGURE 3. Number and hunting harvest of wild boars during the last five hunting seasons in the hunting districts leased by hunting club

supply of venison from this species (Flis 2011b). The risk of potential trichinosis infection cannot be underestimated, especially by hunters, which is confirmed by nationwide survey of this group of people conducted on a sample of 1,027 hunters in 2010–2012. The results of this study indicate that 4.6% individuals had antibodies for *Trichinella* spp., the number of which increased with the age of the examined persons, thus indicating that they were in contact with *Trichinella* (Sadkowska-Todys et al. 2015).

In European Union countries, the epizootic situation has shown considerable variation. In 1996–2001, in some countries (Austria, Denmark, Germany), the trichinosis was reported exclusively

in wild animals. At the same time, in France, Italy, Spain, Sweden, and the Netherlands, *Trichinella* was present in both wild as well as domestic animals. In the same period, the prevalence of trichinella in wild boars has shown significant geographic differentiation. In Finland, the infection rate was 1.3%, in France 0.025%, and in Italy 0.06%. In most European countries, the primary reservoir of *Trichinella* is predatory and wild animals that play a key role in the spread of nematodes within the forest cycle, as well as their meat wild boars is the primary source of human infection (Gołąb and Sadkowska-Todys 2003, Bilska-Zając et al. 2011, Moskwa et al. 2015).

Synanthropic environments

The epizootic situation of *Trichinella* in domestic animals indicates that pigs are the primary reservoir of the virus in Poland as well as in other countries. Between 1995 and 2000 in Finland, the percentage of infected pigs was in the range 0–0.0103% and in Germany in the period 1990–1998 it was lower and did not exceed 0.00008% (Gołąb and Sadkowska-Todys 2003). In our country, at the end of the 1950s, the prevalence of trichinella found in the samples, was about 0.016%, while in 1968 it was 0.005% and in 1985 – 0.002%. In 1987, the proportion of infected pigs was 0.002%, while 10 years later – 0.0004% (Lis 1988, Lis 1999). In 2003–2009, the incidence of trichinella at this species ranged from 0.00008 to 0.00034% of the examined animals, whereas in 2007–2011 the level of trichinosis at pigs was within the range of 0.00004–0.00034% (Flis 2011c, Flis 2012).

Quite disturbing is the fact that the trichinellosis sporadically occurs in other species, both wild and domestic animals, the meat of which is intended for human consumption. This contributes to an increase in the epidemiological risk, which is determined by the lack of mandatory meat testing of these species prior to market entry. Although it is widely believed that herbivorous animals do not play a major role in the epizootiological chain of trichinosis, there has been a problem since the late 1970s associated with the possibility of trichinosis occurrence in horse meat. In 1975–1986 in Italy, there were multiple foci of trichinellosis, which resulted from the consumption of horse. Also in France, two outbreaks occurred in 1985, the direct cause of which was horseflesh containing trichinosis. After this incident, studies carried out in five European countries (France, the Netherlands, Ireland, Greece, Poland) showed that only samples from Poland had no trichinella, and the highest prevalence (4.6%) occurred in horse meat originating from Ireland (Ramisz et al. 2002, Nowakowski 2004, Gawor 2007). In 2008, in the meat of a 10-year-old horse imported from Poland to Italy, both *T. britovi* and *T. spiralis* were found at 4 : 1 proportion. Trichinosis was also found in a horse that was fed with kitchen waste of animal origin in Serbia (Murrell et al. 2004, Liciardi et al. 2009). Veterinary and sanitary assessment of horses in Poland in 2001–2010 revealed a single case of trichinella occurrence in 2010 (Szkucik et al. 2012b). Mutton and goat meat may also be the source of trichinella infection. At the turn of the 1970s and 1980s in China, many cases

of trichinellosis have been reported in mutton (Kocięcka 1996, Nowakowski 2004). Experimental results have shown that sheep are susceptible to trichinellosis infection, and the greatest intensity of invasion has been found in ruminal, diaphragm, and tongue muscles. Goat experiments also demonstrated the susceptibility of this species to trichinella infection, and thus the possibility of further transmission to humans (Pajerský et al. 1996, Reina et al. 1996, Nowakowski 2004, Kořinková et al. 2006). On the other hand, experimental studies conducted at cattle showed low susceptibility to infection (Smith et al. 1990).

Another threat of trichinella transmission may also be wild herbivores, the meat of which can be consumed by human. In 2015, *T. britovi* was found in Latvia for the first time at European beaver (Seglina et al. 2015). Although the proportion among tested animals was 0.5%, it should not be underestimated. Considering the radical changes in the beaver culling reduction policy introduced in the past year in our country, their carcasses will be partly compensated to hunters, and it is expected to consume this meat in various forms (Flis 2016a). Despite the lack of traditions of beaver consumption, the emergence of the parasite in another host in the food chain contributes to an increased risk of public health. Also the experimental infections of other species of wild herbivorous animals (fallow deer, reindeer) indicated their varying susceptibility to particular trichinella species. These animals were most susceptible to *T. spiralis*, *T. britovi*, *T. pseudospiralis* (Moretii et al. 2000, Oksanen et al. 2000).

SUMMARY

The present described situation of the occurrence and development of trichinosis indicates a constant and increasing threat to the food safety of the animals origin. The appearance of new trichinella species in forests, and consequently also in synanthropic environments, in connection with changing epizootiological pattern, makes the possibility of transmitting capabilities of the parasite unlimited. Increasing research on food safety has highlighted the wide spectrum of wild and farm animals that are the reservoir of *Trichinella*. It contributes to the situation when the prevalence of trichinellosis is increased, both in the natural as well as in the synanthropic environment, which in turn implies an increased epidemiological risk. Due to the presence of *T. pseudospiralis* in wild boars, which does not produce muscle envelope, and therefore is not detected by the compressive method, it is now necessary in all preventive and diagnostic procedures to use etching as the most effective diagnostic method for the prevention of trichinellosis. Consideration should also be given to the possibility of introducing the obligatory examination of beaver meat intended for human consumption in the countries, where they are harvested, including etching method.

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Streszczenie: *Włośnica – historia, stan obecny, nowe zagrożenia.* W artykule przedstawiono sytuację epizootyczną włośnicy w ujęciu historycznym, stan obecny, jak również pojawiające się nowe zagrożenia. Na przełomie ostatnich lat pojawiły się nowe gatunki włośni, zwiększeniu uległo także spektrum gatunków zwierząt (zarówno dzikich, jak i domowych) stanowiących potencjalne

źródło transmisji pasożyta. Dość istotne zagrożenie stanowi problematyka związana z coraz powszechniejszym występowanie włośni u zwierząt roślinożernych, które do niedawna traktowane były z urzędu jako wolne od włośni. Zmieniający się obraz epizootiologiczny trichinella, sprawia, że obecnie konieczne wydaje się wzmożenie działań prewencyjnych (wykorzystanie w tym celu metody wytrawiania) w zakresie profilaktycznego badania mięsa wszystkich gatunków podatnych na możliwości występowania włośni. Tego rodzaju działania powinny zagwarantować bezpieczeństwo żywności pochodzenia zwierzęcego wprowadzanej na rynek, a tym samym skutecznie zmniejszać ryzyko zagrożenia epidemiologicznego.

Słowa kluczowe: włośnica, dzik, zagrożenie epizootyczne, bezpieczeństwo żywności

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Applications of continuous body temperature measurements in pigs – a review

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Abstract: *Applications of continuous body temperature measurements in pigs – a review.* The temperature measurement is one of the most important indices in estimating the state of health both in humans and animals. Nowadays along with the development of technical knowledge and its implementation, increasingly more attention is paid to the continuously measurement of body temperature. This solution seems to be especially needed for the animal production. Continuous body temperature measurements provide a better control of the herd and a precise determination of the environmental impact on animal. Moreover this measurement may be very useful method in different animal studies enabling to obtain a large amount of data. The aim of this paper is to review the current state of knowledge on the topic of the most widely used methods of continuous body temperature measurement in pigs and also to show the directions of studies for the future.

Key words: pigs, continuous temperature measurements

INTRODUCTION

The temperature measurement is one of the most important indices in estimating the state of health in humans and animals. Along with the development of technical knowledge and its implementation, increasingly more attention is paid to the continuous measurement of body temperature. Apart from the monitoring of the health state this type of measurement can be of key importance in preventing the outcomes of thermal stress. The measurement can also be an ideal indicator of the applied cooling systems as well as other strategies preventing the overheating of the body. The monitoring of body temperature may also facilitate better diagnostics of oestrus or other physiological conditions, affecting the profitability of production.

The aim of this paper is to review and systematize the current state of knowledge on the topic of the most widely used methods of continuous body temperature measurement in pigs to assess their welfare and also to show the directions of studies for the future.

REGULATION OF BODY TEMPERATURE

The regulation of body temperature is one of the most important mechanisms for maintaining the homeostasis of the whole organism. The proper thermal conditions prevailing inside the body,

guarantee the metabolic proficiency that depends much on the operation of enzymes (Sosnowski et al. 2015).

There are two terms pertaining to body temperature: internal temperature – body core temperature, which determines the thermal conditions prevailing in the regions of abdominal cavity, rib case, and skull, and their external temperature pertaining to the skin surface, subcutaneous tissue, and muscles. The thermoregulatory mechanisms permit keeping a stable level of body core whereas the temperature of the surface shows variability. It is considered that the skin temperature performs as a buffer between the inside of the body and the outside environment. Skin temperature depends on the level of heat exchange between these areas (Lim et al. 2008).

Homoiothermic animals function in various surroundings keeping their core body temperature at a stable level. However it sometimes calls for a major adaptation effort. The thermal comfort zone identifies such thermal conditions of the surrounding in which an animal makes a minimum metabolic effort to maintain a stable internal temperature, where only the skin temperature that fluctuates. Under the condition of a thermal comfort zone, the losses of heat via evaporation are kept to a minimum (Curtis 1983).

The thermal balance is a dynamic process depending on many mechanisms and the work of many organs. It is primarily associated with the reactions of nervous and circulatory systems as well as on the actions of hormones. The centre of thermoregulation is located in the hypothalamus. It receives information on the fluctuations of temperatures in both surface and deep layers of the body. The regulation of temperature consists of three principal stages, i.e. the peripheral and cortical perception, central integration, and the efferent autonomic and behavioural responses (Sosnowski et al. 2015).

The impulses of cold enhance the processes of heat generation as well as block its losses from the body. These mechanisms are chiefly associated with the contraction of blood vessels in the skin and blocking the activities of sweat glands. In turn, the level of heat generation is elevated by the increased tension and metabolic activity of the skeletal muscles. Also activated are the hormones of the thyroid gland affecting, e.g. the acceleration of the metabolism. Furthermore, due to excitement of the adrenergic system, the release of the increase in the release of adrenocortical hormones occurs, which activates the respiratory centre and the blood circulation centre (the medulla oblongata). Catecholamines also affect the cells of liver and, by the same token, the increased intensities of glycogenolysis and glyconeogenesis occur (Sosnowski et al. 2015).

The effects of high temperatures trigger the processes of increased heat loss from the body. The greater flow of blood through skin (the dilation of blood vessels) increase heat loss via radiation, convection, and conduction (Guyton and Hall 2006). The sweat glands are stimulated, and heat is evaporated more intensively. The heat loss is also increased by the acceleration of heart rate and respiratory rate (Renaudeau et al. 2012).

The regulation of body temperature is also performed through modifications of

behaviour. Animals use the surrounding environment deliberately, either as the absorber or source of heat (Huynh et al. 2005). The examples of such behavioural reactions could be seeking the shelter in the shade during hot days, or the willingness to huddle with other individuals in cold surroundings.

The changes in core body temperature in animals can be caused not only by the aforementioned improper environmental conditions involving the increased adaptation effort but also by some of the physiological conditions, such as oestrus, the stage of pregnancy, birth, or the digestion processes (Lefcourt et al. 1999). Furthermore, in animals – like in humans – there is the circadian variability of the internal body temperature. These fluctuations are closely related to the function of the autonomous nervous system, and they reflect the circadian variability in the metabolism of heat production and loss (Słomko and Zalewski 2016). Morbidity is one of the key factors resulting in the increase in body temperature. Fever is a complex physiological reaction serving as a principal diagnostic symptom in the case if viral or bacterial infections (Dalal and Zhukovsky 2006). The rapid diagnostics of the increased body temperature associated with infection is one of the key factors that ensure the preservation of health in the herd.

HEAT STRESS IN PIGS

Reports published by international and government research centers indicate an increasing trend towards the systematic warming of the Earth's climate. Bearing in mind the significant influence of heat waves on the well-being and productivity of livestock (Renaudeau et al. 2012, Herbut and Angrecka 2013).

Heat stress is defined as the sum of external forces acting on an animal that causes an increase in body temperature and evokes a physiological response. It creates the need to meet the requirements of environmental conditions and entails the activation of neuronal and neurohormonal systems, the component of which is the immune system. The degree of stimulation of these systems determines the intensity of the stress response as well as the consequences they bring to the organism (Dikmen and Hansen 2008).

Pigs are particularly sensitive to heat stress. A small amount of sweat glands (about 30 per cm^2 of skin surface), thick fat layer, relatively small heart, and barrel shape make them very susceptible to overheating (Renaudeau et al. 2006, Bracke 2011). Some studies results determined the thermal comfort zone for diverse groups of pigs. Black et al. (1993) reported that the optimum air temperature for piglets is within the range of 30–37°C and for sows is 12––22°C. According to Myer and Bucklin (2012), sows experience negative effects of heat stress at 20°C and temperatures above 26°C are considered as critical for this group of animals (Quiniou et al. 2001). Dallaire et al. (1996) noted a significant increase in mortality among lactating sows, when the air temperature, at which these females had been kept, increased above 30°C. Brown-Brandl et al. (2012), using thermography, measured the skin temperature of the trunk in growing finishing pigs. The authors determined that the upper threshold of

comfort zone for these group was in the range of 20.4–22.8°C.

High ambient temperature affects body temperature in livestock. Renaudeau et al. (2012) claimed that in animals kept under acute heat stress, body core temperature increases rapidly up to critical values. However, maintenance of livestock under a long period of high air temperature causes at first a rise in animal body temperature and then a decrease to a dynamic steady state linked to acclimatization processes. The latest study of Sapkota et al. (2016) showed that after 30 min of exposing pigs to 39.3°C room temperature the core body temperature increases above 1° and this tendency is continued for the next 30 min even under optimal environmental conditions. It worth to mention that under optimal environmental condition normal body temperature in resting piglets is about 39.5°C, in slaughter pigs is 39.3°C, in gilts 38.8°C and in multiparous sows it is about 38.3°C (Soerensen and Pedersen 2015). The authors claimed also that some skin areas such as: ear base, eyes and udder correspond with the body core temperature. These areas can be called thermal windows. Due to well perfusion by blood these body parts are characterized by higher emissivity comparing with other skin locations.

METHODS OF BODY TEMPERATURE MEASUREMENT IN ANIMALS

The blood temperature measured in the pulmonary artery is considered to be the closest to the deep body temperature (Brengelmann 1987). Nevertheless, obtaining such measurements involves the use invasive measuring techniques; therefore, this method is applied only very rarely. A commonly used method of evaluating the health status of livestock animals is the measurement of rectal temperature (measured in the canal of rectum). Some authors note the disadvantages of such a method, principally associated with the necessity of immobilising the animal (Hentzen et al. 2012). Apart from the stress caused by holding, the result of the measurement can be disturbed by digestive processes, peristaltic movements, the presence of faeces, or muscular tone changes (Rexroat et al. 1999).

In connection with mentioned limitations of rectal thermometer, there have been sought the other methods to measure the temperature of animals. Technologies based on remote measurements are the most needed for these studies. An extensive review has been made by Sellier et al. (2014) of the methods of temperature measurements in livestock. The authors enumerated different contact and non-contact measurement medias useful for animal study. The infrared thermography cameras and pyrometers are non-contact and non-invasive tools to measure the skin surface temperature. It has been now increasingly often that thermographic cameras are used in this type of continuous measurements (Cook et al. 2015). The data loggers attached to the body surface can also be used to measure the temperature of skin surface. Some thermometers (pyrometers) are created to measure the heat radiation from the tympanic membrane (eardrum). This areas of taking measurement seems to be a reliable equivalent of internal temperature, principally due to the fact

that the blood flowing through the vessels of the tympanic membrane comes from the branch of carotid artery, which also supplies the hypothalamus (Chue et al. 2012). The measurement of the tympanic temperature can be performed either as single operation or a continuous measurement. The latter method is often used in cattle are ear tags with a probe inserted into the earn (Davis et al. 2003). In everyday practice, such devices may indicate the increase in body temperature in animals through a light signal (Richeson et al. 2012), however, more frequently they are a part of telemetric system (Hamrita and Paulishen 2011). Telemetry (biotelemetry) consists of small size sensors or transducers, which are placed on animal skin or inside of the body. The output of the sensor is modulated and then transmitted over a distance to a receiver. Next the signal is demodulated and after some proceedings it is measured by the data acquisition system. The transmission is most often achieved via radio waves, Internet, cellular network, Bluetooth. This technology enables a continuously monitoring of diverse physiological parameters (including body temperature) without handling or restraining the animal. Moreover it provides to collect a large number of data and through this system a real-time processing data is also possible (Hamrita and Paulishen 2011).

Besides of the tympanic temperature, the telemetric system is used increasingly often to obtain the continuous measuring of temperature in the digestive tract. Rumen boluses containing temperature sensors are very popular in cattle studies. The devices are orally administered to animal and because of the specific features of the stomach in ruminants, these boluses stay in the rumen till the end of animals' life (Bewley et al. 2008). In monogastric animals different kinds of ingestible internal temperature sensors most often remain in the body of animal up to several days, and they are then voided with faeces (Green et al. 2005, Angle and Gillette 2011). The data from these device can be obtained through telemetry system or it can be downloaded after recovering the data loggers (Angle and Gillette 2011, Fox et al. 2014).

Outside the monitoring of body temperature based on the data obtained from continuous measurement of temperature in the digestive tract, the values of vaginal temperature can also be a good parameter in the studies of female animals (Burdick et al. 2012). At present, there are various types of minor devices sensitive to temperature changes, which are periodically placed in the vagina (Bergen and Kennedy 2000). In such cases, there is also the possibility of remote transfer of data (Rorie et al. 2002).

The monitoring of peripheral or mid-peripheral temperature can be also by applying various subcutaneously implanted devices. These are most often data-loggers implemented for the specific period of time or temperature sensors allowing remote transfer of data. Nevertheless, the implantation of such device is an invasive procedure, often involving general anaesthesia (Hentzen et al. 2012).

Other techniques are also characterized by some limitations (Table). Implemented devices or those whose location requires piercing of the pinna, could cause local inflammation (following these procedures) and thus cause an

TABLE. Advantages and disadvantages of using some continuous temperature measurement methods in animals

Method/Device	Advantages	Limitations	References
Skin surface temperature sensor	non-invasive	Difficulty in keeping the sensors on the animal skin/coat for a longer period. Easily affected by environmental conditions.	Sellier et al. 2014 Mostaço et al. 2015
Subdermal temperature sensor	Subcutaneous placement of the devices allows to carry on measurements for a longer period of time.	Invasive procedure of implantation, often involving general anaesthesia. Dependency on changes in environmental conditions.	Lee et al. 2016 Hentzen et al. 2012 Brown et al. 1977
Infrared thermography camera	non-invasive, non-contact Rapid visualization of temperature distribution on the body surface.	Many factors may affect the measurement result (e.g. solar radiation, air dustiness, high humidity).	Church et al. 2014 Cook et al. 2015
Tympanic temperature sensor	Tympanic membrane share an arterial blood supply originating from the carotid artery; therefore, the tympanic membrane is considered to directly reflect body core temperature.	Implementation often requires piercing of the pinna, which may cause local inflammation.	Chue et al. 2012, Bergen and Kennedy 2000
Ingestible temperature sensor	Relatively easily to place the sensor in the digestive tract. This location is considered to be a good indicator of core temperature.	Temperature of digestive tract may fluctuate during water and food intake. In monogastric animals duration of measurements takes only up to several days.	Bewley and Shutz 2010 Angle and Gillette 2011
Vaginal temperature sensor	easy insertion The correlation between the values of the rectal and vaginal temperatures was confirmed.	The sensor often requires a special housing to prevent falling out. Different types of infection – characteristic of this body location may affect the normal temperature monitoring.	Hillman et al. 2009 Suthar et al. 2013

increase in the temperature measured (Bergen and Kennedy 2000). Then, the negative effect of the measurements of body temperature using the boluses in the digestive tract can be associated with the fluctuations associated with food and water intake (Bewley and Schutz 2010). The disadvantage of telemetric systems is often the necessity of maintaining a short distance between the sensor (placed on on/in the body of animal) and the data reader. The recording of temperature on the skin surface is the least invasive method; however, the hair cover, mobility of an animal, as well as high humidity in the environment represent some factors which can considerably hamper the placement and attachment of the sensors on the body surface. The recordings from a thermographic camera also bear some limitations, e.g., solar radiation, air dustiness, or the speed of air movement can disturb the measurement (Church et al. 2014).

THE USE OF CONTINUOUS TEMPERATURE MEASUREMENT IN THE STUDIES ON PIGS

Hanneman et al. (2004) carried on the research to determine the equivalence of pulmonary artery, urinary bladder, tympanic, rectal and femoral artery methods of temperature measurement in pigs. Study was carried out for a few days under clinical intensive care unit conditions. The experimental animals had indwelling bladder and tympanic thermistors and a thermocouple femoral artery sensor. Moreover two individuals had indwelling pulmonary artery thermistors, and two pigs had rectal thermistor sensors. According to the authors, the intravascular instrumentation methods were the optimal for continuous temperature monitoring in pigs. The other methods were characterized by unacceptable bias and were less precision.

Stiehler et al. (2013) measured rectal and vaginal temperature in postpartum sows. The measurement intravaginally was performer continuously for 6 h using a temperature logger. The results showed that rectal and vaginal temperatures were highly correlated ($r = 0.80$, $P < 0.01$). The authors claimed that the measurement of vaginal temperature using a data logger is a reliable method for a continuous and non-invasive monitoring of body temperature. In the other study Stiehler et al. (2015) found a clear circadian rhythm of vaginal temperature. The data was obtained through temperature loggers inserted and remained into vagina for 6 days. The mean minimum of vaginal temperature values was found in the morning hours (5:00–6:00) and the mean maximum temperature values were observed in the afternoon 13:00–19:00).

Fox et al. (2014) and Sapkota et al. (2016) conducted the experiments on heat stress in slaughter pigs, based on data obtained from thermal data-loggers administered orally to the animals. Sapkota et al. (2016) found that, along with the exposure of hogs on a short but intensive heat stress (transferring the animals to a microclimatic chamber with air temperature set at 39.3°C), their internal temperature (in the digestive tract) increased from 39.3°C to 40.3°C, and – contrary to the skin temperature – it did not lower during the time, when the hogs are transferred back to neutral conditions.

Fox et al. (2014) studied the effect of sprinkling on the behaviour and the internal temperature of pigs transported to a slaughterhouse. The skin of animals was sprinkled with water upon loading and again during unloading. Cooling the animals did not affect the differences in internal temperature. Nevertheless, during the transportation of pigs in an air temperature exceeding 25°C, in pigs subjected to sprinkling procedure, lower values of internal temperature were found. Therefore, the authors suggested that the application of sprinklers could have the great importance for the improvement of thermal comfort in pigs transported during high air temperatures (more than 25°C).

Hentzen et al. (2012) dealt with the validation of the telemetric method of body temperature measurement in pigs. In earlier tests, the authors found that handling and restraing young pigs in order to measure the rectal temperature is coupled with the increase in body temperature evoked by stress. Prior to the experiment proper, young meat swine of the experimental group were subjected to habituating lasting from two to three weeks to the handling procedures associated with measuring rectal temperature and thus to the contact with humans. The individuals, who were not given such training, formed the control group. During the proper experiments, the pigs of the control group showed the higher values of rectal temperatures. In view of these results, Hentzen et al. (2012) developed the system of continuous, contactless measurement of temperature in young meat swine based on the operation of four subcutaneously implanted sensors. During more than two weeks, the great amount of data was obtained, which were correlated with the values of rectal temperatures.

Jang et al. (2015) measured body temperature using three infrared sensors. The devices were installed above the animals, inside a special house. Temperature measurements were taken during feeding time. The sensors measured the skin surface temperature of the pig back every second. The results showed a high correlation between the temperature of the pigs skin and the air temperature.

Mostaço et al. (2015) conducted a study on piglets, attaching the data loggers in six locations on their skin. Apart from monitoring skin temperature, the rectal and tympanic temperatures were also measured by the use of digital rectal thermometer and infrared thermometer. In these studies, the respiratory rate was also analysed on the basis of flank movements. The animals were kept in climatic chambers, where air temperature was raised from 14°C to 35.5°C. One of objectives of the study was to determine the region of the body, whose external temperature is best correlated with the values of rectal temperature. The data obtained from the logger placed near the ear canal was the closest to this obtained in measurements of internal temperature.

Cook et al. (2015) used the recordings from an infrared thermographic camera conducted during 24 h to record the changes in the skin temperature of piglets. In this experiment, the first experimental group consisted of weaned piglets subjected to standard inocula-

tion. The second group included piglets injected with physiological saline, whereas the control group was formed by piglets not going through any of the procedures. The heat radiation from each group of piglets was monitored continuously for 24 h by a camera placed above the pen. The highest skin temperatures were found in the inoculated piglets. In this group, the increase in temperature was found from as early as the third hour after the procedure. The highest values of skin temperature were found after 10 h past the procedure, and they were maintained for the next 10 h. The results of these studies confirm the effectiveness of thermographic measurements in detecting febrile conditions in pigs kept in groups.

CONCLUSIONS

The review of professional publications warrants the conclusions that the systems based on continuous temperature measurements acquire ever increasing importance in the studies in livestock. However, there is still a need to develop and implement technologies for remote measurements the body temperature in pigs. In these searches for the best measurement methods it should be noted that these techniques are not free from certain imperfections. In view of all these limitations, it is a strong premise in favour of continuing the research in this domain. The best solution for conducting these kinds of measurements should be sought. The current dynamic developments in technology are undoubtedly a factor boosting such actions.

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Streszczenie: *Zastosowanie ciągłego pomiaru temperatury ciała w badaniach trzody chlewnej*. Pomiar temperatury jest jednym z najważniejszych wskaźników oceny stanu zdrowia u ludzi i zwierząt. Obecnie wraz z rozwojem wiedzy technicznej coraz więcej uwagi poświęca się ciągłemu pomiarowi temperatury ciała. Ten typ monitorowania wydaje się być szczególnie użyteczny w przypadku zwierząt gospodarskich. Pomiar temperatury ciała przeprowadzony w sposób ciągły zapewnia lepszą kontrolę nad stadem, a także wiarygodną ocenę wpływu czynników środowiskowych na zwierzę. Taki sposób monitorowania ciepłoty ciała umożliwia również uzyskiwanie znacznej ilości danych, co jest niezwykle przy-

datne w prowadzeniu badań naukowych. Celem tego artykułu jest przegląd literatury dotyczącej powszechnie stosowanych metod ciągłego pomiaru temperatury ciała u świń, a także wskazanie przyszłych kierunków badań.

Słowa kluczowe: trzoda chlewna, ciągły pomiar temperatury

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Preliminary study of memory processes in Mongolian gerbil (*Meriones unguiculatus*)

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Abstract: *Preliminary study of memory processes in Mongolian gerbil (*Meriones unguiculatus*)*. Mongolian gerbil (*Meriones unguiculatus*), a small rodent living in the wild steppes of Mongolia, was discovered in 1866 and it is becoming increasingly popular as a pet. The present work is an introduction to describe the behaviour of this species and to investigate the influence of gender and age on memory process. Forty gerbils divided into four study groups (young males, young females, adult males, adult females) were twice tested in a modified version of the Lashley III maze (repeat after a week). Using statistical tests memory parameters, the activity of animals and behaviour associated with the level of stress were analyzed. Conducted observations and calculations performed showed no difference between the groups ($P > 0.05$), which leads to the conclusion that in Mongolian gerbils age and sex have no effect on memory.

Key words: behavioural tests, Lashley III maze, age, sex, behaviour

INTRODUCTION

Mongolian gerbils are rodents originating from semideserts and steppes of Mongolia and Northeast China. They are gaining popularity both as pets and subjects of scientific experiments. Gerbils rarely become ill and are able to treat their own wounds after fights with other individuals (Clark and Galef 1977, Shimozuru et al. 2006, Liu et al. 2009). Nevertheless, their vigor may be influenced by both the way they are bred and their habitat. Stress is generally the main cause of gerbil diseases (Kaplan and Hyland 1972, Starkey et al. 2007). According to Agren et al. (1989), Mongolian gerbils are relatively resistant to radiation, and thus they may play a significant role in the studies of tumours. They are also used in research in the field of cytology, histology, parasitology and virology. Due to their exceptionally friendly nature it is suggested that Mongolian gerbils may also be used in the treatment of children with emotional disorders.

The Mongolian gerbil is an excellent subject for various behavioural observations. It is a friendly and very clean animal, which is one of the reasons for its growing popularity and researchers' willingness to work with these animals. Gerbils can learn quickly and are able to cope well with novel (previously unknown) situations (Shimozuru et al. 2006).

The majority of behavioural studies using the Mongolian gerbil as an animal model are focused on the response of its organism to a specific substance or stimulus (Jarbe and Johansson 1977), or concentrate on one sex and one age group only (Stuermer and Wetzel 2006). Therefore, observations that lead to a better understanding of this species' behaviour and take into account both sex and age are of great importance.

At present, there are numerous behavioural tests for rodents; among the most popular and least invasive are tests that verify the animal's willingness to explore, rate of learning and memory (Bressler et al. 2010). Stimulation by rewards is an important aspect of this type of tests. Additional motivation for the animal to explore is sometimes provided by previously restricting its access to food and placing its favourite, known food at the end of the task to be performed.

Studies using various types of mazes have made it possible to uncover general principles of rodents' learning process, which may prove useful for many other species, including humans. It is commonly believed that rats are animals characterized by an exceptional ability to run mazes, which resulted from their traditional habit to find way in underground tunnels, determined by their evolution (Deacon and Rawlins 2006, Dockery and Węsierska 2010).

A classic maze is a large structure with a number of vertical walls and a transparent roof. The animal begins exploration at the start place, moves through the maze and finishes its travel at the end of the route, where a reward is usually waiting for it. This test enables the assessment of animals' learning curve on the basis of subsequent trials during which, each time, the number of errors and time of passage are counted (File 1993, Bridges and Starkey 2004).

One variant is the Lashley III maze, which consists of three connected alleys, a start box and a goal box (Kolata et al. 2008). Numerous studies using this test have demonstrated that the time, in which an animal reaches the goal box, gradually decreases with subsequent trials; likewise the number of errors made, i.e. returns to a previously explored alley. The test may be modified in various ways. For example, the number of alleys to be travelled may be smaller or greater.

MATERIAL AND METHODS

The experiment was carried out on 40 Mongolian gerbils (*Meriones unguiculatus*) bred in the Husbandry of the Department of Genetics and Animal Breeding, the Faculty of Animal Science, Warsaw University of Life Sciences – SGGW. The herd was formed about 10 years ago, with over a dozen unrelated individuals. Since then, animals have been bred with minimizing kinship.

The animals were kept in a controlled environment. The temperature was approximately 23°C, air humidity oscillated around 60%, and light regime was 12 h of light / 12 h of darkness. The animals received food and water *ad libitum*. Additionally, once a week they received a bun with milk and multivitamin.

The experiment was carried out by means of a modified version of the Lashley III maze. Rectangular arena (100 × × 80 cm) with six equal pathways was used in the study. Each animal had two trials in the maze; during the first trial

the animal learned, whereas the purpose of the second trial (performed a week later) was to check the acquired skills and route learning.

The animals were divided according to age (into younger animals – aged 7–8 weeks and older animals – aged 3–4 months) and sex. In this way four groups were formed, each group consisting of 10 animals (younger females, adult females, younger males, and adult males).

The animals' access to food was restricted for about 12 h before the planned observation in order to increase their willingness to explore and motivate them to run through the maze. Additionally, the animals' favourite food was placed at the end of the maze. After a week, the test was repeated and the same parameters were noted. The results of both trials were compared and thus the ability to learn a spatial task in this species were analysed. There is no work in the literature evaluating memory processes in gerbils, therefore, the optimal interval between test's repetitions for this species is not known. Weekly interval was selected for this preliminary study as an initial value and a reference point for future research.

The following parameters were measured during the experiment:

- total time of passage (the maximum time, in which the animal was supposed to reach the end point, was 10 min; after that time the animal was considered to have failed the task);
- number of errors made (returns to a previously explored alley);
- number of defecations and urinations (stress indicator);
- number of rears (standing on hind legs as an indication of exploratory behaviour);
- number of groomings.

After each individual animal's travel, the research apparatuses were thoroughly rinsed with ethanol solution to eliminate the animal's smell and disinfect the arena.

Due to non-parametric character of the analysed variables, hypotheses concerning the influence of sex and age on the general ability of this species to learn were verified by non-parametric tests: the Kruskal–Wallis (the rank sum test) and the Wilcoxon signed-rank test.

All observations were carried out with the consent of Polish III Local Ethical Commission 24/2013.

RESULTS

Nine of all examined animal did not manage to finish the test within the prescribed time of 10 min (one animal failed the test in both trials). The Table shows that the shortest time was achieved by adult females in the second trial (217.10 s). During the first trial, the test was best performed by adults males (282.40 s), whereas the performance of young females was worst in the first trial (411.60 s). An increase in the average total time of passage was observed only in adult males. The remaining groups showed a better performance in the second trial as compared with the first trial. The difference between the two transitions for each of the groups was not statistically significant (in all groups $P > 0.05$).

Among all the surveyed groups, the greatest decrease in the time of passage and number of errors in the second trial as compared to the first trial was observed in the young females (Table). A decrease in activity and level of stress was also noted in this group. These results suggest the ability to learn; however, the differences are not statistically significant.

TABLE. The summary of all parameters observed in the study (number of each group is 10)

Specification		$\bar{x}$	*SD*	Min	Max	*P*
Time of passage – I trial (s)	young males	354.20	222.44	110	600	0.421
	young females	411.60	200.38	163	600	
	adult males	283.40	166.98	75	543	
	adult females	315.30	219.33	91	600	
Time of passage – II trial (s)	young males	248.80	92.26	130	403	0.202
	young females	237.00	143.09	104	574	
	adult males	362.50	217.10	54	600	
	adult females	217.10	202.98	51	600	
Errors – I trial (number)	young males	9.40	6.77	2	21	0.985
	young females	10.70	8.55	0	25	
	adult males	9.10	7.47	2	22	
	adult females	8.50	5.91	2	21	
Errors – II trial (number)	young males	5.50	1.18	4	8	0.181
	young females	6.90	4.01	2	15	
	adult males	11.70	8.82	0	27	
	adult females	5.60	4.22	2	15	
Rears – I trial (number)	young males	57.90	36.23	25	117	0.617
	young females	79.20	39.77	28	133	
	adult males	65.80	31.32	18	126	
	adult females	74.40	50.28	21	159	
Rears – II trial (number)	young males	40.80	18.83	19	75	0.131
	young females	51.80	29.84	22	126	
	adult males	94.60	62.94	6	219	
	adult females	48.50	38.48	5	137	
Groomings – I trial (number)	young males	1.00	1.49	0	4	0.611
	young females	1.30	1.25	0	4	
	adult males	0.70	0.95	0	2	
	adult females	0.70	0.95	0	2	
Groomings – II trial (number)	young males	0.30	0.95	0	3	0.724
	young females	0.60	1.26	0	3	
	adult males	0.30	0.48	0	1	
	adult females	0.30	0.95	0	3	
Defecations – I trial (number)	young males	0.70	0.82	0	2	0.710
	young females	1.00	1.33	0	4	
	adult males	0.70	1.34	0	4	
	adult females	1.80	2.49	0	7	

TABLE – cont.

Specification		$\bar{x}$	*SD*	Min	Max	*P*
Defecations – II trial (number)	young males	1.00	1.15	0	3	0.932
	young females	0.80	1.13	0	3	
	adult males	1.60	2.12	0	5	
	adult females	1.50	2.49	0	7	
Urinations – I trial (number)	young males	0.10	0.32	0	1	1.000
	young females	0.10	0.32	0	1	
	adult males	0.80	2.53	0	8	
	adult females	0.10	0.32	0	1	
Urinations – II trial (number)	young males	0.10	0.32	0	1	0.447
	young females	0.10	0.32	0	1	
	adult males	0.40	0.70	0	2	
	adult females	0.40	0.70	0	2	

Young males showed an improvement in the time of passage and a smaller number of errors compared to the first trial (Table). The number of rears decreased in the second trial, which is also an evidence of decreased activity of the animals. This change however is not statistically significant and is probably connected with shorter time of passage through the maze. The number of defecations and urinations showed that statistically the level of stress was comparable in both trials.

Shorter time of passage and a smaller number of errors in the second trial was also observed in adult females. This result was not statistically significant either.

Adult males were the only group of all the surveyed groups that demonstrated worse performance during the second trial, compared to the first trial (both the number of errors and time of passage increased). Nevertheless, both changes were not statistically significant. An increase in activity was also noted on the basis of the number of rears and grooming behaviours.

Young gerbils exhibited a similar improvement in the average time of passage through the maze in the second trial (the males were faster in the first trial, whereas the females were faster in the second trial). While variability in the performance of young animals were similar, adult gerbils showed greater differences in both trials. Similar results can be observed in respect of the number of errors. The experiment showed greater differences among adult individuals compared to the young animals, and the changes in time were slightly greater in adult animals. The level of both parameters, i.e. the time of passage and number of errors, decreased in the second trial. This is not however a statistically significant change, i.e. no differences in respect of the time of passage through the maze and number of errors were noted between the studied groups. Therefore, we cannot

draw a conclusion that age and sex have an influence on the memory processes of Mongolian gerbils.

Both young and adult females exhibited a similar level of activity (it was slightly increased in the first trial, which is evidenced in the number of rears). Young males dominated in respect of the number of groomings. Adult males showed a greater number of rears compared to young males. Young gerbils performed a greater number of groomings compared to adult individuals. However, these differences are not statistically significant ($P > 0.05$).

Both adult and young males showed an increased number of defecation scores in the second trial, which may be an evidence of an increased stress level in these animals (Table 1). In females defecation decreased in the second trial (supposedly, stress was greater in the first trial). The number of defecations was greater in the case of adult animals compared to the young ones (this may suggest an influence of age on the level of stress). Young males and females showed an identical average number of urinations in both trials (this may suggest that sex has no influence on the stress level in young individuals). In the group of adult animals, a greater number of urine drops left by males was observed (in this group the number was greater in the first trial) compared to the females (more urine drops in the second trial), which may suggest a greater influence of sex on stress level in adult animals). However, the level of significance obtained from the conducted analyses was higher than 0.05, which may demonstrate that sex and age have no influence on the level of stress either.

DISCUSSION

Stuermer and Wetzel (2006) demonstrated that there were no significant differences in the behaviour of wild and domesticated Mongolian gerbils in the open-field test. Wild gerbils in that study made the task a little faster, but the difference was not statistically significant, the same in our experiment. Animals in this experience performed rears much less often than in our own. Only on the 5th day the frequency of this behavior were comparable. Our gerbils presented grooming much less often than quoted Authors for the whole experiment. Domesticated individuals used in that experiment were obtained from Tumblebrook farm, whereas the wild ones were caught in Mongolia. All animals were kept in monogamous pairs in transparent cages. Like in the present experiment the animals had *ad libidum* access to water and pellets. Additionally, they were fed with sunflower seeds. Each of three surveyed groups (wild F0, wild F1 and domesticated) numbered eight adult males.

Spangler et al. (1997) studied the effect of age on the behaviour of gerbils. The observations took 77 individuals at age 14–54 months and their study showed decreased with age activity of animals well as increasing the number of errors committed in the T-maze test. In our experiment, young individuals were more active in the first pass. On the other hand Bridges and Starkey (2004) studied the effect of gender on the level of anxiety. On the basis of the tests (open field, black/white box and elevated plus maze) they claimed that female gerbils are more fearful. The same authors continued

research on anxiety-like behaviour in gerbils developing it on the effect of the dose of progesterone, which has anxiolytic effects. This time there was no difference between the sexes (Bridges and Starkey 2010). Also in our experience, both sexes are not statistically different in terms of the number of stress indicators, defecations and urinations.

Our experience suggests a lack of gerbils ability to learn. However, the experience involved only two repetitions of the test. Collett et al. (1986) examining the spatial memory of the gerbils and their ability to use landmarks, have shown that these animals need as many as 150 trials (about a month) to get straight to the correct spot afther placed in the test arena.

Bressler et al. (2010) investigated the influence of mild stress (water and food deprivation or in the dark phase) on the learning process of house mice in the Lashley III maze. The maze constructed for the purpose of that experiment had four alleys. The structure also included a start box and a goal box which housed a pseudo-home cage similar to the cages in which the animals were kept. One of the studies compared route learning in younger and older animals. The study used 25 mice (10 individuals aged 2 months and 15 individuals aged 24 months). Younger animals proved to be quicker at learning the maze route. The authors concluded that in mice, like in humans, senses deteriorate with age.

They also found that age-related behavioural changes occurring in rodents may be difficult to interpret during the most popular behavioural tests, such as the Morrison water maze. Thanks to possible modifications of the Lashley III maze, animals may be given various clues (odour, sound stimuli) which motivate them for exploration and effective navigation. Also our experience was based on the modified Lashley III maze, but the results we achieved are not supported by the experience quoted.

Vöikar et al. (2001) have studied mice using various behavioural tests which demonstrated that behavioural differences between males and females were incidental. A similar phenomenon has been observed by us in our study of gerbils.

During the study we noted a systematic decrease in the average time of passage through the maze and number of errors made by gerbils (except adult males). Wirth-Dzięciołowska et al. (2005) observed a similar phenomenon in the study of behaviour and learning in three selection lines of mice in the open field test and the Lashley III maze. The experiment used three lines: light line (L), heavy line (C) and control line (K) in three age groups (P-21, P-56 and P-90). In the course of the whole experiment an increase in the activity of L individuals was observed, whereas the heavy-line and control-line mice habituated in the last stage of the experiment.

In our study several individuals tried to jump out of the maze during the experiment. A similar behaviour was observed in the studies by Stuermer and Wetzel (2006) in the open field test. Agren et al. (1993) report that jumps are a typical way of moving in this species.

Węsierska and Turlejski (2000) in their study of rats in the elevated plus maze test found that rats exhibited a typical behaviour, known in the literature, described as passive-defensive. The

animals interpreted a new situation in which they found themselves as a threat and gradually acclimated to the environment. The Mongolian gerbils used in our study exhibited a similar behaviour (improved performance in the second trial). The plus maze experiment also assessed exploratory behaviour on the basis of the number of entries and time spent by the animals in the open arms compared with the enclosed arms.

SUMMARY

The present study demonstrated that the performance of all investigated groups during the test was similar. We concluded that the analyzed parameters (age and sex) did not have a statistically significant influence on any of the studied features of the animals (memory, activity and stress level). Definitely worth repeat the test in the extended schema: more iterations of the test and different intervals between them.

It should be noted that due to the lack of earlier cognitive studies of this species of animals it was not possible to assess the minimum sample size before the observations. Although no significant differences were reported, the present study may be a valuable starting point for future analyses of these species.

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Streszczenie: *Wstępne badanie procesów pamięciowych u myszoskoczka mongolskiego (*Meriones unguiculatus*).* Myszoskoczek mongolski (*Meriones unguiculatus*) to mały gryzoń dziko żyjący na stepach Mongolii, odkryty w 1866 r. i stający się coraz bardziej popularny jako zwierzę domowe. Niniejsza praca stanowi wprowadzenie do opisania zachowania tego gatunku oraz zbadanie wpływu płci i wieku na procesy pamięci. Czterdzieści myszoskoczków podzielonych na cztery badane grupy (młode samce, młode samice, dorosłe samce, dorosłe samice) zostało dwukrotnie zbadanych zmodyfikowaną wersją labiryntu Lashleya III (powtórzenie po tygodniu). Za pomocą testów statystycznych analizowano procesy pamięciowe, aktywność zwierząt oraz zachowania związane z poziomem stresu. Przeprowadzone analizy nie wykazały różnic między grupami ($P > 0,05$), co prowadzi do wniosku, że u myszoskoczków mongolskich wiek i płeć nie mają wpływu na procesy pamięciowe.

Słowa kluczowe: testy behawioralne, labirynt Lashleya III, age, płeć, behawior

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New data for invasive pilengas mullet species *Liza haematocheila* (Temminck & Schlegel, 1845) along Bulgarian Black Sea coast

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Abstract: *New data for invasive pilengas mullet species* Liza haematocheila *(Temminck & Schlegel, 1845) along Bulgarian Black Sea coast. Liza haematocheila* (Temminck & Schlegel) (syn. *Mugil soiuy* Basilewsky) is an invasive mugilid species, native to the Amu Darya River basin. After numerous introduction attempts to support commercial fisheries, this species established a successful breeding population in the Sea of Azov during the early 1980s. This invasive species expanded its areal of distribution and has been recorded for north-eastern Black Sea, Turkish coast, Aegean Sea and Western Meditteranean. The present study covered new data for morphometrical and meristic characteristics of the speciemens caught along Bulgarian Black Sea coast as well as genetic-biochemical evidence for species identification.

Key words: pilengas, invasive species, Black Sea, genetics, morphology

INTRODUCTION

The Black Sea biodiversity has become much more sensitive to emigrants expansion than those in other seas. *Liza haematocheila* (Temminck & Schlegel, 1845) is one of the main invaders established and having the most dramatic impacts on species diversity (Erdogan et al. 2010). The species was anthropogenically introduced to the Azov Sea and Black Sea for aquaculture purposes from the Amur River estuary to Japan Sea in 1972–1982. In the late 1980s an exotic species Pacific mullet appeared in the Black Sea (Okomus and Bascinar 1997). The haarder *Mugil soiuy* became an important commercial species in the Black and Azov Seas; its annual catch in the Black Sea exceeds 10,000 t (Gomoiu et al. 2002).

Mugil soiuy has not been reported for Bulgarian fish fauna until 2003. It should be mentioned that for a long time *M. soiuy* was observed along Bulgarian Black Sea coast, but it was not scientifically described. For the first time Dobrovolov et al. (2003) provided genetic-biochemical evidence for existing of pilengas species as well as data for phylogenetic relationship of all mullet species inhabited Bulgarian Black Sea coast. After that the species was included in the list of species for the Bulgarian fish fauna (Zhivkov et al. 2005, Karapetkova and Zhivkov 2006).

In Bulgaria the statistical information about the presence of the haarder in the catches is insufficient. During the period 2004–2009 catches of mullets in the Black Sea represented 0.7–0.8% of the total catches in Bulgaria. In 2009 catches

(2,243 t) of *M. soiuy* increased from 10 to 24 times, compared with the previous two years (Bekova and Petrova-Rajkova 2011). To the best available knowledge, no further data about species distribution and catches along the Bulgarian Black Sea coast exists since 2009.

Up to now there is no data about morphological and morphometric parameters of the pilengas mullet along Bulgaria marine waters.

The aim of the study is to describe morphological and morphometric paramethers of the invasive pilengas species along Bulgarian Black Sea coast as well as to provide supporting genetical data for accurate species identification.

MATERIAL AND METHODS

The morphometric measurements were taken on two *Liza haematocheila* (= *M. soiuy*) samples (Figs 1 and 2), collected in November 2014 and January 2015, by gillnets from the commercial catch in the Varna Bay (northern Bulgarian Black Sea coast).

FIGURE 1. Pilengas mullet, caught in November 2014, Varna Bay, analyzed electrophoretically (photo by P. Ivanova)

FIGURE 2. Pilengas mullet, caught in January, 2015, Varna Bay, analyzed electrophoretically (photo by P. Ivanova)

The meristic and morphometric features were measured according to Koutrakis and Economidis (2000). The total length of the specimens was measured to the nearest 0.1 mm and total weight nearest 1 g. About 10 scales from each fish were taken for ageing. Annuli of at least three scales from each individual were counted under a light microscope NTB-3A.

For allozyme analyses the samples were stored at a temperature of –20°C until the analyses in the laboratory. Comparison with the flathead grey mullet (*M. cephalus*) and golden grey mullet (*L. aurata*) was done. For analysis of the enzymes and non-enzyme protein systems, a homogenate of white dorsal muscle was used. The proteins were separated by horizontal starch gel electrophoresis. General muscle proteins (PROT) and esterases (EC 3.1.1.1 – EST) were analyzed. The staining of different enzymes was performed according to Shaw and Prasad (1970). The buffer systems of Dobrovolov (1976) and Clayton and Gee (1969) were used for the electrophoresis. The nomenclature of loci and alleles used here followed essentially the recommendation of Shaklee et al. (1990).

RESULTS AND DISCUSSION

The exotic pilengas mullet (Figs. 1 and 2) could be distinguished from other native mullet species in the Black Sea by its slight forked caudal fin, large scales, elongated spindle-shaped body and strongly flattened head.

The detailed morphological description covered with the data pointed from Kaya et al. (1997). The morphological and morphometric measurements for the two pilengas samples analyzed are presented in the Table. They were compared with the data for the same species, from different Black Sea regions. Some differences were found only in two morphometric parameters (number of the rays of anal and pectoral fins), but probably they can be a result from the polymorphism in these meristic characters.

During the ageing of two individuals' scales relatively clear annual rings were visualized. The age composition of the individuals was found 3+ for the first specimen and 4+ for the second one (Fig. 3).

Elucidation of the taxonomic status of the Mugilidae family has been traditionally based on the use of morphological characters. The results obtained were in most cases controversial, failing to provide any conclusive answers (Papasotiropoulos et al. 2007). Thus difficulty is due to the very conservative morphology by all mullets and very few characters are suitable as key characters to establish unambiguously the phylogenetic relationship among species (Stiassny 1993, Caldara et al. 1996).

More recently the phylogenetic relationships of grey mullets have been investigated with the use of non-morphological characters, employing biochemical and nucleic acid markers. Pilengas mullet is morphologically similar to other mullet species, and genetical analyses are necessary for its clear identification.

On the base of allozyme analyses carried out, the species identity of the two mullet samples, caught form the Varna Bay in 2014 and 2015 was specify as pilengas mullet species.

TABLE. Morphometric and meristic characters (in mm) of *Liza haematocheila* (= *M. soiuy*) specimens caught in front of Varna Bay (1 and 2), the data for Ismarida lagoon and Thracian Sea [3 and 4, according to Koutrakis and Economidis (2000), and from Aegean Sea, Homa Lagoon, according to Kaya et al. (1997) – 5 and 6]

Date Location	Speciemen					
	1	2	3	4	5	6
	03.11.2014 Varna Bay	12.01.2015 Varna Bay	05.01.1998 Ismarida lagoon	19.03.1998 Thracian Sea	Homa Lagoon	Homa Lagoon
Total length	330.0	375.0	488.0	606.0	550.2	453.2
Standard length	290.0	330.0	411.0	502.0	478.7	378.6
Body height	56.0	68.0	92.0	112.0	90.3	65.0
Head length	60.0	70.0	96.0	121.0	115.0	97.0
Pectoral length	50.0	55.0	65.0	83.0	22.0	23.3
Eye diameters	12.0	13.0	12.0	13.5	20.0	18.3
First dorsal fin	IV	IV	IV	IV	IV	IV
Second dorsal fin	I 8	I 8	I 8	I 8	I 8	I 8
Anal fin	II 8	II 8	II 8	III 9	I 8	I 9
Pelvic fin	I 5	I 5	–	–	I 5	I 5
Pectoral fin	14	14	14	14	I 14	I 14
Forck length	470.0	520.0	469.0	578.0	–	–
Predorsal 1 distance (PdI)	125.0	143.0	187.0	235.0	–	–
Predorsal 2 distance (PdII)	214.0	243.0	305.0	377.0	–	–
Preanal distance	198.0	232.0	293.0	367.0	–	–
Peduncle hight	31.0	35.0	–	–	–	–
Preventral distance	106.0	122.0	147.0	182.0	–	–
Upper lip high	5.5	6.0	7.0	7.2	–	–
Weight (g)	345.0	490.0	–	–		
Longitudinal number of scale series	42	44	40	42	45 (±2)	45 (±2)
Age	3+	4+	–	–	–	–

The data received for some genetical markers as general muscle proteins (PROT) and non-specified esterases (EST) were compared with our previous allozyme data for all mullets species along the Bulgarian Black Sea coast, identified by Dobrovolov et al. (2003) and our unpublished data. As a result of the comparison it is found that the allozyme spectra of enzyme and non-enzyme systems received (Fig. 4) are typical for the pacific mullet *M. soiuy.*

FIGURE 3. Scales of *Liza haematocheila* from the Bulgarian Black Sea coast with visible growth rings, marked with dots

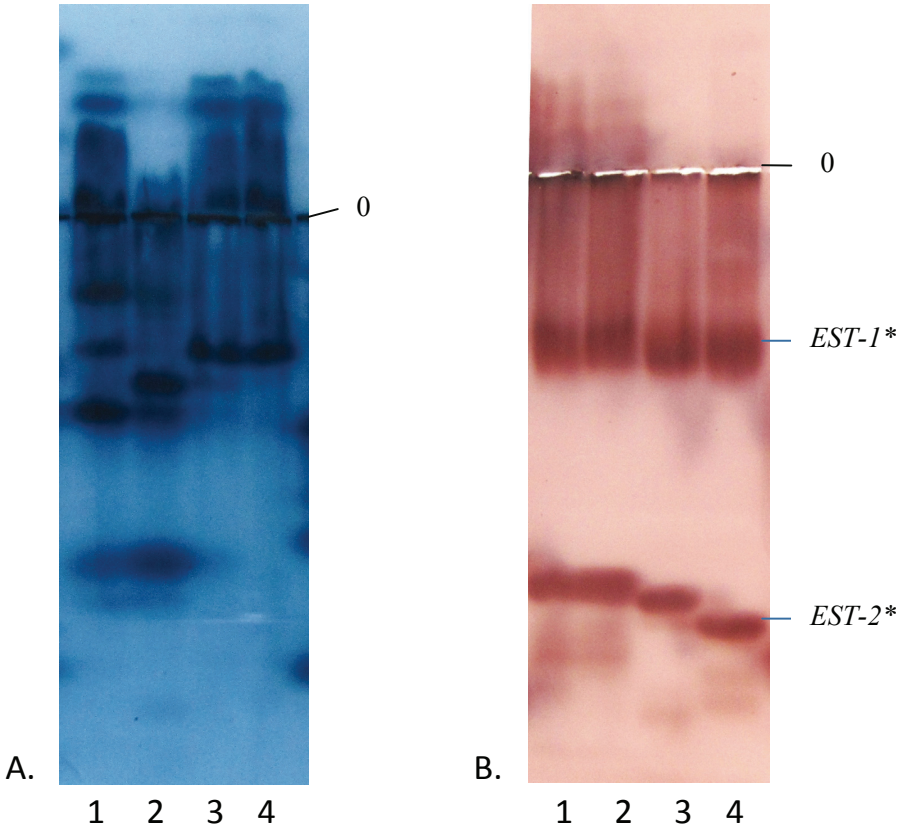

FIGURE 4. Electrophoregrams on: A – general muscle proteins (PROT): 1 – striped mullet (*M. cephalus*), 2 – pilengas mullet, 3–4 golden grey mullet (*L. aurata*); B – esterases (EST): 1–2 – golden grey mullet (*L. aurata*), 3 – pilengas, 4 – striped mullet (*M. cephalus*), 0 – origin

There is a need of additional investigations of more samples about genetic diversity caused by acclimatization and successful adaptation on the new range along Bulgaraian Black Sea coast.

The statistics information about catches of pilengas in the Bulgarian Black Sea waters is scarce. The maximum level of *M. soiuy* catches along the Bulgarian Black Sea coast was registered in 2009. The level of catches decrease from 2010 (0.33 t), 2011 (0.43 t) and 2012 (0.18 t) according to Bekova and Raikova-Petrova (2011). Unlike the very common occurrence of the species in the Black Sea, a relatively rare population can be observed in the northern Bulgarian Black Sea. According to personal communications with local fishermen the pilengas become a rare along Bulgarian Black Sea coast nowadays.

CONCLUSIONS

Genetic-biochemical and morphological data presented could be used as useful markers for distinguishing the invasive pilengas (*Liza haematocheila*) from the other mugilids inhabiting Bulgarian Black Sea coast. Regardless of the fact that during the last years the species become a rare for our coast, the data received can be used to monitor and to find the actual status of its stocks as well to assess its possible impact on native grey mullet species.

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Streszczenie: *Występowanie inwazyjnego gatunku ryby* Liza haematocheila *(Temminck & Schlegel, 1845) wzdłuż bułgarskiego wybrzeża Morza Czarnego. Liza haematocheila* (Temminck & Schlegel) (syn. *Mugil soiuy* Basilewsky) jest jednym z inwazyjnych gatunków ryb z rodziny mugilowatych, naturalnie występujących w basenie rzeki Amu-daria. Po licznych próbach introdukcji tego gatunku do Morza Azowskiego, w celu wzbogacenia połowów, gatunek ten na początku lat 80. XX wieku stworzył stabilną populację. Obecnie gatunek ten rozszerzył zasięg występowania i jest stwierdzany w północno-wschodniej części Morza Czarnego, wzdłuż wybrzeża Turcji, w Morzu Egejskim oraz w zachodnim obszarze Morza Śródziemnego. Obecne badania, obejmujące nowe dane merystematyczne i morfometryczne, jak również analizy genetyczno-biochemiczne osobników złowionych wzdłuż bułgarskiego wybrzeża Morza Czarnego, są dowodem potwierdzającym występowanie tego inwazyjnego gatunku w Bułgarii.

Słowa kluczowe: *Liza haematocheila,* gatunek inwazyjny, Morze Czarne, genetyka, morfologia

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The use of *sternomandibularis* muscle for beef quality assessment

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Abstract: *The use of sternomandibularis muscle for beef quality assessment.* The goal of the research was to check the possibilities of using *sternomandibularis* muscle instead of the *longissimus lumborum* muscle to assess the physico-chemical traits and the chemical composition of cattle meat. Thirty carcasses were chosen for testing (10 carcasses of each of the following categories: bulls, heifers and cows). The *longissimus lumborum* muscle (LL) and *sternomandibularis* muscle (StM) samples were taken 72 h post mortem for the quality evaluation. It was found that the pH of the StM muscle dropped down slower than in the LL muscle. What is more, the StM muscle had a better water holding capacity, higher water content and lower fat content than the LL muscle. No significant differences were found between the muscles when it comes to lightness and pH_{72}. In the heifers and bulls carcasses no differences were found in terms of shear force. A significant interdependence was demonstrated between muscles in the following traits: pH_{48} ($r = 0.77$), fat content ($r = 0.68$) and water content ($r = 0.47$). Due to this fact and based on the measurements of the StM it was possible to develop regression equations to estimate the values of the above mentioned traits for the LL. So it is possible to substitute the LL with the StM in the beef quality evaluation especially in terms of the following traits: pH_{48}, pH_{72}, lightness, fat and water content for all of the cattle categories. It is also possible to substitute the LL muscle with the StM muscle in the beef quality tests in terms of protein content and meat tenderness measured with the shear method for bulls and heifers.

Key words: beef, meat quality, muscle comparison

INTRODUCTION

In the research concerning beef quality the *musculus longissimus dorsi* was used as a sample representing the top part of the carcass, and the *biceps femoris*, *semitendinosus* or *semimembranosus* muscle was used as a sample that represents the biggest round muscles and the *triceps brachii* was used as a sample representing the shoulder muscles (Carmack et al. 1995, Grześkowiak et al. 2002, Kirchofer et al. 2002, Lennon et al. 2002, Nowak et al. 2005, Baublits et al. 2006).

The availability of the above-mentioned muscles for tests in scientific experiments is a common problem because of economic reasons (Tume et al. 2010). Beef carcasses are an expensive product and slaughterhouses usually refuse to take samples from the main back muscles, leg muscles or shoulder because it means financial losses for them, e.g. such carcasses cannot be exported. That is why it is important to find an alternative muscle that can be used as a sample in tests and which is not as troublesome as the other important beef muscles. This muscle can be found

in the neck part of the carcass, which is usually not for export and is cut for further processing. Very easily accessible for tests is the sternum-mandible muscle (*sternomandibularis*). This muscle has already been used by some authors like Davey and Gilbert (1977), Bendall and Restall (1983), Yook et al. (2001), Terrescano et al. (2003), and Sikes et al. (2014). The samples of this muscle were usually taken at the very early stage, right after bleeding and decapitation and were used for biochemical studies of the after slaughter changes, e.g. changes, in enzyme activity. The muscle weight and its anatomical uniformity (Tume et al. 2010) give the possibility to use it for meat quality tests. The goal of this research was to check the possibility of using the *sternomandibularis* muscle instead of the *longissimus lumborum* muscle to determine physico-chemical traits and the basic chemical composition of beef.

MATERIAL AND METHODS

Experiment scheme

The research material comprised 30 carcasses of Polish Black and White Holstein Friesian cattle from 3 categories, i.e. 10 bulls, 10 heifers and 10 cows. All of the carcasses were chosen in the slaughterhouse in Chróścina. All animals were slaughtered after 24 h of resting. Radical device was used for stunning. Only carcasses of the R conformation class and third class of fatness, in EUROP system classification (EC 1249/2008), were chosen for testing. The carcasses were chilled with a one stage system in the chamber with 2–4°C and relative humidity about 90%. After the 24-hour-chilling the pH_{24} was measured in the *musculus longissimus lumborum* (LL) and the *musculus sternomandibularis* (StM). Then, the samples with weight approx. 1 kg of the LL and the StM muscles were removed from the left carcasses. The samples were delivered in thermos boxes to the biotechnology lab of the Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology in Poznań. The samples were stored in refrigerators at approx. 4°C up to 72 h post mortem. After that time the physical and chemical analysis were performed.

Measurements and analysis

The following quality traits were analysed:

- pH_{24}, pH_{48}, pH_{72} after slaughter using a Radiometer PHM 80 Portable pH-meter;
- meat colour with a Chroma Metter CR400 device by Konica Minolta, determining L*a*b* colour parameters in the CIE Lab system (the source of light D65, observer 2°, the opening of the measuring head 8 mm, calibration on the white standard: L* = 97.83, a* = 0.45, b* = 1.88);
- water holding capacity with the Grau and Hamm method modified by Pohja and Niinivaara (1957);
- water content according to the PN-ISO 1442:2000, fat content according to the PN-ISO 1444:2000, protein content according to the PN-75/A-04018 and ash content according to the PN-ISO 936:2000.

Meat samples were weighed with 1 g accuracy and cooked at 85°C for approx. 40 min. After cooling down the samples

were weighed again in order to determine their cooking losses. Shear force was measured on cooked muscle samples which were cut along the fibres by 2.5 cm diameter cylinder. The measure was made using Zwick Roell 0.5 device equipped in 0.5 kN head and Warner––Bratzler unit. Speed of the blade was 100 mm/min.

Statistical analysis

The obtained results were statistically analysed using the Statistica 6.0 software. The mean values and standard deviations were calculated and the date were analysed by analysis of variances using ANOVA procedure. The significance of the differences between the means was established using the Tukey test (Stanisz 1998). A P value of less than 0.05 was considered significant. The correlation coefficients were calculated and the regression equation was developed for the chosen quality traits.

RESULTS

Physical and chemical traits

The significant influence of muscle on physico-chemical properties and chemical composition beef was found (Tables 1 and 2). Higher pH was observed in

TABLE 1. Values of pH and water holding capacity (WHC) of *musculus longissimus lumborum* (LL) and *sternomandibularis* (StM) depending on cattle category

Quality traits	Beef category	LL		StM		Category		Significance level (P)
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
pH_{24}	bulls	5.73	0.07	6.20	0.26	5.96	0.30	muscles 0.01
	heifers	5.68	0.08	6.14	0.22	5.91	0.28	category NS
	cows	5.74	0.07	6.14	0.20	5.94	0.26	interaction NS
	muscle	5.72^{A}	0.08	6.16^{B}	0.22	–	–	0.01
pH_{48}	bulls	5.67	0.04	5.72	0.07	5.69	0.06	category NS
	heifers	5.67	0.06	5.73	0.08	5.70	0.08	interaction NS
	cows	5.70	0.11	5.81	0.19	5.75	0.16	muscles 0.05
	muscle	5.68^{a}	0.08	5.75^{b}	0.13	–	–	0.05
pH_{72}	bulls	5.74	0.07	5.75	0.04	5.75	0.06	category NS
	heifers	5.76	0.11	5.84	0.12	5.80	0.12	muscles NS
	cows	5.75	0.14	5.82	0.19	5.79	0.17	interaction NS
	muscle	5,75	0,10	5.80	0,11	–	–	NS
WHC (%)	bulls	32.45	2.07	29.96	2.84	31.20^{a}	2.74	muscles 0.05
	heifers	30.18	2.47	30.87	2.91	30.52^{a}	2.65	category 0.05
	cows	30.45	3.01	27.57	3.02	29.01^{b}	3.29	interaction NS
	muscle	31.02^{a}	2.67	29.47^{b}	3.16	–	–	0.05

Mean values in rows and columns denoted by different letters differ significant: $^{A, B}$ at $P \leq 0.01$; $^{a, b}$ at $P \leq 0.05$, NS – not significant.

TABLE 2. Colour parameters, shear force and cooking loss of investigated LL and StM muscles depending on cattle category

Quality traits	Beef category	LL		SM		Category		Significance level (*P*)
		$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	
Lightness	bulls	36.26	2.71	36.83	2.66	36.54	2.60	muscles NS
	heifers	37.35	2.61	35.60	1.53	36.48	2.00	category NS
	cows	34.24	2.51	35.77	1.97	35.00	2.24	interaction NS
	muscle	35,95	2,60	36.05	2,05	–	–	
Redness	bulls	19.27	1.08	16.08	1.03	17.67	1.01	muscles 0.01
	heifers	17.67	1.17	16.27	1.63	16.97	1.35	category NS
	cows	19.44	2.68	15.46	1.87	17.44	2.20	interaction NS
	muscle	18.79^{A}	1.92	15.94^{B}	1.54	–	–	0.01
Yellowness	bulls	2.27	0.81	1.21	1.11	2.24	0.85	muscles 0.01
	heifers	2.66	0.84	1.37	0.66	2.01	0.70	category NS
	cows	2.17	1.05	1.67	1.02	1.92	1.03	interaction NS
	muscle	2.37^{A}	0.90	1.42^{B}	0.94	–	–	0.01
Shear force (N/cm^2)	bulls	22.90^{A}	5.73	23.89^{A}	5.30	–	–	interaction muscle × category 0.01
	heifers	23.83^{A}	7.94	27.70^{A}	7.37	–	–	
	cows	21.29^{A}	10.98	37.75^{B}	7.31	–	–	
Cooking loss (%)	bulls	26.22	2.08	27.34	2.49	–	–	interaction muscle × category 0.01
	heifers	22.48^{A}	2.34	26.56	2.90	–	–	
	cows	22.97^{A}	1.57	29.06^{B}	2.74	–	–	

Explanatory notes as in Table 1.

the StM but only 24 ($P \leq 0.01$) and 48 h ($P \leq 0.05$) post mortem. After 72 h the differences between pH of LL and StM were not significant. The differences in the lightness (L*) were not significant. The LL muscle was redder (a*) and more yellow (b*) than the StM ($P \leq 0.01$).

Water holding capacity depended on both the muscle ($P \leq 0.05$), and the cattle category ($P \leq 0.05$). The water holding capacity observed for the StM was by 1.5 p.p. higher than for the LL. This trait reached better value in cows than in the carcasses from heifers and bulls. Significant interaction was found between the muscles and the cattle categories ($P \leq 0.01$) in cooking losses. It proved that the StM obtained approx. 4 to 6 p.p. higher cooking losses than the LL but only in cows and heifers. The bulls' muscles showed similar but not significant cooking losses.

Shear force was significantly affected by the interaction between muscles and cattle categories ($P \leq 0.01$). The cows' LL proved to be tenderer than the StM. The muscles of heifers and bulls did not show significant differences in terms of meat tenderness. When comparing the cattle categories it was found that they have an impact on StM muscle tenderness. The cows' StM is much tougher

than from bulls and heifers (the shear force was greater from 10 to 14 N/cm^2).

Chemical composition

The muscle had the significant impact on the chemical composition of muscles (Table 3). The cattle category had no impact on the water and fat content in beef. The water content in the StM was by approx. 3.5 p.p. higher ($P \leq 0.01$) and the fat content was by approx. 3.2 p.p. higher ($P \leq 0.05$) than in the LL. For protein and ash contents the significant ($P \leq 0.01$) interaction (muscle × cattle category) was found. In cows' StM the protein content was lower than in bulls and heifers muscles, whereas in the LL such a relationship was not observed. Only in cows' muscles the protein content differs significantly between muscles. The differences in the protein content in the bulls' and heifers' muscles were not significant.

The same multirelations were observed for the ash content. The differences in the ash content in the StM were not significant between the cattle categories. In the LL the ash content in the bulls' muscles was significantly lower than in the heifers' muscles. No significant differences in the ash content were found between the bulls' muscles, but there were significant differences between the muscles of cows and heifers.

Correlations

Out of the 13 quality traits, significant correlations between the muscles were found only for 5 traits, i.e. pH_{48}, pH_{72}, lightness, water and fat contents (Table 4). No significant relationships between muscles were found for protein

TABLE 3. Chemical composition of *longissimus lumborum* (LL) and *sternomandibularis* (StM) muscles

Qaulity traits	Beef category	LL		StM		Category		Significance level (P)
		$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	
Water content (%)	bulls	71.54	1.98	74.52	1.56	73.03	1.76	muscles 0.01
	heifers	70.37	1.38	73.14	1.28	71.75	1.30	category NS
	cows	69.20	3.81	73.92	3.99	71.56	3.85	interaction NS
	muscle	70.37^A	2.69	73.86^B	2.56	–	–	0.01
Fat content (%)	bulls	5.57	2.34	2.21	0.80	3.89	1.50	muscles 0.01
	heifers	6.93	1.77	3.72	1.30	5.33	1.49	category NS
	cows	7.09	4.06	4.07	3.99	5.58	4.01	interaction NS
	muscle	6.53^A	2.87	3.33^B	2.52	–	–	0.01
Protein content (%)	bulls	21.81^A	1.18	22.14^A	1.02	–	–	interaction muscle × category 0.01
	heifers	21.42^A	1.09	21.99^A	0.60	–	–	
	cows	22.44^A	1.61	20.88^B	0.66	–	–	
Ash content (%)	bulls	1.08^A	0.05	1.14^A	0.06	–	–	interaction muscle × category 0.01
	heifers	1.28^B	0.12	1.15^A	0.08	–	–	
	cows	1.27^B	0.09	1.14^A	0.08	–	–	

Explanatory notes as in Table 1.

TABLE 4. The correlation coefficients between the results of the assessment and indications of physico-chemical characteristics of LL and StM muscles (n = 30)

Physico-chemical characteristic	Correlation coefficient	Significance level (P)
Water content (%)	0.47	0.01
Fat content (%)	0.68	0.00
Protein content (%)	–0.25	0.19
Ash content (%)	0.04	0.82
pH_{24}	–0.07	0.70
pH_{48}	0.77	0.00
pH_{72}	0.46	0.01
WHC (%)	0.30	0.10
Cooking losses (%)	0.13	0.50
Lightness	0.38	0.04
Redness	–0.02	0.91
Yellowness	0.08	0.68
Shear force (N/cm^2)	–0.02	0.92

and ash contents, water holding capacity, redness and yellowness as well shear force.

Among the five above-mentioned correlated traits, only between the two of them there were no significant differences between the muscles i.e. pH_{72} and the lightness (L*). These traits were excluded from statistical analysis. Whereas for pH_{48} (Y_1), fat content (Y_2) and water content (Y_3) the regression equations were developed in order to estimate the value of these traits in the LL muscle based on the StM muscle measurements. The regression equations are as follows:

$Y_1 = 3.09 + 0.45x$
where x – pH_{48} of StM muscle;
$RSD = 0.05$; $R^2 = 0.59$;
$Y_2 = 0.88 + 1.95x$
where x – StM muscle fat content;
$RSD = 1.67$; $R^2 = 0.64$;
$Y_3 = 19.77 + 0.27x$
where x – StM muscle water content;
$RSD = 2.36$; $R^2 = 0.26$.

The third equation for water content is not precise enough because the determination coefficient is low $R^2 = 0.26$.

DISCUSSION

The chemical composition of LL muscle presented in this paper is very similar to the data that can be found in the literature. Grześkowiak et al. (2002) reported on average 75.11% of water content and 3.48% fat content in the LD muscle of young cattle. Other authors indicated 5.98% of fat and 71.88% of water in a rump cut (Modzelewska-Kapituła et al. 2009). In Limousine bulls the fat content was very low reaching only 0.80% whereas the water content was 75.77% (Daszkiewicz and Wajda 2002). High interdependences were found in present study between the fat content in LL and StM (r = 0.68). This fact allowed developing the regression equation Y_2 used to assess the fat content in the LL muscle

based on the measurements taken on the StM muscle. A similar equation Y_3 for the water content has not been precise enough because the estimation error was 2.36 and determination coefficient was only 0.26.

In the research on beef quality the authors usually concentrated on the meat colour and muscles' pH. The pH ultimate value of the *musculus longissimus dorsi* has been quoted in the literature starting from 5.4 (Terrescano et al. 2003, Goni et al. 2007) up to approx. 6.0 (Grześkowiak et al. 2002). The pH ultimate range is so wide because it depended on many factors during an animal life (e.g. breed, feeding, welfare) as well as many post slaughter factors (e.g. meat chilling rate). The average value of the pH_{72} in the LL muscle was. 5.7 and was placed in the middle of the literature range. The average value pH_{72} in the StM was very similar (5.8). Some authors reported even lower pH (5.4) in the StM (Yook et al. 2001). Page et al. (2001) proved that in the cattle populations in three US states (Illinois, Texas and Ohio) in over 80% of the beef carcasses the *longissimus dorsi* muscle pH varied from 5.40 to 5.59. Wajda et al. (1999) reported that the crossbred bulls of lowland black and white with beef cattle breeds, slaughtered after 40 h rest led to 5.61 pH in the *longissimus dorsi* muscle (LD).

The differences between LL and StM for pH were also confirmed by other authors. Torrescano et al. (2003) showed that the pH_{24} of the Swiss Brown bulls' LL muscle was on average 5.49 whereas the pH of the StM muscle was on average 5.77 ($P \leq 0.05$). The reported high correlation (r = 0.77) between pH_{48} of the LL and the StM muscle enabled to develop a Y_1 regression equation to assess pH_{48} in the LL muscle based on the pH_{48} measurements of the StM muscle. The pH measurement taken 48 h post mortem has been commonly used in industrial selection and in the scientific experiments. That is why the developed regression equation may be of high practical and cognitive importance.

Similar LL muscle lihgtness (L* = 37) to the one reported in this paper was reported by Sikes et al. (2014). A slightly brighter colour (L* = 39) was reported by Page et al. (2001) and Torrescano et al. (2003) and higher lightness (L* = 44.21) was reported by Goni et al. (2007).

Torrescano et al. (2003) found no significant differences between LL and StM for colour, lightness, and redness. It was however proved that the LL muscle was of higher yellowness than the StM muscle. It was also partially confirmed by the results of this research, apart from the redness, because it was higher in the LL muscle than in the StM muscle.

Yook et al. (2001) for young Korean cattle showed that the shear force of the unaged StM after 24 h post mortem muscle was 32.06 kg/cm^2. These values indicated that meat from Korean young cattle has similar tenderness as StM muscle from cows in present study. The tenderness of bulls' and heifers' meat was similar. These results have been compatible with the lack of relations between the shear force of the cooked LD muscle and the lightness (L*) of the fresh bulls' muscle (r = 0.19) reported by Goni et al. (2007). Both traits were not significantly different between the tested muscles. The similar shear force values of heifers' LD muscle (24 N/cm^2) were obtained by Borzuta (1995/1996).

CONCLUSION

The presented study demonstrates that almost quality traits were significantly different between LL and StM muscle, with exception of lightness (L*) and the pH_{72}. The significant interdependence was found between the tested muscles within the following traits: pH_{48}, fat and water content. This fact enabled to develop regression equations to estimate the values of these traits for the LL muscle based on the measurements and analyses of the StM muscle. So there is a possibility to assess two StM muscle's quality traits that get the same results as in the LL muscle (i.e. lightness and pH_{72}). There is also a possibility to assess three StM muscle's quality traits based on which the values of the same traits for the LL muscle might be calculated using the developed regression equations (pH_{48}, fat and water content).

The above-mentioned conclusions should be treated as contributory because the studied issue requires further analysis and experiments with more numerous materials.

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Streszczenie: *Przydatność mięśnia* sternomandibularis *do oceny jakości mięsa wołowego*. Celem badań było sprawdzenie możliwości wykorzystania mięśnia *sternomandibularis* zamiast mięśnia *longissimus lumborum* do określenia cech fizykochemicznych i składu podstawowego mięsa wołowego. Do badań wybrano 30 tusz wołowych (po 10 szt. buhajów, jałówek i krów), z których pobierano próby mięśni *longissimus lumborum* (LL) i *sternomandibularis* (StM) do badań jakościowych po 72 h *post mortem*. Stwierdzono, że mięsień StM charakteryzuje się wolniejszym tempem spadku pH oraz lepszą wodochłonnością, a także większą zawartością wody i mniejszą tłuszczu niż mięsień LL. Nie stwierdzono statystycznie istotnych różnic między mięśniami w ocenie jasności barwy i pH_{72}, a u jałówek i buhajów nie wystąpiła różnica w ocenie kruchości mierzonej szerometrycznie. Wykazano istotną współzależność między badanymi mięśniami w takich cechach, jak: pH_{48} (r = 0,77), zawartość tłuszczu (r = 0,68) i zawartość wody (r = 0,47), co umożliwiło opracowanie równań regresji do szacowania wartości tych cech dla mięśnia LL na podstawie pomiarów i oznaczeń mięśnia StM. Istnieje zatem możliwość zastąpienia w badaniach jakościowych wołowiny mięśnia LL mięśniem StM, szczególnie w takich cechach, jak pH_{48}, pH_{72}, jasność barwy, zawartość tłuszczu i wody dla wszystkich kategorii bydła oraz zawartość białka i ocena kruchości mierzonej szerometrycznie dla buhajów i jałówek.

Słowa kluczowe: mięso wołowe, jakość, porównanie mięśni

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Preliminary observations of the behaviour in two wild species of equids: Przewalski's horse (*Equus ferus przewalskii*) and Hartmann's zebra (*Equus zebra hartmannae*) kept in socially changed groups at Warsaw Zoo

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Abstract: *Preliminary observations of the behaviour in two wild species of equids: Przewalski's horse (*Equus ferus przewalskii*) and Hartmann's zebra (*Equus zebra hartmannae*) kept in socially changed groups at Warsaw Zoo.* Investigations were carried out at Warsaw Zoo to examine general behaviour in two wild equids after social changes. This changes were caused by management procedures. Three Hartmann's zebra and four Przewalski's horses were observed for 117 h throughout the whole year. Three methods of sampling were used: *ad libitum*, scan and focal. Abnormal behaviour in these wild equids was not found. There were several significant differences in frequency of behaviour between Przewalski's horse and Hartmann's zebra. In both species coordination of behaviour also occured. Social interaction frequency was very low and gave little support for the speculation about social structures in these groups.

Key words: Hartmann's zebra, Przewalski's horse, behaviour, zoo

INTRODUCTION

Przewalski's horse (*Equus ferus przewalskii*) and mountain zebra (*Equus zebra*) are species of hoofed mammals to the great degree similar. Both are large ungulates build for speed and longdistance movement. Although they are distributed in separate continents both are adapted to open grasslands. Both species are endangered and they were pushed away from their former distribution area to the harsher environment. Przewalski's horse is actually extinct in the wild and it has been reintroduced to some areas of Mongolia and China from 1986. Mountain zebra, with two subspecies, mountain zebra (*Equus zebra zebra*) and Hartmann's zebra (*Equus zebra hartmannae*) is confined to the restricted areas in the south Africa. The last stand of wild Przewalski's horse was also called Dzungarian Gobi, in fact large desert surrounded by mountains covered with dry steppes. Mountain zebra is found in the wild in a subdesert plains and barren rocky uplands (Bouman 1986, Estes 1992).

Przewalski's horse and mountain zebra live in one male (harem) groups. The number of mares per stallion is 3–4 in average in both cases. The young stallions (and fillies in mountain zebra) after abandoning family group form so-called bachelor herds (Bouman 1986, Penzhorn and Novelle 1991). Other behaviours of

both equids are also similar. For example the study of McDonnell and Havilland showed that from 45 forms of agonistic behaviour observed in the equid bachelor herd over 60% was common for Przewalski's horse and zebras (McDonnell and Havilland 1995).

There are adjacent equid exhibits at Warsaw Zoo: for Przewalski's horse (*Equus ferus przewalskii*) later abbreviated as PH and for Hartmann's zebra (*Equus zebra hartmannae*) abbreviated as HZ. These animals were kept in normal social "one male" group. However, in summer 2016 HZ stallion was transferred to the separate enclosure because of its aggressiveness towards mares. On the other hand, to the PH group third mare was introduced. Thus, both groups of equids have changed and could be seen as to some degree socially disturbed. The social manipulation in zebra species could be potentially dangerous for example leading for example to male infanticide (Pluhacek and Bartos 2000).

The purpose of this study was to compare behaviour of captive PH and HZ performed at Warsaw Zoo including the relations with the visitors. Authors particularly interested in the effect of social change in both groups and on the of abnormal behaviour occurence in these animals.

MATERIAL AND METHODS

The observations were carried out at Warsaw Zoo during the whole year between autumn 2015 and summer 2016. Detailed information on studied individuals are shown in Table 1.

The enclosures for both species at Warsaw Zoo were localized side by side near the main alley designed for visitors. The enclosures were also localized far away from the main zoo gate. Enclosure for HZ was rectangular and 3,500 m^2 in area. In the case of pH the size of enclosure was 4,500 m^2 and its shape resembled letter "J". Both exhibits seemed to be fairly spacious and their designs enabled performance of various locomotory behaviour. Animal had also access to stables heated in winter. Their stables

TABLE 1. The basic information concerning observed animals at Warsaw Zoo

Przewalski's horse			Hartmann's zebra		
Sex and ID	Birth date/ /acquisition	Birth location	Sex and ID	Birth date/ /acquisition	Birth location
Stallion	26.07.1998	Warsaw Zoo	Stallion***	22.07.2009	Reserve Africaine de Sigean
Mare 1	15.05.1994 24.01.2005*	Rotterdam Zoo	Mare 1	27.06.2011 05.06.2014*	Dvur Kralove Zoo
Mare 2	26.09.2000 19.12.2007*	Cologne Zoo	Mare 2	14.07.2011 05.06.2014*	Dvur Kralove Zoo
Mare 3**	02.08.2011 30.09.2015*	Prague Zoo	Mare 3	13.07.2013 09.10.2014*	Wrocław Zoo

*Acquisition by Warsaw Zoo; **introduced during observation period; ***exclude from group.

were 46 and 30 m^2 in area for HZ and PH, respectively. From the alley the visitors had opportunity to observe animals present at all parts of enclosure. Equids were out of view only when they came to stables. Moreover, PH could hide away to some degree in vegetation concentrated near the fence. In the HZ and PH enclosures there were several scattered trees and logs. In both enclosures animals could move on the concrete surface as well on the softer (sand and grass covered) floor. Additionally there is also small pond in the HZ enclosure. The diet of both equids was standard and comprised of oats with other ingredients

Prior to the study observation period three pilot observations of 1 h each were carried out. During the study period observer has occupied determined place near exhibit which enabling the best possible view of animals. One observation lasts for 65 min for each species. In each hour the percentage of time, when animal disappeared from observer sight, was noted. Various characteristics (e.g. size, stripe pattern etc.) were used to differentiate individuals. Specialized equipment in observation (e.g. camera) was not used. Statistical calculations of χ^2–test were performed using a statistical software pacage SPSS Statictics 23 Pl.

During single observation following methods of sampling were employed:

- *ad libitum* – for 30 min to examine activity and behaviour repertoire; each occurence of behaviour performed by each individual were recorded in this case;
- scan – for 15 min – to measure synchronization of behaviour in the group; instantaneous samples were taken on individuals at the same time;
- focal – for 20 min – to investigate occurence of social interactions in the group; with this sampling one individual was focus of observation for given time, then the second, third etc.

Above-mentioned methods of sampling were in detail described by Altmann (1974).

The list of possible behaviour categories observed in equids was prepared on the basis of literature (McDonnell and Havilland 1995, McDonnell and Poulin 2002) and the pilot observations.

RESULTS AND DISCUSSION

There were 106 observations, which were distributed evenly throughout observation period (28 in summer, 26 each in other seasons). The observation took place usually around noon or in early afternoon. In the winter the animals were observed as the weather allowed, usually in the morning. For the sake of relatively small number of observations in each season in this study the comparative analysis of behaviour was not prepared.

The total data obtained was 117 h of behaviour recording (58.5 h for each species). There was possible to observe animal behaviour for 95.2% of total time in the case of PH and for 88.5% in the case of HZ. In the rest of time animals have disappeared from the observer's sight (came to their stables). The difference in disappearance was highly significant ($P < 0{,}01$, χ^2-test was used).

The following categories of behaviour were ascertained in both equids at Warsaw Zoo:

- locomotory behaviour – walk, trot;
- alert – attention of animals was directed to certain stimuli;

- exploration – looking for food, olfactory investigations of surroundings;
- resting – standing in relaxed posture, recumberent resting;
- feeding – grazing, nibbling various plants;
- excretion – defecation and urination;
- comfort-seeking behaviour – scratching self, rubbing, stretching, rolling, response to insect harassment (e.g. stamping); to this category social grooming was included;
- non-aggressive interactions – any affiliate interaction involving physical contact, play;
- agonistic interactions – threat, attack, bite, kick, fight, chase (only incidents with occurence of all these sequences were recorded);
- vocalization – neigh, snort (only recorded in PH);
- relations with visitors – approach fence via straight path without sign of aggression and exploring.

This behaviour of equids at Warsaw Zoo is presented only in outline. Authors decided to show rather the categories of behaviour at the beginning and their accordance with ethograms from literature. The frequency of particular kinds of equids behaviour is shown in Table 2.

As may be expected in the case of behaviour repertoire in equids movement, feeding and resting were predominant. This pattern is typical for the wild equids (Souris et al. 2007). On the other hand, there was relatively small number of social interactions in observed individuals. Both, PH and HZ showed positive reactions towards visitors. Authors did not observe any sign of territorial marking. On the other hand, comfort seeking behaviour was fully expressed. In vocalization usually the neigh was recorded probably used by PH to maintain contact between members of group. All above-mentioned behaviours performed by wild equids housed at

TABLE 2. The frequency of behaviour (*N*) and activity (%) of total time of observation recorded in Przewalski's horse and mountain zebra

Behaviour category	Przewalski's horse		Hartmann's zebra		Diffference (significance)
	N	%	*N*	%	NS
Locomotory	230	15.0	266	17.4	NS
Alert	33	2.2	102	6.7	**
Exploration	14	0.9	154	10.1	**
Rest	143	9.3	319	20.8	**
Feeding	786	51.4	384	24.4	**
Excretion (defecation/urination)	18	1.2	6	0.4	*
Comfort-seeking behaviour	112	7.3	32	2.1	**
Non-aggressive interaction	45	2.9	2	0.1	**
Agonistic interaction	8	0.5	12	0.8	NS
Vocalization	24	1.6	0.0	0.0	**
Relations with visitors	42	2.7	87	5.7	**

* Difference significant at $P > 0.05$; ** highly significant at $P < 0.01$.

Warsaw Zoo are well described in literature (McDonnell and Havilland 1995, McDonnell and Poulin 2002, McGreevy 2004). The reproductive activity and abnormal behaviour especially stereotypic behaviour (aimless repeating of behaviour sequences) were not recorded in this study. It is worth to mention that the stereotypical behaviour is frequently observed in both domesticated and wild equids (Sarrafchi and Blokhuis 2013).

In spite of rather low frequency of many behaviours there was marked difference between their frequency in PH and HZ. For example HZ more time spent resting, exploring and performing alert reaction. In PH feeding was more frequently observed as well as comfort seeking, non-aggressive behaviour acts, excretion and vocalization (not observed in HZ). Locomotory activity and aggressive interactions were at the same level in the both species. Although HZ showed higher frequency as regards relations with the visitors than PH, zebras seemed to be more fearful and rather easily took flight. This observation confirmed general opinion than zebras, particularly mountain zebra is rather fierce animal (MacClintock 1976). On the other hand, PH at Warsaw Zoo appeared more tame and confident towards visitors.

Some findings in this part of study could be explained by the observations in the wild. For example it is known that mountain zebra is less social than other species (plain and Grevy zebra). Herd members maintain a greater individual distance and mutual grooming in this species is infrequent. Individuals rarely formed large aggregations. Mountain zebra is also less vociferous than other zebra species (Estes 1992). Of course it is need to confirm this conclusion by further observations of captive individuals in zoological garden.

The second method of sampling, used in the study of equids behaviour at Warsaw Zoo, was scan. This method was used to examine coordination of behaviour of the group members. There was interesting to study, if certain behaviours were performed by all individuals at the same time. The results of scan measurement are shown in Table 3.

Eight behaviour were to some degree synchronized, but only in the three cases of behaviour their frequency were visibly high: feeding in PH, locomotory and rest behaviour in HZ. The similar pattern of PH social activity was also obtained in the case of wild individuals (Souris et al. 2007). Moreover, in both species there was also highly coordinated reaction of coming to the stable. The analysis using χ^2-test revealed that differences in coordinated behaviour between both equids were highly significant ($P < 0.01$) in the case of feeding, rest and coming to the stable.

The focal sampling turned out to be inconclusive in this study. As it is shown in Table 1 there was relatively small number of interactions (aggressive and non-aggressive) in both equids. From the authors point of view more interesting were aggressive interactions, because they offered some insight into hierarchy. In HZ all agonistic interactions were recorded between Mare 1 and younger Mare 3. Mare 1 initiated the sequence of aggressive behaviour acts described above. It was clearly interaction which signal dominant position of Mare 1. In PH only small number of agonistic interactions was observed between stallion and

TABLE 3. Frequency of all recorded behaviour performed simultaneously in both species of eqiuds at Warsaw Zoo

Behaviour category	Przewalski's horse				Hartmann's zebra		
	simultaneous behaviour frequency observed in number of individuals						
	2	3	4	Σ	2	3	Σ
Feeding	109	213	172	494	102	83	185
Locomotory	36	12	10	58	20	8	28
Rest	23	5	1	29	43	42	85
Comfort-seeking behaviour (not in social context)	16	1	0	17	1	0	1
Alert	0	1	0	1	4	2	2
Exploration	0	0	0	0	1	0	1
Vocalization	1	0	1	2	0	0	0
Coming to the stable	40	18	2	60	164	20	184

freshly introduced Mare 3. Both specimens initiated this interaction, which was limited to threat, bite and kick. On the basis of these data there is hard to speculate about social hierarchy. This finding is partly confirmed by observations of Feh (1988) on PH in semi-reserve conditions revealing agonistic interaction between stallion and mare leading even to subordination of male horse.

Findings concerning PH agonistic behaviour and reaction towards visitors recorded at Warsaw Zoo are contrary to certain speculation that this horse in captive environment have higher level of aggression than domesticated or feral horse (McGreevy 2004).

CONCLUSIONS

1. After the social manipulation which changed the structure of Przewalski's horse and Hartmann's zebra groups at Warsaw Zoo these animals did not show abnormal behaviour during the observation period.
2. As a whole ethogram of captive individuals at Warsaw Zoo resembled patterns of behaviour described in the wild equids.
3. Observed individuals showed also some differences in behaviours and their frequencies between Przewalski's horse and Hartmann's zebra at Warsaw Zoo. They were present in spite of similar husbandry practices, enclosure design and localization, feeding etc. There is need for further more detailed examination of social behaviour in Hartmann's zebra and Przewalski's horse.

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Streszczenie: *Wstępne obserwacje dotyczące zachowania się dwóch dzikich koniowatych: konia Przewalskiego (*Equus ferus przewalskii*) i zebry Hartmanna (*Equus zebra hartmannae*) utrzymywanych w zmienionych pod względem społecznym grupach w Miejskim Ogrodzie Zoologicznym w Warszawie.* Badania przeprowadzono w Warszawskim Ogrodzie Zoologicznym, aby zbadać ogólne zachowanie się dwóch dzikich koniowatych po zmianie społecznej. Zmiany te podyktowane były procedurami zarządzania zwierzętami w zoo. Trzy zebry Hartmanna i cztery konie Przewalskiego obserwowano przez 117 h przez cały rok. Zastosowano trzy metody próbkowania: *ad libitum*, scan i focal. Nie stwierdzono występowania zachowania anormalnego. Odnotowano natomiast kilka bardzo istotnych różnic między częstością występowania form zachowania między zebrą Hartmanna a koniem Przewalskiego. U obydwóch gatunków zaobserwowano również koordynację behawioru w obrębie grupy. Częstość zachowań społecznych u obu gatunków była mała i nie dała podstaw do rozważań na temat struktury społecznej.

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Effect of training on the heart rate in mares

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Abstract: *Effect of training on the heart rate in mares.* The purpose of this paper was to determine the effect of training on the heart rate in mares and analyse the adaptation of examined specimens to training loads. The tests were carried out on the basis of measurements of the post-effort resting heart rate and restitution time at various training stages in the Training Centre in Bielice. The measurements were taken with an electric heart rate monitor in 56 mares. The resting heart rate in mares was higher than in the final training stage. The heart rate restitution was quicker at the end of the training period. A significant influence of the length of training of mares selected for tests on the way they had reacted to the same load was observed. Horses with a longer preparation period had better efficiency parameters, both at rest and after training. An influence of the level of preparation of mares on their results of the final test in the training centre was also demonstrated – the average grade of mares following an extended preparation period was higher by 2 points.

Key words: training centres, mare performance test, heart rate

INTRODUCTION

Among all species of animals bred, maintained and used by humans, the horse shows the best adaptation to effort. The evolution determined by the lifestyle (a constant threat posed by predators resulting in the need for escape) had to develop such adaptation mechanisms (in terms of anatomy, physiology and behaviour) to ensure quick and often long escape in the face of danger. These adaptation features of the horse often exceed capabilities of other species, not to mention humans (Higgins 2013).

Effort adaptation capabilities of the horse provided by the cardiovascular and respiratory systems are manifested, among other things, by (Akajewski 1997):

- The difference between resting and maximum heart rate values. The number of heart beats per minute in a horse can increase eight times (from 20–46 – resting heart rate, up to 220–240 – maximum heart rate; this value in a trained person can increase only four times).
- The maximum aerobic capacity (VO_2 max), also referred to as the peak oxygen uptake. It is an amount of oxygen, which can be taken up by an organism. For a trained horse, this value increases 35 times on exertion (up to 150 ml of O_2/kg BW/min in comparison to the resting state). Peak

oxygen uptake of the best endurance athletes is approx. 70–90 O_2 ml/kg BW/min, while this value for moderately active people is approx. 50 O_2 ml/kg BW/min. It has been shown that VO_2 max capabilities are genetically determined; however, they can be developed by training to some extent.
- The surface of the lungs (gas exchange surface) of over 500 m^2. For comparison, the same surface in cattle of a similar weight is approx. 360 m^2.
- The anatomic structure of the trachea and larynx – a much wider lumen than in cattle; the volume of air flow in cattle of the same weight corresponds to one third of the air flow in horses.

One of the most important stages of horse breeding is the assessment of performance characteristics for motor activity and individual traits manifesting themselves in a horse's responsiveness etc. For years, leading riding horse breeding associations have been trying to develop a uniform test model for these traits. One of the tools necessary for this purpose, in relation to female material, is a mare performance test. It is a single test conducted in accordance with a specific scheme (http://pzhk.pl/hodowla/programy-hodowlane).

The monitoring of the heart rate and the entire cardio-respiratory system is essential in professional training. According to Szarska (2006), the systematic monitoring of a horse's heart rate in training allows for:
- objective assessment of a horse's exercise capacity;
- early detection of any organism disorders;
- simple observation of any increase of a resting heart rate, which may be caused by a possible infection or a symptom of overtraining a horse;
- determination of individual, precise ranges of heart rate values for respective gaits, speeds or specific exercises;
- optimal interval training performance and planning of subsequent training sessions.

The purposes of the test were:
- assessment of the influence of a specific standardised training effort on clinical parameters, i.e. the heart rate value, during a 60-day training test for mares;
- an analysis of the adaptation of mares to training loads on the basis of measurements of resting heart rate and restitution time values, following efforts at different training stages;
- an attempt to find a relation between the degree of mare performance preparation and the results of their performance tests.

MATERIAL AND METHODS

The tests were conducted on a group of mares participating in a training test in Bielice, in four consecutive sessions. A set of tests constitutes a list of measurement results of 56 mares. The mares were divided into two groups: trained up to 30 days before the qualification procedure – the first group (25 horses), and trained for more than one month – the second group (31 horses). The tested mares were mainly of the following breeds: the Wielkopolski (17 horses) and the Polish Half Bred Horse (34 horses). One mare was a Małopolski horse, another 4 mares were the representatives of 2 foreign

breeds: the Holsteiner (3 horses) and the Hanoverian (1 horse). The mares were different ages. The average mare age was three years. Due to different degrees of preparation of the mares taking part in the tests, the proposed exercise stress test could not be too intensive (preparation of the 'weakest' mare had to be taken into account in order not to push it too hard with the training).

The following cardiac monitor was used for heart rate measurements: Trainer Pulsometr HRM – Timex T5H881. The strong points of this device are its resistance to environmental factors and its user-friendliness. A clear display is designed for easy measurement reading. The body temperature of the horses was measured by the rectangle method, with a mercury free thermometer – PiC Solution, with an electronic display.

The initial resting heart rate (IRHR) was measured for seven consecutive days, starting from the third day of stay in the training centre, the same time of the day. An average of seven measurements was taken as a value of the resting heart rate (RHR). Each time, measurement results were read out after approx. 1 min from the application of the transmitter to the area around the heart. In the event a result of any measurement deviating from other results significantly, it was repeated after approx. 5–10 min to exclude the influence of a 'single, unpredictable external stimulus'.

The final resting heart rate (FRHR) was measured on the same way.

The heart rate reserve was measured as per the following scheme: the first measurement in a peak moment of the effort – HR; next 1 min after the HRE measurement – HR1; next 10 min after the HRE measurement – HR10; next 15 min after the HRE measurement – HR15. Measurement results were read by a rider of a given horse, directly from display readings at a specific moment.

The obtained results were analysed statistically with the SAS Enterprise Guide statistical package, ver. 4.3 ('Local', XP_PRO). Variables, in terms of their distribution, were analysed with the Shapiro–Wilk test. The Fisher test demonstrated the equality of variances of comparable traits. Student's t-distribution was applied, formulating a null hypothesis about the equality of average comparable parameters at statistical significance of $P = 0.05$. Pearson correlation coefficient was calculated.

RESULTS AND DISCUSSION

The average values of the resting heart rate in the group of mares with a shorter period of preparation for training tests were higher than in mares which had received expanded training, both at the beginning (IRHR) and at the end (FRHR) of the training tests. A statistical analysis has shown the significance of differences between the above average values (Table). A drop of resting heart rate value during training sessions in the training centre was 0.80 beats per minute for the first group and 0.74 beats per minute for the second group. Both drops turned out to be statistically significant. The difference between the drop values (IRHR-FRHR) for both groups was statistically insignificant. A drop of RHR during training in the centre was observed in all groups. The Pearson correlation coefficient for RHR and the result was statistically significant.

TABLE. Average heart rate values and differences between the mean values for both groups (symbols explained in the text)

Group	Beginning of the training					At the end of the training				
	IRHR	IHRE	IHR1	IHR10	IHR15	FRHR	FHRE	FHR1	FHR10	FHR15
Medium value of the heart rate										
I	36.9*	131.2	125.0*	83.4	72.5	39.1*	127.4	115.0	75.8	64.8
II	38.4	125.5	119.0	74.9	63.1	37.7	123.6	114.4	70.6	59.5
$\bar{x}$ (I & II)	39.1	128.1	121.5	78.7	67.3	38.3	125.3	114.9	72.9	61.9
Differences toward median for both groups										
I–$\bar{x}$	0.8	3.2	3.1	4.7	5.2	0.8	2.1	0.6	2.9	2.9
II–$\bar{x}$	–0.7	–2.6	–2.5	–3.8	–4.2	–0.7	–1.7	–0.5	–2.3	–2.4
I–II	–1.5	–5.7	–5.6	–8.5	–9.4	–1.5	–3.7	–1.1	–5.2	–5.3
Standard deviation of value of the heart rate										
I	1.4	11.8	12.4	13.7	7.3	1.0	13.0	8.9	7.9	4.8
II	1.0	12.8	13.7	7.9	5.2	0.8	17.5	18.9	6.5	4.7
II–I	–0.4	1.0	1.2	–5.9	–2.2	–0.2	4.6	10.0	–1.4	–0.1

*Statistically significant difference at $P \geq 0.05$.

The heart rate reserve of mares with a shorter preparation period was higher than that in mares receiving extended training, both at the beginning and the end of training tests. In fact, the differences were found to be statistically insignificant.

There is a high correlation between the HR0 an HR1 values (at the beginning ($r = 0.77$) and the end of training ($r = 0.82$).

The t-test has shown differences between the average values of the heart rate measured in 10 min of the effort – for both mare groups. The higher HR10 value was always observed in the group of mares with a shorter period of preparation for training tests. Differences between the initial HR10 and the final HR10 were also found to be significant for both groups, whereas the difference between the initial HR10 and the final HR10 in respective groups was the same.

The difference between the initial HR15 and the final HR15 turned out to be significant for both groups. The value of this difference was significant as well. As a result of the training, the HR15 heart rate dropped by 7.7 beats per minute in the first group and 3.6 beats per minute in the second group. There is a significant statistical relation between HR15 and other heart rate measurements, though it is a low or moderate relation (acc. Guillford scale). A connection of HR15 with IHRH (correlation coefficient of approx. 0.5) seems to be vital.

An analysis of standard deviations has shown that at the end of the training they were lower than at the beginning. For parameters: RHR, RH10, TH15 (parameters crucial for horse training assessment). The scatter of results for HR0 and HR1 was higher at the end of the training (Table).

Changes indicating the heart rate restitution times were similar in all mare

groups. In the initial training period, differences between heart rate values in individual groups were more distinct than at the end of the training (Fig.).

For mares better prepared for training tests it was definitely easier to drop below a HR of 64 at measurements of HT15. The number of mares with a heart rate below 64 beats per minutes increased in both groups at the end of the training tests – from 12 to 60% in the first group and from 61.29 to 90.32% in the second group.

The average mare performance test grades in the group of the worse prepared mares was 56.7 (Group 1) and 58.7 (Group 2) – the average higher by 2 points. This difference has no statistical meaning.

The grades obtained by all mares fell within the range of 67.8 to 44.3 points. In the group of 56 tested mares, the first 14 positions were occupied by mares with better preparation; the last 20 positions included 10 of them. The training tests were completed with a very good grade by 9 mares (only three of them came from the first group), whereas 10 mares completed the tests with a satisfactory grade (6 mares from the first group). One mare from the first group received a negative grade. The remaining mares were assessed 'good' (15 from the first group and 16 from the second one).

Vincen et al. (2006) explains that resting heart rate values depend on many factors, to include age, breed, a horse's excitability determined by its temperament, its health conditions and training progress. Jezierski (1993) and Szarska (2003) describe that the heart rate value is a crucial physiological factor indicating a horse's reaction to the training process. Training is to be understood as an association of methods, sets of exercises and various actions necessary for maximum adaptation aimed at achieving the level of supreme performance when taking a specific task/effort (Kaproń 1999, Kowalska and Sadkowski 2008, Ogiński at al. 2010).

The value of the resting heart in ponies is higher than in horses. However, due to the origin and size and regardless of its sex, temperament and other factors which diversify individual animals, the resting heart value drops with an increase of horse capacity (Clayton 1991, Szarska 1999, Gill 2003). This dependence has been observed in horses from the very first sessions of regular training (Kaproń et al. 1997). At the same time, the high

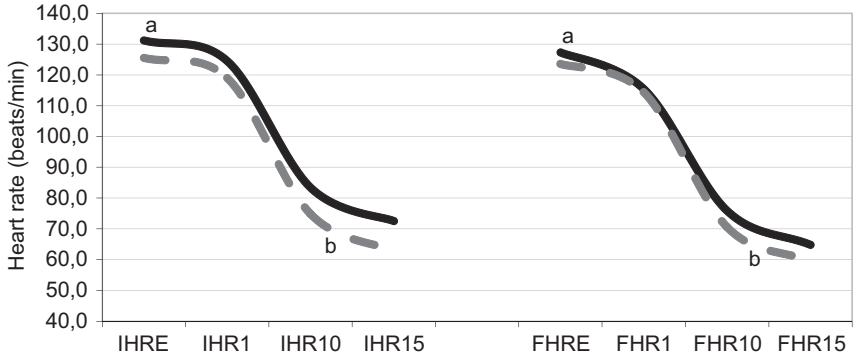

FIGURE. Measurement of heart rate (a – Group I, b – Group II). Symbols explained in the text

resting heart rate may suggest insufficient preparation of the organism for physical activity, poor condition, injury or other health problems or a stress response (Courouce 1999, Kaproń 1999, Stucke et al. 2015).

The reference of the resting heart rate value according to Szarska (1999) ranges from 20 to 46 beats per minute.

Although training loads in the training centre are not too intense and the period of 60 days is not long in relation to the whole horse training process, a proper direction of adaptation changes was observed with regard to the resting heart rate in almost all horses. Statistically significant drops in average resting heart rate values for both groups clearly indicate the influence of training on horse capacity and the meaning of its monitoring in the training practice. The observed correlation between the resting heart rate value and the result of the mare performance test were too small to be treated as a reference for horse assessment. However, they show the significance of physical preparation, even in easy tests.

It should be emphasised that no particular signs of fatigue have been observed in the training set manager or the riders on the day of the test and the consecutive days, though the restitution time of some horses was not entirely satisfactory. Szarska (1999) states in her paper that if the same effort, e.g. trotting in the field, is made by a trained and untrained horse, post-effort heart rate values in such horses will differ substantially: 80–100 beats per minute for the trained horse and 120–150 beats per minute for the untrained horse. Considering the above, the untrained horse may switch to anaerobic workout at such an effort. It means that it will exceed a so-called lactate threshold which is approx. 150 beats per minute. Four mares got very close to this threshold in a test at the beginning of the training (HR0 – 145 beats per minute) – two mares from the first group and two mares from the second group. Five mares at the end of the training – three from the second group, two from the first group, whereas two mares that were close to the lactate threshold participated also in the first test (one mare from the first group and one mare from the second group).

Controlling a heart rate restitution time curve is a very helpful element of well-balanced horse training and it gives an opportunity to assess training progress in an objective way – not only in the discipline of endurance riding but in all other competitions (Podolak at al. 2004).

When analysing heart rate restitution time curves of individual mares, one can observe that they are similar. Therefore, in order to assess their progress on an individual basis, they should be referenced to average values of a specific group (or average values of the whole tested population).

Averages of tested parameters indicating the heart rate restitution time were always lower in mares with a longer preparation time. However, the scatter of all results was lower at the end of the training. This confirms the thesis about the dependence of training values on the training progress. The difference of HR15 values between the beginning and the end of the training tests in the first group was 7.7 beats per minute and 3.6 in the second group which means that mares receiving a shorter preparation period before the tests made greater progress in relation to Group II. The average HR15

parameter at the beginning and the end of the training tests was lower in the group of mares of more advanced training preparation. To achieve the similar training test effect in the second group, greater training loads should be applied, to which the group was prepared according to the tests. In this group, HR15 below 64 beats per minute was observed already in the first test in more than half of the mares. In the final test, this value was as much as 90.32%. Kaproń et al. (2000) believe that a properly selected and conducted training programme results in a progressive decrease of the heart rate value – including on exertion – and the resting heart rate.

Definite conclusions can be drawn on the basis of horse heart rate analyses that a two-month training period in the training centre has a significant influence on mares' capabilities. This is manifested by post-effort heart rate values, the average drop of the resting heart rate value and an increase of the restitution time. Szarska (1999) gives estimate data on heart rate ranges at individual training stages. After 5–10 min following aerobic workout, the heart rate value should not exceed 60–64 beats per minute. If the heart rate value exceeds 64 beats per minute after 15 min of training, it means that too much effort was experienced by a specific horse at this training level. If, however, the heart rate value is below 52 beats per minute after 10 min following the training, it means that the load applied was insufficient to achieve a positive effect of effort-adaptation changes.

According to the test results, horses with a longer preparation period have a greater chance to achieve better results in the final training test. In a way, mares which had received more extensive training before the actual training tests were of a better physical condition which was then reinforced during the subsequent two-month training session. The final training results of mares with a shorter preparation period to the training tests fell short of the capacity indicator parameters in the mares of the second group. However, their progress was clear.

CONCLUSIONS

- Mares properly prepared to training tests obtain higher final results.
- The proposed effort measurement, modified on the basis of previous observations, could be used as a tool for checking training effectiveness in tests, as well as for assessing horse capacity, which could in turn constitute an additional selection criterion.
- Performance of such tests and compilation of results thereof in training centres would be a very valuable material for horse breeders and owners. Information obtained this way, combined with a performance test, could help make a decision on further training steps or a horse breeding career.

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Streszczenie: *Wpływ treningu na wartości tętna u klaczy.* Celem pracy było określenie wpływu treningu na wysokość tętna u klaczy oraz analiza przystosowania badanych osobników do obciążeń treningowych. Badania prowadzono na podstawie pomiarów tętna spoczynkowego i tempa restytucji tętna po wysiłku na różnych etapach treningu w Zakładzie Treningowym w Bielicach. Pomiary u 56 klaczy wykonywano za pomocą elektronicznego pulsometru. Stwierdzono, że na początku treningu tętno spoczynkowe klaczy było wyższe niż w fazie końcowej przeprowadzonego treningu. Tempo restytucji tętna było szybsze na końcu okresu treningowego. Wykazano istotny wpływ długości treningu poprzedzającego przystąpienie klaczy do zakładu treningowego na sposób, w jaki reagowały one na to samo obciążenie. Konie dłużej przygotowywane miały lepsze parametry wydolnościowe zarówno w spoczynku, jak i po treningu. Wykazano również wpływ stopnia przygotowania klaczy na uzyskane przez nie oceny w teście kończącym zakład treningowy – średnia ocena klaczy dłużej przygotowanych była wyższa o 2 punkty.

Słowa kluczowe: zakład treningowy, próba dzielności klaczy, tętno

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Genetic associations of reproductive traits in pigs

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Abstract: *Genetic associations of reproductive traits in pigs.* In the field of genetics, one of the main research area in relation to animal reproduction is the identification of genes or genomic regions influencing reproductive phenotypes. The genes analysed for the determinants of their fertility are among other: LEP, PRL, PRLR, RBP4. With the use of genetic markers, it is possible to identify of both males and females carrying beneficial alleles, and choose reproduce high-quality individuals, which in turn accelerates the genetic improvement of the examined feature. According to literature, about 30% of culling in pig production systems has been primarily due to reproductive problems. Litter size is very important and easily measured reproductive trait, and often included in scientific researches, and defined as the total number of piglets born (TNB) and the number of piglets born alive (NBA). Selection of individuals carrying favourable alleles has the potential to improve reproductive traits and in this connection also sow productive life (SPL). It is a measure of the longevity and reproductive performance of a sow and is directly related to the number of viable piglets produced during its lifespan. Because reproductive traits are so multifaceted, researchers are able to consider many different facets of the organism biology to come up with candidate genes and QTLs genes.

Key words: QTLs, candidate gene, WGAS

INTRODUCTION

Reproduction is an essential process for the maintenance of a species. It has to be genetically controlled to ensure that the reproductive process is repeated with a strong degree of precision (Rothschild and Ruvinsky 2011). Overall processes of sexual reproduction are relatively conserved and well defined, but the underlying molecular and genetic basis of each of the numerous steps involving these general processes is much less known. The complexity and transient nature of specific reproductive processes are the cause of reduced knowledge concerning their molecular basis (Óvilo and Valdovinos 2012).

Genetic markers allow identification of both males and females carrying beneficial alleles early in life, thereby improving accuracy, reducing the generation interval, and accelerating the genetic improvement of the trait. For instance, average litter size of mature sows varies from 4 to 16 piglets per litter among breeds. These differences, combined with the appreciable genetic variation that also exists within breeds, have given the opportunity for substantial genetic improvement of sow prolificacy over the last 15 years (Rothschild and Ruvinsky 2011). Furthermore the pig, being a highly prolific mammal, could be one of the best species to study the genetic complexity of lowly heritable reproductive traits. Around 30% of culling in pig production systems has

primarily been because of reproductive problems (Serenius and Stalder 2004).

Many genes are researched by scientists to find the effect on reproductive traits. Terman et al. 2011 detected polymorphism in the *RBP4* gene and the analysis showed that sows with BB genotype had significant bigger litter size. Korwin-Kossakowska et al. (2003) analyzed the *PRL* locus genotype and were found to have a significant effect ($P \leq 0.01$) on litter size based on analysis of sows records from second and later parities. The effect of *PRLR* genotype was significant ($P < 0.05$) for the numer of pigs born alive for sows during first parity. Also Babich et al. (2008) analyzed *PRL* gene and a novel mutation, insertion/deletion (InDel) was found. The examples above are just some of the many publications about genes that affect litter size.

From a productive point of view, reproductive efficiency is one of the most important factors affecting productivity in livestock industries. Reproductive traits, especially those related with fertility, litter size and pre-weaning viability, are important components for reducing the costs of producing animal meat (Rothschild and Ruvinsky 2011). Therefore, much effort is made to identify the ways for improving these traits. This improvement of reproductive traits is related to different fields, with management, nutrition and genetics having a great impact.

REPRODUCTIVE TRAITS IN PIGS

In the field of genetics, the main research area in relation to animal reproduction is the identification of genes or genomic regions influencing reproductive phenotypes. The reproductive function consists of a complex mosaic that combines different male and female processes, and interactions among them. The first process is the development of competent gametes. Interaction between male and female cells starts with the ability of the female tract to transport, select and prepare spermatozoa for fertilization, then to ensure a maternal environment that facilitates fertilization and early embryo development. The cross talk between an embryo and its maternal environment leads then to successful early embryo development and implantation. In the whole reproductive process it is difficult to isolate and measure precise events and functions involved in pregnancy success or failure (Óvilo and Valdovinos 2012). Thus, in the genetic study of reproduction, these complex reproductive processes are measured in the form of objective records, which reflect the success/efficiency of the several stages of the reproductive process. These reproductive phenotypic records are diverse and the common feature is that all the processes measured correlate in some way with reproductive efficiency.

Phenotypic records include endocrine measures (plasmatic hormonal levels), litter measures (embryo survival, counts of live, dead, mummified, weaned descendants), morphologic measures of reproductive organs (weights of testicles or ovaries, scrotal circumference, teat number, length and placement, udder characteristics, uterine capacity and length), semen quality (sperm, semen and ejaculation characteristics), fertility related traits (heat intensity, fertilization rate, non-return rate, ovulation

rate, inseminations per conception) and other general reproductive traits as age at puberty or gestation length. The relevance of the different reproductive traits is not the same and also differs among species. For this reason, most genetic studies have focused on some reproductive traits of utmost relevance (Óvilo and Valdovinos 2012).

In pig breeding, as in other polytocous species such as rabbits, the litter size is probably the most relevant and easily measured reproductive trait, and the one to which most attention is devoted. The usual measures of litter size used are the total number of piglets born (TNB) and the number of piglets born alive (NBA). Value of TNB is the sum of NBA and the number of stillborn piglets (NSB). Litter size is determined by the interaction of numerous physiological components, such as the number of ovulated eggs (ovulation rate, OR) which determines the maximum number of possible offspring, and the rate of prenatal survival (Haley and Lee 1993).

SOW PRODUCTIVE LIFE (SPL)

Length of productive life (LPL) is an important trait to consider from a productivity, profitability, and animal welfare perspective in modern pork production systems. Net-present value increases with the LPL of the sow (Serenius and Stalder 2004). Animal welfare concerns may arise when sows cannot remain productive for more than one or two parities. Thus, the interest to select for LPL in breeding programs has increased in recent years. Alternative breeding value estimation methods have been presented for LPL and similar traits. Survival analysis is a well-accepted method for estimating breeding values for LPL, similar traits, or both. However, it has not been possible to conduct a multiple trait analysis that estimates breeding values for survival traits and other types (such as Gaussian and categorical) of traits simultaneously (Ducrocq and Sölkner 2001). Recently, Damgaard and Korsgaard (2006) introduced bivariate survival and Gaussian trait analysis, where both environmental and genetic correlations are modeled.

Previous literature heritability estimates for LPL have ranged from 0.02 to 0.34, being population and trait definition dependent (Serenius and Stalder 2004). Previous research results have not provided a consensus regarding the genetic associations between measures of sow longevity and other efficiency related traits. Reliable estimates of these correlations are needed to develop breeding value estimation routines for sow longevity.

Moreover, some evidence exists which indicates that sow longevity, prolificacy and/or conformation traits are genetically associated. Additionally, for example the U.S. pork industry is experiencing additional animal welfare pressure due to a variety of factors including the type of housing that our sow's experience during the gestation period. When housing issues are combined with poor sow longevity, welfare concerns may arise because a large portion of sows in commercial breeding herds do not remain productive for more than one or two parities. Thus, there is interest among swine breeders and commercial pork producers to select for improved LPL (or sow longevity as it has been more commonly referred to)

in breeding programs, and this interest has increased in recent years (Óvilo and Valdovinos 2012).

In this connection sow productive life (SPL) is an important trait in modern pork production. Sow productive life is a measure of the longevity and reproductive performance of a sow and is directly related to the number of viable piglets produced during its lifespan. Sows with reduced piglet production are removed early in their productive lifetime. Approximately 15 to 20% of the sows removed have produced only single litter, and more than 50% are removed before their fifth parity (Engblom et al. 2008). The increased removal rate of young animals is of considerable concern. Reproductive traits are known to be of poor heritability (Ehlers et al. 2005); however, these traits are correlated with the length of SPL (Serenius and Stalder 2004). The economic cost and ethical issues associated with a short SPL has increased the interest in selecting for traits affecting the SPL in pig breeding programs.

REPRODUCTIVE PERFORMANCE OF PIGS AND ECONOMICS VALUES FOR PRODUCERS

Number of pigs per sow per year is one of the most important factors influencing profitability of pig production. Currently, 43% of the worldwide population consumes pork, making it the major red meat. Demand for swine has risen, not only as a food source, but also as a model system for human health, causing a need for increased swine production. Foodstuffs are considered a fixed cost for producers. Therefore, increasing litter size would reduce cost of feed per pig, thus, greatly increasing economic returns with minimal additional inputs (Huges and Varley 1980).

Economical pork production requires that pigs grow fast and efficiently, have high carcass merit and good meat quality, are disease resistant, have high levels of reproductive success, and increased survivability. The most important requirement of successful swine production is reproductive success, with litter size being the major component of sow productivity. Reproductive performance of sows is critical to the economic survivability of producers. The lifetime production of a sow is defined as the number of pigs weaned per sow during lifetime. Rodriguez-Zas et al. (2006) reported that a sow must produce on average four litters for optimal economics. It has been estimated that 40–50% of sows are culled annually with over one-third of these removals attributed to reproduction inadequacies (D'Allaire et al. 1987). Roughly 50% of culls attributed to reproduction are from parity one or less gilts (Engblom et al. 2007). With a large number of animals being culled at younger ages, there is an increased economic demand for females with greater stay ability, the potential for a sow to remain in the herd (Serenius and Stalder 2004).

Primiparous gilts have greater energetic demands associated with growth and energy utilization during critical periods of time such as gestation and lactation, which may have an impact on reproductive and litter trait performances (Britt 1986). An ideal situation would be to utilize genetic screening to identify those animals at greater risk of failing in reproductive performance over

the average life of the animal or to find those animals that may have the genetic potential for increased longevity.

CANDIDATE GENE APPROACHES AND QTL FOR REPRODUCTIVE TRAITS IN PIGS

In order to detect loci for a special trait, there are in principle two strategies possible: QTL analysis and the candidate gene approach (Rothschild et al. 2000, Omelka et al. 2001). There is a general debate about which of these two strategies is more powerful in order to detect genes for reproductive traits in pigs (Buske et al. 2006).

CANDIDATE GENE APPROACHES

The rationale of candidate gene approach states that a major component of quantitative genetic variation of phenotype under investigation is caused by functional mutation of putative gene. Candidate genes are generally the genes with known biological function directly or indirectly regulating the developmental processes of the investigated traits, which could be confirmed by evaluating the effects of the causative gene variants in an association analysis. Candidate gene approach has been ubiquitously applied for gene-disease research, genetic association studies, biomarker and drug target selection in many organisms from animals to humans (Zhu and Zhao 2007).

The proportion of pig genes that have been mapped is still small and consequently, the number of positional candidate genes is limited (King et al. 2003). It is often mentioned that in principle every animal from any population can be investigated. Basically, this is feasible, and many research groups genotyped a candidate gene for a chosen trait at a given standardized F1- or F2--population, depending on the breeding system. According to Rothschild et al. (2000), one requirement of the candidate gene approach is to test the gene variants in several populations to detect general effects. In general, the resource populations and the number of tested pigs in different experiments varied to a considerable amount. Some research groups used reference families, some used commercial pig populations, but only a few of them let the animals house in a commercial farm. Because of differences concerning housing, feeding and other environmental influences, results of these studies are difficult to compare (Buske et al. 2006).

Candidate gene approach has been proven to be extremely powerful for studying the genetic architecture of complex traits, which is a far more effective and economical method for direct gene discovery. Nevertheless, the practicability of traditional candidate gene approach is largely limited by its reliance on existing knowledge about the known or presumed biology of the phenotype under investigation, and unfortunately the detailed molecular anatomy of most biological traits remains unknown. It is quite necessary to develop new strategies to break the restriction of information bottleneck, although considerable candidate genes have already been identified (Zhu and Zhao 2007).

QUANTITATIVE GENETICS – QTLS

Quantitative genetics is the branch of genetics that studies quantitative traits and has as main objective the study of their inheritance. Any phenotypic trait that takes different values in different individuals and does not follow a pattern of simple mendelian inheritance is a quantitative or polygenic trait. Most of the traits of interest in livestock are included in this category (e.g. growth, physiological functions, milk production). Other traits, such as litter size or conception versus non-conception, present only few discrete categories, and are named "threshold traits". However, we can assume that there is an underlying continuous distribution, such that they can be treated as continuous traits (Óvilo and Valdovinos 2012).

The basic idea around QTL is that a specific gene is responsible for a portion of genetic variation. A primary feature of this type of technology is its early availability in life of the individual, thus allowing an earlier, more accurate selection. It is believed that QTL will play a major role in animal breeding, especially if their use in future breeding programs can be optimized. Examination of how marker technology can influence the genetic gain formula reveals several things. Enhancing reproductive efficiency can increase selection intensity, and providing additional information can increase accuracy of individual evaluations. Reducing genetic diversity could reduce genetic variance, and allowing selections to be made earlier could decrease generation interval. There are some major advantages to incorporating QTL or marker usage in breeding programs. The first advantage is increase in accuracy of selection due to addition of information directly related to genotype. A second advantage is the possibility of reducing generation interval by allowing selection to be made at an earlier age because this technique is not sex or age dependent (Blowe 2003).

Advances in molecular biology and genomics over the past 20 years have profoundly changed our knowledge of the genetic determination of economically important traits in major livestock species. In particular, the development of panels of genetic markers covering the whole genome has allowed the individual loci underlying the genetic variance of quantitative traits of economic importance to be systematically detected and mapped. A large number of experiments aiming at detecting these QTLs have led to the identification of a large number of such loci. Hu et al. in 2007 in his publication states that more than 5600 QTLs are currently referenced in the PigQTLdb (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index) now on 8 October 2017 this is 25,610 QTLs associations from 593 publications and those QTLs associations represent 646 different traits. It has to be noted that these QTLs have essentially been detected using low density maps based on panels of microsatellite markers and not the high-density linkage maps based on single nucleotide polymorphism (SNP) markers that have recently become available (Ramos et al. 2009). QTLs for female pig reproductive traits detected so far are summarized in Table 1. QTLs affecting litter size traits have been detected on 13 different chromosomes, but most of them

TABLE. Quantitative trait loci (QTLs) for female reproductive traits

Trait	Pig chromosome number	Population	Size	Variance	Reference
Age at puberty	1, 4, 6, 7, 13	LW × MS	476	3.0–10.0	Bidanel et al. 2008
	7, 8, 12	LW × LR	295	2.7–9.7	Cassady et al. 2001
	15	LW × LR	295		Holl et al. 2004
	1, 10	WC × MS	344		Rohrer et al. 1999
	1, 7, 8, 17 11	DU × ER DU × YO × LR	454	2.0–8.0 13.13	Yang et al. 2008 Nonneman et al. 2014
Ovulation rate	4, 5, 7, 9, 13,	LW × MS	502	3.9–5.9	Bidanel et al. 2008
	9	LW × LR	295	3.4	Cassady et al. 2001
	3, 8, 9, 10, 15	WC × MS	344		Rohrer et al. 1999
	7, 8, 15	YO × MS	104		Wilkie et al. 1999
Numebr of embryos	9, 12, 18	LW × MS	468	2.8–7.2	Bidanel et al. 2008
Uterine capacity	8	WC × MS	187		Rohrer et al. 1999
Gestation lenght	1, 9, 15 1	YO × MS LW × LR	104	9.4–23.6 82.96–83.56	Wilkie et al. 1999 Onteru et al. 2012
Number of mummified	2, 6, 12 13	LW × LR LW × LR	279	 21.78–21.90	Holl et al. 2004 Onteru et al. 2012
Total number of born	11	LW × LR	279	5.1	Cassady et al. 2001
	1, 7, 14, 17, 18	LW		4.20–25.50	Coster et al. 2012
	8	LW × MS	152		Holl et al. 2004
	7, 15	DU × ER	299	2.8–4.3	Li et al. 2009
	7, 14, 15, 17	DU × YO × LR		16.00–96.92	Schneider et al. 2012
	6	YO × MS	104		Wilkie et al. 1999
Number of stilborn	5, 13	LW × LR	279	7.9	Cassady et al. 2001
	12, 14	LW × LR	279		Holl et al. 2004
	7, 8	DU × ER	299	3.7–5.0	Li et al. 2009
	6, 11, 14	LW, LR			Tribout and Bidanel 2008
	4 15	YO × MS DU × YO × LR	104	 64.63–65.36	Wilkie et al. 1999 Schneider et al. 2015
Number of born alive	11	LW × LR	279		Cassady et. al. 2001
	6, 15	DU × ER	299	3.7–5.0	Li et al. 2009
	1	BP		97.6	Laliotis et al. 2016
	7, 16, 18	LW, LR			Tribout and Bidanel 2008

LW – Large White, MS – Meishan, LR – Landrace, WC – White Composite, DU – Duroc, YO – Yorkshire, ER – Erhualian, BP – Black Pigs.

Source: Own elaboration on the basis of Rothschild and Ruvinsky (2001).

are putative results and there is limited overlap between studies.

THE PIG GENOME PROJECT AND WHOLE-GENOME ASSOCIATION STUDIES (WGAS)

The pig genome project (http://www.sanger.ac.uk/Projects/S_scrofa/) and the development of the Illumina PorcineSNP60 BeadChip (Ramos et al. 2009) via the efforts of the International Swine Genome Sequencing Consortium have provided an opportunity to carry out WGAS in the pig. Advanced statistical methods (Kizilkaya et al. 2010) and tools (GENSEL software at http://bigs.ansci.iastate.edu) based on Bayesian approaches are available to analyse the large quantities of SNP chip data for genomic selection and WGAS in domestic animal populations (Fernando and Garrick 2008).

Onteru et al. (2012) report that in the WGAS study were: (I) pig reproductive traits were lowly (for TNB, NBA and MUM) to moderately (for SB and GL) heritable under farm management and environmental influences; (II) different chromosomal regions and thus different genes appear to be associated with each reproductive trait in different parities, which supports the presence of temporal gene effects in different parities; (III) pathway analyses identified some modest underlying biological pathways associated with pig reproductive traits in different parities; and (IV) future validation studies in very large populations were recommended for pig reproductive traits, as these were lowly heritable traits regulated by many genes, and the interaction between genes and environment may be significant.

CONCLUSIONS

Optimal reproductive function is a necessary process for maintaining a species. Reproduction must be under precise genetic regulation. Breed differences and within breed genetic variability implies that genetic improvement in reproductive traits in pigs is possible. Selection of individuals carrying favourable alleles at QTL based directly on DNA evaluation is called marker assisted selection and has the potential to improve traits like litter size. Because reproductive traits are so multifaceted, researchers are able to consider many different facets of the organism biology to come up with candidate genes.

Besides candidate gene study established that the genes associated with pig reproductive traits are primarily involved in energy metabolism. However, genomic improvement in pig reproductive traits requires detailed whole-genome association studies to explore the chromosomal regions and genetic markers that explain the variation in these traits. In conclusion SPL is a measure of the longevity and reproductive performance of a sow and is directly related to the number of viable piglets produced during its lifespan. The economic cost and ethical issues associated with a short SPL has increased the interest in selecting for traits affecting the SPL in pig breeding programs.

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Streszczenie: *Podłoże genetyczne a cechy rozrodcze u świń.* W dziedzinie genetyki jednym z głównych obszarów badań w aspekcie cech reprodukcyjnych zwierząt jest identyfikacja genów lub regionów genomowych mających wpływ na fenotyp reprodukcyjny. Z puli genów analizowanych w aspekcie determinacji ich plenności i płodności należy wymienić między innymi: LEP, PRL, PRLR, RBP4. Dzięki wykorzystaniu markerów genetycznych możliwe jest wczesne rozpoznanie zarówno samców, jak i samic (nosicielki korzystnych alleli) i wybór do rozrodu osobników cechujących się wysokimi parametrami, co z kolei przyspiesza genetyczną poprawę badanej cechy. Z danych literaturowych wynika, iż około 30% ubojów w systemach produkcyjnych świń spowodowanych jest głównie przez problemy związane z cechami reprodukcyjnymi. Wielkość miotu jest bardzo istotną i łatwo mierzalną cechą reprodukcyjną. Często

jest ona uwzględniana w badaniach naukowych, w których używa się na nią określeń całkowita liczba prosiąt urodzonych (TNB) oraz liczba prosiąt żywo urodzonych (NBA). Wybór osobników wykazujących korzystne allele ma potencjalny wpływ na poprawę cech rozrodczych i użytkowych. Ich wykładnikiem jest tzw. życie produktywne świń (SPL), które jest miarą długości życia oraz zdolności reprodukcyjnych lochy i jest bezpośrednio związane z liczbą prosiąt żywo urodzonych, przypadających na daną maciorę przez całe życie. Podsumowując, cechy reprodukcyjne dotyczą tak wielu aspektów, że należy przebadać wiele różnych cech organizmów w celu wyłonienia genów kandydujących oraz genów noszących miano QTLs.

Słowa kluczowe: QTL-y, gen kandydat, WGAS

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Influence of housing system on selected quality characteristics of duck meat. Chapter 2. Muscovy duck

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Abstract: *Influence of housing system on selected quality characteristics of duck meat. Chapter 2. Muscovy duck.* The objective of this study was to determine the effect of housing system on the selected quality characteristic of breast muscles of Muscovy (MR71) ducks. The ducks were divided into four experimental groups according to their sex and housing system: intensive system (IS) and outdoor system (OS). Analysis was performed for a total of 48 breast muscle (12 in each experimental group: 2 × gender; 2 × rearing system). For test samples of meat there were determined: chemical composition, physicochemical properties and the sensory attributes of the breast muscles. There was no effect of housing system on the proximate composition of breast muscles of both MR71 ducks. Rearing system of ducks MR71 had significant ($P < 0.05$) effect on cooking loss. The principal component analysis (PCA) showed, that meat of MR71 ducks from free range system is better perceived by the consumers than the meat of MR71 ducks from the intensive system, mainly for its greater tenderness and flavor.

Key words: Muscovy duck, rearing system, meat quality

INTRODUCTION

Therefore the importance of duck breeding has increased gradually as well (Rodenburg et al. 2005, Solomon et al. 2006, Erisir et al. 2009). For Muscovy duck, the market is estimated by us at 45 million Muscovy ducks produced in Europe per year, with a major part produced in France by around 37 million ducks. The rest is produced in Poland, Germany, Italy and some also produced in the Czech Republic, Hungary and Spain (http:www.grimaudfreres.com). Concerning Asia, some Muscovy are produced in China or Vietnam, estimated sometimes at 10 million ducks in Vietnam. The number of ducks in Turkey is about 0.4 million according to data of 2016. It is possible to encounter duck farming in every region of Turkey (Tüik 2017).

There is an increasing demand for animal protein, and duck production may be able to help meet this demand. Muscovy ducks are able to adapt to a wide range of environmental and natural conditions, which may be the reason for the increasing importance, and popularity of the duck industry.

The aim of this study was to determine the effect of housing system on the

selected quality characteristic of breast muscles of Muscovy (MR71) ducks.

MATERIAL AND METHODS

Experimental material and procedures

The study material consisted of the breast muscles of Muscovy ducks (Grimaud, Rossy, France) of the R71 line (MR71) (http:www.grimaudfreres.com). To three weeks of age all birds (180 males and 180 females) were kept according to the guidelines for intensive production system on deep litter and at a stocking density of 2.9 birds per m^2. Thereafter, half the ducks and drakes were allowed to use free ranges, at a stock density of 0.08 birds per m^2 (outdoor system – OS). The remaining birds were kept under conditions of intensive system production (IS) throughout the rearing period. The ducks were fed *ad libitum* with standard feed concentrates for particular rearing periods (Table 1). Production results of ducks related to the growth characteristics, fodder use, mortality and slaughter efficiency have already been published (Damaziak et al. 2014). The MR71 ducks were kept they reachad an age of until 10 weeks, and MR71 drakes

TABLE 1. Nutritive value of basal diet fed to Muscovy (MR71) ducks

Parameter	Unit	Weeks 0–3	Weeks 4–10	Weeks 11–12 (males)
EM_N	kcal	2 850	3 000	3 100
EM_N	MJ	11.9	12.5	12.9
Crude protein	%	20.5	19.0	17.5
Crude fibre	%	4.0	5.0	6.0
Crude fat	%	5.0	6.0	7.0
Amino acid				
Lysine	%	1.0	0.9	0.8
Methionine	%	0.5	0.4	0.4
Mineral				
Calcium (Ca)	%	1.1	0.9	0.8
Phosphorus (P)	%	0.5	0.4	0.4
Sodium (Na)	%	0.2	0.2	0.2
Vitamin				
A	IU	15 000	10 000	10 000
D_3	IU	3 000	2 000	2 000
E	mg	20	20	15

EMN – is the gross energy of the feed consumed minus the gross energy contained in the faeces, urine, and gaseous products of digestion. For poultry EM represents the gross energy of the feed minus the gross energy of the excreta. A correction for nitrogen retained in the body is usually applied to yield a nitrogen-corrected EM (EMN) value.

until they reachad an age of 12 weeks. Owing to differences in duration of rearing, the placement of ducklings was also conducted under different terms, which enabled the synchronizing of the slaughter, so that all birds were slaughtered on the same day. After rearing was finished, 12 males and 12 females with mass similar to the mean for a given sex were selected for slaughter from each production group. Slaughter of ducks and post-slaughter processing were conducted using the industrial method as in the first part of the publication (Michalczuk et al. 2016).

Analysis

Chemical composition were determined in meat samples according to the Association of Official Analytical Chemists (AOAC 2005). All chemical analyses were carried out in duplicate. Moisture content by drying about 5 g test sample at 105°C (±2°C) until reaching a constant weight; ash content by incinerating about 3–5 test sample in a muffle furnace at 550°C until light grey ash results; protein content by the classical Marco-Kjeldahl method using Kjeltec System 1026 Distilling Until (Foss Tecator, Höganäs, Sweden); and fat content by petroleum ether extraction using a Büchi Extraction System B-811 (Büchi Labortechnik AG, Flawil, Switzerland). The pH (pH_{24}) was value of meat samples with a CP-411 pH-meter (Elmetron, Zabrze, Poland), using a combined glass-calomel electrode. The electrode was calibrated against buffers of pH 4.00 and 7.00 at 20°C. Color parameters (a*, b*, L*) were analyzed with a Minolta CM-2600d (Konica Minolta LTD, Tokyo, Japan: light source D65, observer 10°, a measuring head hole 8 mm) calibrated to the white plate (L* = 99.18, a* = −0.07 and b* = −0.05). Each measurement was carried out in five replications, taking their average value as the result. Parameter L* (color brightness) can assume values from 0 to 100. Parameters a* (redness) and b* (yellowness) are trichromaticity coordinates. They can assume positive and negative values; +a* corresponds to red, –a* to green, +b* to yellow, and –b* to blue.

The cooking loss of intact fillets were determined by weighing the fillets and then cooking in a convection oven on aluminum trays at 180°C to an internal temperature of 75°C. The fillets were drained, allowed to equilibrate to room temperature, and weighed. Cooking loss was determined by expressing cooked sample weight as a percentage of pre-cooked sample weight following the procedure of Bianchi et al. (2005). Twenty--four hours after heat treatment, from each muscle five samples with cross section (1 × 1 cm) were cut out along the muscle fibers. They were used for the measurement of share force. Shear force was measured with the tensile tester ZWICK 1120 using a Warner–Bratzler blade. Maximum share force (F_{max}) was read at the head speed of 0.83 mm/s. The experiment was conducted until the decrease of the share force, after cutting the sample, reached 75% of F_{max}. As the result of the determination mean from five measurements was adopted. Sensory evaluation of the breast muscles was conducted by a 12-member team with the appropriate qualifications. Equal bite size from each treatment was coded, replicated thrice and served in odorless plastic plates. Each sample was evaluated independent of the other. The samples were evaluated

on a nine-point hedonic scale for color, flavor, tenderness, juiciness, stringiness and overall acceptability (nine is extremely desirable and one extremely undesirable).

Statistical analysis

Prior to the statistical analysis, data were checked for normality by the Shapiro–Wilk test. The homogeneity of variance across treatments was assessed by Levene's test. The statistical analysis was conducted with the software system Statistica 10. StatSoft Inc. For the critical level of significance $P \leq 0.05$ was adopted. Because of differences in rearing time of males and females, MR71 ducks, one-way analysis of variance (one-way ANOVA) was used including the housing system according to the formula:

$$Y_{ijk} = \mu + S_i + e_{ijk}$$

where:
Y_{ijk} – trait;
μ – general mean;
S_i – effect of *i*-the housing system;
e_{ijk} – random error.

To verify differences between average values t-Student test was applied. Principal component analysis (PCA) with the classification was performed on values: tenderness, juiciness, stringiness, overall acceptability, cooking loss, share force, L* and fat.

RESULTS AND DISCUSSION

Keeping conditions did not significantly influence the proximate chemical composition of the breast muscles of MR71 ducks (Table 2). Similar results were obtained by Erisir et al. (2009) and Michalczuk et al. (2016), who studied the effect of housing system on the chemical composition of Pekin duck (P-44 and Star 52) meat. Our results of moisture and protein contents in breast meat (73.6–75.1; 22.2–23.1 respectively) are similar to those reported by other authors (Baéza et al. 1997, Schiavone et al. 2007). Meat of Muscovy ducks is usually characterized by larger protein content and lower water and fat content than in Pekin duck meat, which is beneficial from a nutritional point of view (Michalczuk et al. 2016). In breast muscle, the fat content

TABLE 2. Effect of housing system on the proximate composition of the breast muscles of MR71 ducks (each value is presented as mean ±*SD*, *n* = 12)

Treatment	Sex	Chemical composition (%)			
		moisture	protein	fat	ash
IS	♂	75.0 ±0.2	22.4 ±0.3	1.1 ±0.2	1.5 ±0.1
OS		75.1 ±0.4	22.2 ±0.4	1.2 ±0.1	1.5 ±0.1
Housing system	×	**	**	**	**
IS	♀	73.6 ±0.3	23.1 ±0.4	1.8 ±0.2	1.5 ±0.1
OS		73.8 ± 0.2	23.1 ±0.2	1.7 ±0.2	1.4 ±0.2
Housing system	×	**	**	**	**

** Not significant at $P > 0.05$.

can by twofold higher in Pekin ducks than in Muscovy ducks (Chartrin et al. 2006a, b). Higher fat content in tissues of ducks derived from *Anas platyrhynchos* is the consequence of adaptation of this group of ducks to temperate climatic conditions. Adipose tissue constitutes insulation during winter and provides large amount of energy during migration, while at the same time protects the bird against utilization of muscle protein. On the other hand, the Muscovy ducks come from a tropical climate and have a rather sedentary lifestyle; therefore, storing excess of the input energy constitutes an unnecessary burden for these birds (Kijanko et al. 1999).

No significant effect of housing conditions on pH_{24} of breast muscles of Muscovy ducks (Table 3) was determined. The mean pH value of duck meat is generally between 5.7 and 5.9 (Larzul et al. 2006, Witak 2008). The amount of cooking loss during heat treatment of breast muscles of MR71 OS ducks was significantly lower ($P \leq 0.05$). Smaller amount of cooking loss during heat treatment of MR71 OS ducks breast muscles could be related to slightly higher pH_{24} (Table 3). Breast muscles of MR71 drakes were characterized by higher amount of cooking loss than in females, regardless of housing conditions. Due to this fact their meat was tougher, which is exhibited by higher values of the share force. No effect of housing conditions on breast muscle texture of Muscovy ducks (Table 3) was determined. Significantly higher values of the share force was determined in the case of breast muscles of MR71 ducks, and at the same time in the sensory evaluation they received lower scores for such characteristics as tenderness, flavor (Tables 4 and 5). It probably stemmed from the much longer rearing period of MR71 ducks compared to P-44 ducks (Michalczuk et. al. 2016). Age has a major effect on muscle development. As part of the aging process the collagen content in breast muscle decreases, while collagen solubility remains unchanged, thus the decrease in meat juiciness and tenderness, and the increase in stringiness may be related to fiber size (Baéza

TABLE 3. Effect of housing system on the physicochemical properties of the breast muscles of MR71 ducks (each value is presented as mean ±*SD*, $n = 12$)

Treatment	Sex	pH_{24}	Cooking loss (%)	Shear force (N)	Color		
					lightness	redness	yellowness
IS	♂	5.8 ±0.1	35.8 ±1.6	57.5 ±4.1	32.5 ±0.6	13.7 ±0.1	13.9 ±0.1
OS		5.9 ±0.1	31.1 ±1.4	55.4 ±1.2	32.2 ±0.9	13.9 ±0.6	14.3 ±0.5
Housing system	×	**	*	**	**	**	**
IS	♀	5.8 ±0.1	25.5 ±0.3	45.2 ±2.3	32.1 ±0.2	14.2 ±0.5	14.4 ±0.1
OS		5.9 ±0.1	24.8 ±0.4	43.1 ±1.8	31.6 ±0.7	14.6 ±0.2	14.4 ±0.2
Housing system	×	**	*	**	**	**	**

* Difference significant at $P \leq 0.05$; ** not significant at $P > 0.05$.

TABLE 4. Effect of housing system on the sensory attributes of the breast muscles of MR71 ducks (each value is presented as mean ±*SD*, *n* = 12)

Treatment	Sex	Color (points)	Flavor (points)	Tenderness (points)	Juiciness (points)	Stringiness (points)	Overall acceptability (points)
IS	♂	4.6 ± 0.2	5.6 ± 0.1	3.4 ± 0.1	3.4 ± 0.2	3.9 ± 0.2	5.8 ± 0.1
OS		4.7 ± 0.1	5.6 ± 0.2	3.5 ± 0.1	3.0 ± 0.2	4.0 ± 0.2	5.9 ± 0.1
Housing system	×	**	**	**	**	**	**
IS	♀	4.7 ± 0.1	5.7 ± 0.1	4.7 ± 0.1	3.0 ± 0.2	4.6 ± 0.3	5.8 ± 0.1
OS		4.9 ± 0.1	6.2 ± 0.1	5.3 ± 0.2	3.1 ± 0.2	5.0 ± 0.3	5.9 ± 0.1
Housing system	×	**	*	*	**	**	**

* Difference significant at $P \leq 0.05$; ** not significant at $P > 0.05$.

TABLE 5. Loadings for the first two PCs of the breast muscles of MR71 ducks (male and female)

Traits	PC1	PC2
Tenderness	0.162	0.001
Juiciness	0.149	0.007
Stringiness	0.011	0.657
Overall acceptability	0.161	0.006
Cooking loss	0.169	0.020
Share force	0.166	0.002
L*	0.039	0.305
Fat	0.147	0.001

2006). The Muscovy duck has a higher muscle weight and greater fiber area and length than the Pekin duck. The muscle collagen content is also higher in this species, and the solubility of collagen is lower (Baéza 2006, Larzul et al. 2006). These differences may explain why Muscovy duck breast meat is judged less tender and stringier than Pekin one.

Color of MR71 duck breast muscles was not differentiated by the housing conditions, which was demonstrated on the basis of instrumental color measurement (Table 3), as well as sensory evaluation (Table 5). Studies conducted in various European countries demonstrated a large value span of duck breast muscle color parameters, especially the L* parameter – from 28.6 to 46 (Chartrin et al. 2006b, Larzul et al. 2006, Ali et al. 2007, Witak 2008, Wołoszyn et al. 2009). A variety of factors could have an influence on this fact, including genotype, age of birds and food. Moreover, the methods of measurements or equipment used in the studies are not always the same, which can explain the different values obtained (Wołoszyn et al. 2011).

Breast muscles of MR71 ducks were darker than in P44, which is demonstrated by a lower L* color parameter and higher a* color parameter (Michalczuk et al. 2016). It most likely stems from a longer housing period and greater muscle mass. According to Baéza et al. (2002), the level of heme pigments increases with age and increase of muscle mass.

In the sensory evaluation (Table 4) breast muscles of MR71 OS duck females received higher scores, especial-

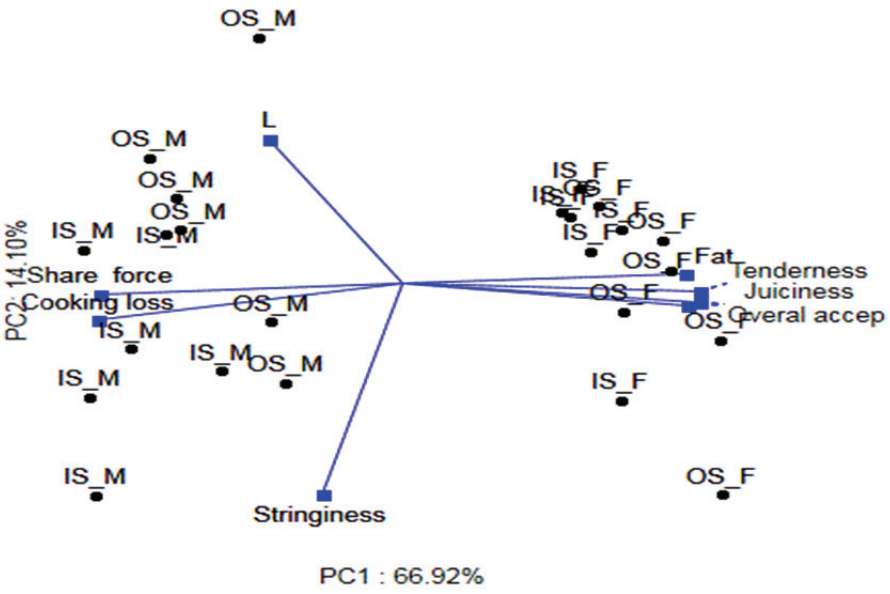

FIGURE 1. PCA biplot of breast muscle samples of MR71 ducks: IS_F – female, IF_M – males kept in intensive system; OS_F – females, OS_M – males kept in outdoor system

ly for flavor and tenderness ($P \leq 0.05$), than MR71 IS. According to Baéza et al. (1998), flavor increased with age, probably linked to the quality of lipids. Table 4 contains the loadings for the first two principal components (PCs) with their variances of the breast muscles of MR71 ducks – male and female (Fig. 2). The tenderness, juiciness, overall acceptability, cooking loss, share force and fat created the first component (PC1), whereas the second component (PC2) was created by the stringiness and L* (Table 5). Selected PC1 and PC2 components together explained 81% of variation with the concurrent loss of 19% of information. The first component explaining 66.9% of total variance was formed by tenderness, juiciness, overall acceptability, cooking loss, share force and fat. The second component explaining 14.1% of total variance was formed by stringiness and L* (Table 5). No effect of housing system (IS and OS) on the quality of breast muscles of MR71 ducks was determined. Meat samples of MR71 duck on the PCA map arranged themselves in two groups, differentiated in terms of sex. On the right side of the PCA map female meat samples were located, which were characterized by higher sensory quality than meat of males, which was primarily influenced by greater tenderness. Similar results were obtained by Chartin et al. (2006b) who studied the effect of intramuscular fat levels on sensory characteristics of Muscovy duck breast meat. Authors' study showed the lowest for tenderness, juiciness, and flavor, and the highest for stringiness in sensory analysis. Genotype exerted a higher effect on the sensory quality of breast muscle than did feeding levels.

CONCLUSIONS

This study demonstrates that to obtain high quality meat, MR71 ducks can be successfully kept in the outdoor system. Rearing system of ducks had no significant effect on chemical composition and physicochemical properties without cooking loss. Better tenderness and flavor were the features of the meat from the ducks reared in free range system than the ones fed intensively.

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Streszczenie: *Wpływ system utrzymania na wybrane cechy jakościowe mięsa kaczek. Część 2. Kaczki piżmowe.* Celem niniejszych badań było określenie wpływu systemu utrzymania na wybrane cechy jakościowe mięśni piersiowych kaczek piżmowych (MR71). Kaczki podzielono na cztery grupy doświadczalne w zależności od płci oraz systemu utrzymania: system intensywny (IS) i system z dostępem do wybiegu (OS). Analizy przeprowadzono łącznie dla 48 mięśni piersiowych (po 12 dla każdej grupy doświadczalnej: 2 × płeć; 2 × system utrzymania). Dla badanych prób mięsa oznaczono: skład chemiczny, właściwości fizykochemiczne oraz parametry oceny sensorycznej mięśni piersiowych. Nie stwierdzono wpływu systemu utrzymania na skład chemiczny mięśni piersiowych kaczek MR71. System chowu kaczek MR71 miał znaczący ($P < 0,05$) wpływ na straty podczas obróbki cieplnej mięsa. Statystyczna wielowymiarowa analiza składowych głównych (PCA) wykazała, że mięso kaczek piżmowych utrzymywanych w systemie wolnowybiegowym było lepiej postrzegane przez konsumentów w porównaniu z mięsem kaczek MR71 utrzymywanych w systemie intensywnym, głównie ze względu na czułość i zapach.

Słowa kluczowe: kaczka piżmowa, system utrzymania, jakość mięsa

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Effect of dehelminthization on milk yield and milk composition in dairy cows

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Abstract: *Effect of dehelminthization on milk yield and milk composition in dairy cows*. The aim of this study was to assess the effectiveness of action of selected antiparasitic preparation on milk productivity of cows and milk composition. In coproscopic analyses the qualitative methods by Willis, Żarnowski and Josztowa were used, as well as the quantitative McMaster method. A preparation containing eprinomectin was used for dehelminthization. The study involved 100 females after first lactation, which were divided into 2 groups, 50 heads each: experimental (D) – dewormed in May, 2 weeks before going to pasture, control (K) – not dewormed. From the experimental group 15 animals were selected (D_1) which were dewormed 2 days before parturition. Analogous group of cows (K_1) was selected from the control group (at the same stage of lactation, but not dewormed). It was proved that eprinomectin has a positive effect on milk yield, fat level and dry matter in milk, whereas the effect of this preparation on protein content was not confirmed. It was found that the most favorable time of deworming cows is perinatal period.

Key words: cattle, milk yield, nematodes, dehelminthization, eprinomectin

INTRODUCTION

Both practical observations and results of scientific research indicate that increasing milk productivity in herds of dairy cattle is accompanied inseparably by negative phenomena. These include common occurrence of infestation with gastrointestinal nematodes which according to the studies conducted in Poland and abroad play a crucial role in cattle production (Pilarczyk et al. 2002, Piekarska et al. 2013).

Negative effect of parasites on animal organism is expressed by losses caused by invasions proceeding subclinically. Latent character of infection is the reason for little interest of veterinary and zootechnical services, and ignoring this factor in the breeding environment may have serious consequences, such as: delay in growth and development of young animals, decreasing milk capacity and the quality of products of animal origin, less body weight gains and a fall in animal fertility (Nodtvedt et al. 2002, Sanchez et al. 2002, Liedtke et al. 2013).

Incomprehensible attempts to increase milk yield and prevent metabolic diseases and mammary gland diseases, cattle breeders as well as veterinary services put less attention to fighting gastrointestinal nematodosis as well as infection with

Protozoa, fluke worms and tapeworms in dairy cows. Only in recent years a positive effect of using antihelminthic preparations was observed. Negative effect of parasitic diseases on milk production and its composition as well as reproductive indexes was proved. However, many aspects of using antiparasitic preparations is still poorly understood and unexplained (Shoop et al. 1996a, b, Gross et al. 1999, Sanchez et al. 2004).

The aim of this study was to assess the effectiveness of action of a selected antiparasitic preparation on milk productivity of cows and milk composition.

MATERIAL AND METHODS

The study was conducted in five cow houses of dairy cows in the area of the Kuyavian and Pomeranian voivodeship. Experimental material were cows of the Polish Holstein-Friesian breed of black and white variety. Animals were kept in confinement system, whereas from May to September they used pasture. In the previous year, before the analysis concerning the impact of deworming of dairy cows on milk yield and composition of milk, parasitological pilgrimage studies on farms were conducted. Cows with a similar degree of infection, species composition and similar characteristics of milking for the first lactation, what was described on the basis of milk yield tables were selected for research.

The study was carried out in two stages: the first involved parasitological analyses, while in the other period the effect of deworming on milk yield and composition was evaluated. There was determined invasion prevalence (extensiveness), which was assumed as the percentage of hosts infected with the given parasite, as well as invasion intensity, which was assumed as the number of parasites from the given species found in one host. In coproscopic analyses the Willis and Żarnowski as well as Josztowa qualitative methods were used simultaneously, and also the quantitative McMaster method (Ziomko and Cencek 1995).

The study included 100 females after the first lactation, which were divided into 2 groups, 50 heads each:

- experimental (D) – dewormed in May, 2 weeks before going to pasture;
- control (K) – not dewormed.

The preparation used for deworming contained eprinomectin. The water solution of preparation (substance) was poured on the animal's back with a narrow strip along the spine, from the withers to the tail in an amount of 1 ml per 10 kg of body weight (1 ml of the preparation contains 5 mg of the active substance). This substance characterize with a zero grace period for milk.

All the cows from the experimental and control groups calved in the period from March to the beginning of May. From the experimental group 15 animals were selected (D_1) which were dewormed 2 days before parturition. Analogous group of cows (K_1) were selected from the control group (at the same lactation stage, but not subjected to deworming).

Milk yield was analyzed on the basis of the farm documentations. The breeding documents were used for the analysis – RW1 and RW2 result tabs. The following indices were taken into consideration: milk yield (kg), protein content (%), protein yield (kg), fat content (%), fat yield (kg), dry matter (kg, %). All the

cows involved in calculations of milk yield had full lactation, did not show clinical symptoms of mastitis. Calculations referred to 305-day lactation. In the group of cows dewormed in drying the yield was analyzed also at monthly intervals. The results of the study were verified statistically by Duncan's multiple range test and critical values using the Statistica 10.0 software.

RESULTS

At the beginning of the experiment (January), the extensiveness of gastrointestinal nematodes invasion in the experimental and control groups of cows was similar and amounted to 70% and 74%, respectively (Table 1). For the first 5 months, the percentage of animals infected with nematodes increased to the level 94% in the experimental group and 100% in the control. As a result of the dehelminthization treatment carried out in the experimental group, the extensiveness of invasion decreased to 32% in June and stayed on a similar level until December (28%). In the same period of the study in the control group prevalence of nematodes invasion was high and amounted to 94% in June. In next months of the study a slight fall of the infection extensiveness index was observed in this group of cows, while the lowest percentage of infected animals was recorded in November (68%). Statistically significant differences were recorded in all the months of the second half of the year, both at the level $P \leq 0.05$ and $P \leq 0.01$.

TABLE 1. Prevalence of gastrointestinal nematodes invasion (%) in dewormed 2 weeks before pasturing (n = 50) and not dewormed cows (n = 50) in year cycle

Month	Statistical measures	Experimental group (D)	Control group (K)
I	$\bar{x}$	70.00	74.00
	SD	5.70	4.70
II	$\bar{x}$	76.00	82.00
	SD	5.20	5.20
III	$\bar{x}$	90.00	92.00
	SD	6.70	5.40
IV	$\bar{x}$	94.00	92.00
	SD	5.00	4.90
V	$\bar{x}$	94.00^{a}	100.00^{b}
	SD	4.70	5.90
VI	$\bar{x}$	32.00^{A}	94.00^{B}
	SD	4.70	5.00
VII	$\bar{x}$	40.00^{A}	88.00^{B}
	SD	5.70	5.10
VIII	$\bar{x}$	36.00^{A}	96.00^{B}
	SD	4.10	4.30
IX	$\bar{x}$	38.00^{A}	86.00^{B}
	SD	4.80	4.30
X	$\bar{x}$	28.00^{A}	78.00^{B}
	SD	4.90	5.40
XI	$\bar{x}$	32.00^{A}	68.00^{B}
	SD	4.90	5.40
XII	$\bar{x}$	28.00^{A}	70.00^{B}
	SD	4.50	5.60

Values in lines denoted with different capital letters differ significantly at $P < 0.01$; whereas with small letters at $P < 0.05$.

As a result of dehelminthization treatment in cows (Table 2) the number of eggs of gastrointestinal nematodes in the experimental group decreased from a level of 489.4 in May to 54.7 in June, and in the following months after slight

TABLE 2. Intensity of gastrointestinal nematodes invasion (EPG) in dewormed 2 weeks before pasturing (n = 50) and not dewormed cows (n = 50) in year cycle

Month	Statistical measures	Experimental group (D)	Control group (K)
I	$\bar{x}$	143.30	140.50
	SD	16.10	5.80
II	$\bar{x}$	162.60^{a}	168.60^{b}
	SD	5.30	4.20
III	$\bar{x}$	257.90^{a}	261.30^{b}
	SD	4.20	5.40
IV	$\bar{x}$	458.60^{A}	431.70^{B}
	SD	5.30	4.90
V	$\bar{x}$	489.40^{A}	454.30^{B}
	SD	5.30	4.90
VI	$\bar{x}$	54.70^{A}	385.40^{B}
	SD	4.90	4.30
VII	$\bar{x}$	27.90^{A}	321.90^{B}
	SD	4.70	5.20
VIII	$\bar{x}$	26.60^{A}	456.30^{B}
	SD	3.90	4.40
IX	$\bar{x}$	72.00^{A}	230.00^{B}
	SD	3.80	4.80
X	$\bar{x}$	71.30^{A}	241.60^{B}
	SD	4.10	4.60
XI	$\bar{x}$	56.60^{A}	183.30^{B}
	SD	4.50	5.10
XII	$\bar{x}$	47.90^{A}	177.50^{B}
	SD	5.30	4.70

EPG – eggs per gram.
Values in lines denoted with different capital letters differ significantly at $P < 0.01$; whereas with small letters at $P < 0.05$.

fluctuations it reached the value 47.9 (December). In the analysed period of the study the EPG index in the control group was high and ranged from 456.3 (August) to 177.5 eggs in 1 g of faeces (December). Favourable effect of dehelminthization was confirmed statistically at the significance level $P \leq 0.05$ and $P \leq 0.01$.

TABLE 3. Lactation yield of dewormed 2 weeks before pasturing (n = 50) and not dewormed cows (n = 50) in relations to 305-day lactation

Item	Statistical measures	Experimental group (D)	Control group (K)
Milk (kg)	$\bar{x}$	8 266.00^{a}	7 945.00^{b}
	SD	138.90	113.40
Fat (%)	$\bar{x}$	4.06	4.00
	SD	0.37	0.33
Fat (kg)	$\bar{x}$	343.00	335.00
	SD	28.60	26.20
Protein (%)	$\bar{x}$	3.24	3.26
	SD	0.42	0.46
Protein (kg)	$\bar{x}$	267.00	272.00
	SD	18.90	19.80
Dry matter (%)	$\bar{x}$	12.87	12.30
	SD	0.74	0.46
Dry matter (kg)	$\bar{x}$	1065.90	1015.70
	SD	85.50	37.30

Values in lines denoted with small letters differ significantly at $P < 0.05$.

In Table 3 the milk yield of dewormed (D) and control (K) cows was presented in relation to 305-day lactation period. This was respectively for dewormed animals 8,266 kg, for not dewormed 7,945 kg (significant difference at the level $P \leq 0.05$). Deworming cows resulted in an increase in amount of produced milk on average by 321 kg for all lactation period. In dewormed cows, higher level of fat and dry matter in comparison to untreated animals was also found, but the results were not confirmed statistically. As can be seen in Table 4, milk yield of cows in the experimental group

TABLE 4. Milk yield of dewormed 2 days before parturition (n = 15) and control cows (n = 15) in individual months of the study

Month	Statistical measures	Milk (kg)		Fat (%)		Fat (kg)		Protein (%)		Protein (kg)		Dry matter (%)		Dry matter (kg)	
		D_1	K_1	D_1	K_1	D_1	K_1	D_1	K_1	D_1	K_1	D_1	K_1	D_1	K_1
VI	$\bar{x}$	777.00	776.00	4.29	4.27	34.00^{a}	27.20^{b}	2.88^{a}	3.28^{b}	25.20	26.40	12.93^{a}	12.33^{b}	100.00	90.00
	SD	5.60	4.40	0.74	0.70	4.30	4.30	0.15	0.19	6.80	3.05	0.13	0.25	2.20	4.10
VII	$\bar{x}$	1 679.00	1 685.00	4.13	4.31	68.00^{A}	54.00^{B}	2.92^{a}	3.30^{b}	48.80^{A}	58.80^{B}	12.51	12.31	211.60	207.20
	SD	7.90	4.90	0.58	0.68	3.20	3.10	0.19	0.19	2.70	2.40	0.16	0.16	3.13	5.40
VIII	$\bar{x}$	2 588.00^{a}	2 756.00^{b}	4.12	4.06	106.00^{A}	88.00^{B}	3.02	3.30	78,00^{A}	92.80^{B}	12.68	12.25	330.00	336.40
	SD	89.20	36.00	0.38	0.38	3.50	7.60	0.23	0.19	2.90	4.00	0.23	0.11	6.00	5.90
IX	$\bar{x}$	3 565.00^{a}	3 715.00^{b}	4.19	4.06	152.00^{A}	120.00^{B}	3.05	3.27	110.00^{A}	125.40^{B}	12.77^{a}	12.17^{b}	461.60^{a}	451.20^{b}
	SD	95.90	60.80	0.36	0.33	4.70	4.70	0.27	0.19	3.50	3.20	0,12	0.15	6.00	5.20
X	$\bar{x}$	4 644.00	4 556.00	4.03	4.05	191.00^{A}	149.00^{B}	3.08	3.31	141.80^{A}	157.40^{B}	12.71	12.22	585.60^{A}	560.20^{B}
	SD	106.70	112.60	0.37	0.55	5.20	4.30	0.23	0.31	4.50	3,40	0.16	0.16	4.60	5.20
XI	$\bar{x}$	5 502.00	5 472.00	4.02	4.08	222.00^{A}	186.00^{B}	3.11	3.38	167.00^{A}	190.40^{B}	12.47	12.30	696.20^{A}	676.80^{B}
	SD	141.50	103.30	0.50	0.38	4.70	5.10	0.19	0.27	2.20	4.00	0.49	0.14	4.30	4.40
XII	$\bar{x}$	6 439.00^{A}	6 229.00^{B}	4.09	3.97	254.00^{A}	217.00^{B}	3.05^{a}	3.44^{b}	197.60^{A}	217.60^{B}	12.60	12.40	804.40^{A}	776.60^{B}
	SD	94.70	99.80	0.37	0.22	4.50	5.40	0.22	0.18	3.80	2.70	0.46	0.43	5.50	3.00
I	$\bar{x}$	7 139.00	7 042.00	4.01	4.04	290.00^{A}	246.80^{B}	3.07^{A}	3.51^{B}	217.20^{A}	248.60^{B}	12.66	12.58	905.40^{A}	876.40^{B}
	SD	136.60	110.40	0.26	0.32	6.04	4.32	0.22	0.19	1.92	3.00	0.50	0.44	6.42	5.50
II	$\bar{x}$	7 744.00	7 660.00	4.12	4.03	318.00^{A}	274.00^{B}	3.00^{A}	3.52^{B}	234.20^{A}	273.60^{B}	12.68	12.59	981.60^{A}	960.20^{B}
	SD	105.30	163.60	0.41	0.50	3.94	4.00	0.14	0.19	4.02	3.78	0.53	0.44	5.03	5.67
III	$\bar{x}$	8 302.00^{A}	8 037.00^{B}	4.06	4.03	342.80^{A}	288.00^{B}	3.00^{A}	3.56^{B}	251.40^{A}	287.80^{B}	12.68	12.58	1 051.20^{A}	1 009.20^{B}
	SD	153.70	119.50	0.29	0.15	6.42	5.70	0.14	0.23	2.07	4.44	0.48	0.53	11.40	7.33
IV	$\bar{x}$	8 870.00^{A}	8 329.00^{B}	4.05	4.05	371.20^{A}	297.80^{B}	3.04^{A}	3.56^{B}	268.20^{A}	301.60^{B}	12.52	12.40	1 029.40^{A}	1 044.60^{B}
	SD	108.00	140.30	0.31	0.26	6.61	5.36	0.11	0.18	3.27	5.59	0.45	0.42	12.82	9.00
V	$\bar{x}$	9 054.00^{A}	8 534.00^{B}	4.05	4.00	398.40^{A}	304.20^{B}	3.11^{A}	3.58^{B}	287.40^{A}	307.60^{B}	12.53	12.47	1 100.20^{A}	1 071.00^{B}
	SD	113.00	154.90	0.20	0.21	4.33	4.66	0.23	0.24	3.85	2.30	0.46	0.37	7.08	4.85
305-day lactation	$\bar{x}$	8 502.00^{A}	8 056.00^{B}	4.15	4.01	350.80^{a}	294.30^{b}	3.05^{a}	3.50^{b}	255.00	291.50	12.66	12.49	1 094.50	1 051.70
	SD	35.10	155.70	0.26	0.23	36.10	30.20	0.21	0,32	32.60	24.90	0.39	0.36	128.60	134.10

Values in lines denoted with different capital letters differ significantly at $P < 0.01$; whereas with small letters at $P < 0.05$.

(D_1) was lower at the initial stage of lactation and ranged from 777 kg (June) to 3,565 kg (September) in relation to the control group (K_1), which achieved the yield of 776 (June) and 3,715 (September). From October, a clear increase in milk produced by cows from the group subjected to dehelminthization (D_1) was observed. Analyzing the results of production of both evaluated groups of animals (Table 4), an increase in amount of milk for 305-day lactation by 446 kg in favor of group D_1 was observed, and the differences proved to be statistically significant ($P < 0.01$).

Amount of kilograms of fat contained in milk in the experimental group (D_1) was higher in each month and increased in the course of lactation (Table 4). In consequence, for 305-day period 56.5 kg more fat was obtained in comparison to not dewormed cows (K_1) (significant difference at $P < 0.05$). In the conducted experiment a positive effect of eprinomectin on the content of protein in milk was not observed (Table 4). Dry matter content in milk in the experimental (D_1) increased in the course of the experiment and was higher in comparison with the group K_1 on average by 42.8 kg for 305-day lactation, but the results were not statistically significant (Table 4).

DISCUSSION

Numerous studies indicate that deworming cows has a favorable effect on milk production (Hawkins 1993, Sanchez et al. 2004). Gross et al. (1999) made a review of more than 80 various clinical trials evaluating the effect of administering antihelminthics in dairy cows and its potential effect on milk production, and they observed a favorable effect of such therapy. At the same time, American authors focus attention to the fact that dehelminthization in the prenatal period is more favorable than during lactation, since milk production begins from a high level, earlier comes to the peak of milk capacity and remains on a high level to the end of lactation (Bliss and Tood 1973). The same authors in the study of the effect of deworming on milk production found that as a result of dehelminthization treatment in a herd of cows it is possible to obtain an increase in milk yield on a level of 0.63 kg daily. Most experiments where giving anthelmintics were used during the drying period or at the moment of calving showed an increase in milk yield in dewormed cows in comparison with cows which were not subject to this treatment. Similar observations were conducted in Atlantic Canada by Hovingh (1998) and Guitian et al. (1999).

In the present study, similar relationships were found, where the experimental group (D) of cows calved from March to May was characterized by milk production higher by 321 kg, whereas animals dewormed at the drying stage (D_1) showed a higher milk yield on average by 446 kg during 305-day lactation (Tables 3 and 4). In local conditions, few studies were carried out confirming the fact of increasing productivity in dewormed animals. Balicka-Laurans et al. (1994) reported an increase in the amount of produced milk by 449 kg. Foreign experiments with eprinomectin (Huckle et al. 2001, Nodtvedt et al. 2002, Reist et al. 2002) are in accordance with the quoted above, thus confirming the results of the present study, which

indicated an increase in milk yield at different stages of lactation. Analysis of productivity of cows dewormed at the drying stage showed an increase in milk yield by 88 kg, from the 147th day of lactation (October), which increased along with the following months up to the end of the experiment (Table 4).

Ploeger et al. (1989) suggested that higher economic profit could be achieved, when cows are treated during calving or early lactation, while physiological needs and energy requirements of cows are the highest. On the other hand, Block and Gadbois (1986) proved importance of treating all the cows simultaneously in order to limit the level of reinfection.

Ploeger et al. (1990a, b) in experiments with the use of albendazol and ivermectin found an increase in milk production, whereas there was no effect on the content of fat and protein. Charlier et al. (2007) observed that the use of antihelminthics in cows results in an increase in milk yield by 1.2 kg per cow daily, whereas they did not record its effect on the percentage of fat in milk. Similar relationships were shown by McPherson et al. (2001) and Reist et al. (2002), who in different herds estimated an increase in milk yield of cows dewormed with eprinomectin on levels: 0.4; 0.9 and 2.4 kg per cow daily, while the result was not correlated with the percentage content of fat and protein in milk. However, experiments conducted by Gross et al. (1999) indicate an increase in percentage of fat in 26 of 35 analyzed samples, where milk composition was evaluated. This is confirmed by the results of the present study, where the fat level in milk in the experimental group was higher in relation to the control group by 8 kg (Table 3), whereas in cows dewormed in drying on average by 56.5 kg in 305-day lactation (Table 4). The same group was characterized by an increase in the amount of produced fat in milk, which from July to May differed highly significantly. Also favorable effect of animals dehelminthization was reported by Balicka-Laurans et al. (1994) in the experiment where the fat level in milk of dewormed animals increased by 19.6 kg. Among foreign authors also Bliss and Tood (1974) show that along with increasing milk yield by 192 kg an increase in fat level by 7.6 kg per cow occurred.

In the present study, similarly to some foreign authors (Charlier et al. 2007), no effect of dehelminthization on protein level in milk was observed; on the contrary, its content was higher in the control group (Table 4). Different results were obtained by Balicka-Laurans et al. (1994). Those authors showed an increase in protein content on average by 18 kg in animals subjected to deworming.

Not all milk components are subject to thorough analysis in the aspect of cow dehelminthization. In Polish and foreign literature, there is no data informing about the effect of deworming on the level of dry matter in milk. On the basis of the presented study it may be concluded that the action of antihelminthics has a favorable effect on the content of this component in cow milk. In the group of animals dewormed in the perinatal period the percentage of dry matter was higher during the whole lactation, but the difference in favor of the experimental group was not significant (Table 4).

CONCLUSIONS

1. It was proved that eprinomectin has a positive effect on milk yield, the level of fat and dry matter in milk, whereas the effect of this preparation on protein content was not confirmed.
2. The most favorable time for cow dehelmintization is perinatal period.

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Streszczenie: *Wpływ odrobaczania krów mlecznych na ich wydajność mleczną i skład mleka.* Celem badań była ocena skuteczności działania wybranego preparatu przeciwpasożytniczego na wydajność mleczną krów oraz skład ich mleka. W badaniach koproskopowych posługiwano się metodami jakościowymi: Willisa oraz Żarnowskiego i Josztowej, oraz ilościową McMastera. Do odrobaczania użyto preparatu zawierającego w swoim składzie eprinomektynę. Badaniami objęto 100 samic po pierwszej laktacji, które podzielono na 2 grupy po 50 sztuk każda: doświadczalną (D) – odrobaczoną w maju, 2 tygodnie przed wyjściem na pastwisko, kontrolną (K) – nieodrobaczoną. Z grupy doświadczalnej wytypowano 15 sztuk (D_1), które były odrobaczone 2 dni przed porodem. Analogiczną grupę krów (K_1) wybrano z grupy kontrolnej (w tej samej fazie laktacji, jednak niepoddanych odrobaczaniu). Wykazano, że eprinomektyna wpływa korzystnie na wydajność mleczną, poziom tłuszczu i suchą masę w mleku, nie potwierdzono natomiast działania tego preparatu na zawartość białka w mleku. Stwierdzono, że najkorzystniejszym terminem dehelmintacji krów jest okres okołowycieleniowy.

Słowa kluczowe: bydło, wydajność mleczna, nicienie, odrobaczanie, eprinomektyna

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Effect of liquid acidifiers on rearing performance of suckling piglets

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Abstract: *Effect of liquid acidifiers on rearing performance of suckling piglets*. Crossbred piglets from 36 litters (12 litters each in control group C and in experimental groups E1 and E2) were investigated. From birth to weaning at 28 days of age, piglets from group E1 received drinking water with a 2% solution of lactic, formic and orthophosphoric acids, and those from group E2 a 0.05% solution of Baracid P, which contained phosphoric, citric, lactic, formic and tartaric acids. No acidifier was used in group C. The piglets were monitored for body weight, mortality, and the incidence and duration of diarrhea. Production parameters were average in all the groups. Daily weight gains from 1st to 28th day of life were comparable in the groups ($P > 0.05$). The additives increased piglet survival (by 1.0 percentage point in group E1 compare to group C and by 1.8 percentage point in group E2 compare to group C) and decreased the number of days with diarrhea in experimental compared to control piglets. The differences in daily gains between the groups were statistically not significant, but the improved health and higher survival of piglets in the experimental groups compared to the control group indicate the appropriateness of using acidifiers in rearing of young pigs.

Key words: piglets, acidifiers, daily gains, diarrhea days, survival

INTRODUCTION

The withdrawal of antibiotics from pig feeds has increased the interest in alternative feed additives (Mroz 2005, Gajewska et al. 2008, Vondruskova et al. 2010, Hanczakowska and Szewczyk 2011, Papatsiros et al. 2011, Ahmed et al. 2014). The effect of acidifying additives on piglet rearing performance, health, intestinal microflora and gut morphology has been studied for many years (Rekiel and Kulisiewicz 1996, Biagi et al. 2006, Piva et al. 2007, Mazzoni et al. 2008, Budvytis and Włodarczyk 2012, Suryanarayana et al. 2012, Kowalczyk et al. 2013, Liu 2015, Upadhaya et al. 2016). The results of experiments were not always consistently positive (Partanen and Mróz 1999). Biagi et al. (2006) reported no differences in jejunal, ileal and cecal morphology between animals from the groups fed increasing supplements of gluconic acid. However, Rajchert at al. (2011) showed feed acidifiers to improve health and reduce the frequency and severity of diarrhea in piglets. Studies also demonstrated better growth rate and improved feed conver-

sion in piglets that received organic acids (Batorska and Mieńkowska-Stępniewska 2000, Biagi et al. 2006, Rajchert et al. 2011). Most organic acids and their salts are an energy source for animals, which according to Lückstädt (2009) improves the productivity of young pigs.

The aim of the study was to determine the effect of liquid acidifiers fed to suckling piglets during the rearing period on their health expressed as the frequency and severity of diarrhea, mortality, and daily weight gains.

MATERIAL AND METHODS

The study was conducted in a commercial pig house and involved 418 crossbred piglets from 36 litters (12 litters each in control group – C, experimental group 1 – E1, and experimental group 2 – E2). Sows and their progeny were randomly allocated to groups C, E1 and E2 at the time when late-pregnant sows were moved to the farrowing sector.

Thirty-six sows gave birth to 418 piglets, of which 385 were weaned. The total number of piglets born in groups C, E1 and E2 was 135, 140 and 143, respectively, with 123, 129 and 133 piglets weaned on day 28 in respective groups (Table 1).

The all-in all-out management system was used in the experimental piggery. Animals were kept on solid flooring with shallow litter and had access to feed and water from automatic waterers. Lactating sows were fed a complete loose-mix diet based on ground grains and protein concentrate. Starting from 5th–7th day of life, suckling pigs were fed a complete

TABLE 1. Characteristics of the test animals

Parameter	Descriptive statistics	Total	Group C	Group E1	Group E2
Body weight of one-day-old piglet (kg)	*n*	418	135	140	143
	$\bar{x}$	1.56	1.55	1.59	1.55
	min	1.08	1.12	1.18	1.08
	max	1.90	1.79	1.80	1.90
	SD	0.127	0.126	0.124	0.128
Body weight of one-day-old piglet (piglets surviving at age to 28 days) (kg)	*n*	385	123	129	133
	$\bar{x}$	1.58	1.57	1.60	1.57
	min	1.21	1.21	1.37	1.27
	max	1.90	1.79	1.80	1.90
	SD	0.111	0.104	0.111	0.116
Body weight of one-day-old piglet (piglets that died at age up to 28 days) (kg)	*n*	33	12	11	10
	$\bar{x}$	1.37	1.33	1.41	1.37
	min	1.08	1.12	1.18	1.08
	max	1.68	1.52	1.68	1.55
	SD	0.132	0.130	0.135	0.132
Body weight of 28-day-old piglet (kg)	*n*	385	123	129	133
	$\bar{x}$	8.51	8.48	8.65	8.42
	min	6.64	6.69	7.22	6.64
	max	10.01	10.01	9.62	9.55
	SD	0.594	0.622	0.549	0.589

pelleted prestarter diet. Newborn piglets from all groups were subjected to routine veterinary and management practices during the first week of life.

From birth to weaning at 28th day of life, piglets from group E1 received drinking water with a 2% solution of lactic, formic and orthophosphoric acids, and those from group E2 a 0.05% solution of Baracid P, which contained phosphoric, citric, lactic, formic and tartaric acids. No acidifier was used in the control group C. The piglets were monitored for body weight (individual weighing at 1st and 28th day of life), mortality, and the incidence and duration of diarrhea.

The results were statistically analysed by SPSS Statistics 24 (2016). The statistical model included the birth weight of piglet as a concomitant variable (ANOVA).

RESULTS AND DISCUSSION

The mean number of piglets born alive per litter showed slight differences, ranging from 11.25 in group C to 11.67 and 11.92 in groups E1 and E2, respectively. During the maternal nursing period, mortality was 12 piglets (8.9%) in group C, 11 piglets (7.9%) in group E1, and 10 piglets (7.1%) in group E2, but only a small proportion of deaths was due to gastrointestinal problems accompanied by diarrhea. The mean number of piglets weaned per litter in the respective groups was 10.25, 10.75 and 11.08, which translated into survival of 91.1% (group C), 92.1% (group E1) and 93.0% (group E2). This parameter was 1.0 percentage point higher in group E1 compared to group C, and 1.8 percentage points higher in group E2 compared to group C. The difference in the value of this parameter between groups E2 and E1 was 0.8 percentage point to the advantage of group E2. The differences in suckling survival were small but beneficial for the groups in which reared piglets received water supplemented with an acidifier. The number of diarrhea days was smaller in groups E1 and E2 than in group C (4 and 2 versus 6). This finding suggests the appropriateness of using acidifiers, although the lack of economic analysis does not allow for a conclusive determination of benefits.

The results of our study show that differences in the birth weight of piglets were considerable (up to 0.69 kg), which means that the birth weight of the heaviest piglet was higher by 59% compared to the lightest one (Table 1). The mean body weight of the heaviest piglet at age of 28 days was 3.37 kg higher than that of the lightest piglet (difference of 50.8%). The difference in body weight between 28-day-old and one-day-old piglets decreased by 8.2 percentage points, possibly indicating that the piglets with the lowest birth weight began to exhibit compensatory growth. Rehfeldt et al. (2012) claimed that compensatory growth is possible in older piglets. Weight gains differed by as much as 2.93 (55.3%) between piglets showing the extreme values of this parameter. Differences in the daily gains of suckling piglets reached 104 g (55%).

The mean birth weight of all piglets was high (1.56 kg) and can be considered as optimal for newborn piglets. It also indicates a good nutritional status of the sows, especially those in advanced pregnancy. The mean body weights of piglets in the groups were also high, but

despite the small differences between the groups, the differences were statistically significant ($P \leq 0.05$ and $P \leq 0.01$) – Table 2. Inclusion in the statistical model of initial body weight of piglets as a covariable allowed us to show that the body weight of piglets on day 28 of age as well as total body weights and daily gains (days 1–28) of piglets from groups C vs E1 and E2 did not differ significantly ($P > 0.05$).

The beneficial effects of acidifiers in reared piglets were reported by Rajchert et al. (2011), who gave the experimental piglets twice as much acidifier in the second observation stage (days 10–28) compared to the first (days 1–10). The mean daily gains in group E (262 g) were higher by 11% compared to group C. The mean body weight of 28-day-old piglets was 6.92 kg in group C and 7.50 kg in the acidifier-supplemented group E (difference of 8.4% in favour of group C). In the study by Urbańczyk and Hanczakowska (1995), a fumaric acid supplement caused piglet weight gains to improve by 7.5%. Similar to Rajchert et al. (2011), Biagi et al. (2006), who studied the effect of gluconic acid on piglet weight gains, gut microflora and intestinal wall morphology, observed higher growth rate in piglets from groups E1 and E2 compared to group C. In earlier studies by Urbańczyk and Hanczakowska (1995) and Kamyczek and Kujawiak (1999), the use of acidifying additives had a beneficial effect on the mean growth rate of piglets. Roth and Kirchgessner (1998), who supplemented suckling piglets with formic acid and sorbic acid, increased their gains by 20% in comparison with the control group. Mazzoni et al. (2008) concluded that acidification of feed with sodium butyrate works best if it is administered during the maternal nursing period, because this positively affects production results in the later stages of rearing and during fattening.

In our study, the birth weight of piglets exceeded 1.5 kg. Budvytis and Włodarczyk (2012) concluded that the birth weight of piglets above 1.2 kg is conducive to a considerable decrease in the mortality of suckling piglets, which

TABLE 2. Results of the experiment

Parameter	Group C $n = 123$		Group E1 $n = 129$		Group E2 $n = 133$		P
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	
Body weight of one-day-old piglet (kg)	1.57a	0.009	1.60aA	0.010	1.57A	0.006	0.010
Body weight of 28-day-old piglet* (kg)	8.52	0.025	8.54	0.025	8.48	0.024	0.263
Total weight gain* (1–28 days of life) (kg)	6.94	0.025	6.96	0.025	6.90	0.024	0.263
Daily weight gain* (1–28 days of life) (g)	248	0.896	248	0.880	247	0.863	0.263

* The birth weight of piglet as a concomitant variable (1.5804).
A, A – means in the rows with the same capital letters differ significantly at $P \leq 0.01$.
a, a – means in the rows with the same small letters differ significantly at $P \leq 0.05$.

should not exceed 9%. Supplementation of acidifiers may also reduce the losses, as confirmed by the study of Batorska and Mieńkowska-Stępniewska (2000). One example of the beneficial effect of acidifiers on survival of suckling piglets are the results of Rajchert et al. (2011), who reported a mortality of about 14.2% in group C compared to only 7.9% in the acidifier-supplemented group E (a difference of 6.3 percentage points).

The experimental piglets were healthy. In group C, there were only 6 diarrhea days in 2 litters, in which 3 piglets died of diarrhea. In group E1, there were 4 diarrhea days in 2 litters, but no mortality due to gastrointestinal disturbances was observed. In group E2, there were 2 diarrhea days, with no incidence of mortality due to diarrhea.

CONCLUSIONS

The acidifiers used in the present experiment had no statistically significant effect on the daily gains of piglets. Production parameters were satisfactory. The use of the additives slightly increased piglet survival (by 1.0 percentage point in group E1 compared to group C and by 1.8 percentage point in group E2 compared to group C) and decreased the number of days with diarrhea in experimental compared to control piglets. The results obtained confirm the positive effect of the acidifiers on the health of suckling piglets.

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Streszczenie: *Wpływ płynnych zakwaszaczy na wyniki odchowu prosiąt ssących.* Badaniami objęto prosięta mieszańce z 36 miotów (po 12 miotów w grupie kontrolnej C i doświadczalnych E1 i E2). Od urodzenia do odsadzenia w 28. dniu życia prosięta z grupy E1 otrzymywały z wodą do picia 2-procentowy roztwór kwasów mlekowego, mrówkowego i ortofosforowego, a prosięta z grupy E2 0,05-procentowy roztwór preparatu Baracid P, który zawierał kwasy: fosforowy, cytrynowy, mlekowy, mrówkowy i winowy. W grupie C nie stosowano zakwaszacza. Kontrolowano masę ciała prosiąt, ich śmiertelność oraz występowanie i czas trwania biegunek. Wskaźniki produkcyjne we wszystkich grupach były na średnim poziomie. Przyrosty dobowe prosiąt w okresie od 1. do 28. dnia życia były porównywalne w grupach ($P > 0,05$). Zastosowanie dodatków zwiększyło przeżywalności prosiąt – o 1,0 p.p. w grupie E1 w porównaniu do grupy C i 1,8 p.p. w grupie E2 w porównaniu do grupy C oraz zmniejszyło liczbę dni biegunkowych u prosiąt doświadczalnych w porównaniu z kontrolnymi. Różnice w przyrostach dobowych nie były istotne statystycznie między grupami, poprawa zdrowia i większa przeżywalność prosiąt w grupach doświadczalnych w porównaniu z kontrolną wskazuje jednak na zasadność stosowania zakwaszaczy w odchowie młodych świń.

Słowa kluczowe: prosięta, zakwaszacze, przyrosty dobowe, dni biegunkowe, przeżywalność

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Effect of naked oats used for gilts on the state of their reproductive organs and selected reproductive indicators of sows

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Abstract: *Effect of naked oats used for gilts on the state of their reproductive organs and selected reproductive indicators of sows.* The study was carried out on 180 Polish Landrace gilts. The animals were assigned to three groups, two experimental and one control, with 60 individuals in each. The gilts were fed complete mixed rations in amounts consistent with the norms given in "Swine feeding standards" (published by Institute of Animal Physiology and Nutrition of Polish Academy of Sciences in 1993). The mixture fed to the experimental groups contained 40% (D_1) and 20% (D_2) naked oats of the Akt variety. Sexual activity was observed in the gilts during the second and third oestrus. The standing reflex was tested twice a day, in the morning and afternoon, and its duration was determined by timing the positive reaction to a boar, to touch and to mounting. Half of the gilts, selected in equal numbers from the control group and the experimental groups, were slaughtered between fifth and 10th day of the luteal phase of the second or third oestrous cycle. The number of corpora lutea was determined in these gilts. The reproductive organs were evaluated as well, taking into account the weight of the uterus, the length of the uterine horns and the weight of the ovaries. The remaining gilts (90) were mated during the second (45 gilts) and third (45 gilts) oestrus. Then the age at first farrowing, the weaning-to-conception interval between the first and second and the second and third litters, and the length of the farrowing interval between the first and second and the second and third litters were calculated. The highest uterine weight was noted in the gilts from the experimental groups. In both periods the weight of the uterus ranged from 372.8 (D_2, mated during the second oestrus) to 398.4 g (D_1, mated during the third oestrus). The weight of the ovaries was within the normal range, but significant differences were noted between the experimental and control groups. The highest sexual activity during oestrus (2.4–2.7 pts) in the first, second and third reproductive cycles was observed in the sows that received naked oats in their feed ration. Gilts mated during the second oestrus of the first reproductive cycle had litters earliest, on average at the age of 314.7 days (group D_1), compared to 335.3 days in the case of gilts bred in the third oestrus of the first reproductive cycle (group D_1). The most beneficial farrowing interval in each reproductive cycle was noted in the groups of sows receiving mixtures containing naked oats.

Key words: naked oats, gilts, sexual activity, reproductive organs, reproductive indicators

INTRODUCTION

Success in animal reproduction depends in large measure on how they are prepared for it. Normal development of the reproductive organs in gilts ensures

their future productivity. The reproductive system of gilts undergoes intensive changes, both morphometric and histological, from birth to full maturity (Maciołek 1999). The development of the reproductive organs in gilts is influenced by endogenous and exogenous factors. Many authors have found a correlation between the weight and dimensions of the uterus and potential and actual fertility in sows (Maciołek 1979, Kiss and Bilkei 2000). The effectiveness of breeding for reproductive traits is often impeded by insufficient uterine volume in sows, which reduces the increase in ovulation rate obtained through selection. Research is carried out both in Poland and abroad to determine the effect of various genetic and environmental factors on the development of the reproductive organs in gilts (Klocek et al. 1998, Maciołek 1999). Studies by many authors show that the development of the reproductive organs in gilts is affected by factors associated with the environment in which they are raised, including feeding. Maciołek (1979) observed that in pigs fed dry, granulated feed in an industrial system new anatomical traits arise that are not found in pigs fed in a traditional manner. According to Koczanowski (1986), a reduction in protein and calories or in one of these components in the feed ration negatively affects the development of individual parts of the reproductive system, particularly the ovaries, oviducts and uterus.

Opinions regarding the effect of the level of feeding on the development of the reproductive organs in gilts vary among researchers. Some state that *ad libitum* feeding is beneficial (Migdał 1996), while others recommend the use of limited feed rations (Prunier et al. 2001, Rekiel 2002). A greater focus on research concerning the effect of nutrition on anatomical and functional traits of the reproductive tract in gilts and sows and statistical analyses may be of great importance in improving reproduction in pigs.

The aim of the study was to determine the effect of naked oats used in feed rations on the state of the reproductive organs of gilts and on selective reproductive indicators of sows.

MATERIAL AND METHODS

The study was carried out on 180 Polish Landrace gilts. The animals were assigned to three groups, two experimental and one control, with 60 individuals in each. The animals were kept in group pens – 6 heads per pen. The surface of pen was 1.5 m^2 per head. The gilts were fed complete mixed rations in amounts consistent with the norms given in "Swine feeding standards" (IFiZZ PAN 1993). The mixture fed to the experimental groups contained 40% (D_1) and 20% (D_2) naked oats of the Akt variety. The composition and nutritional value of the mixtures is presented in Table 1; the values given in "Swine feeding standards" (IFiZZ PAN 1993) for barley, wheat and post-extraction ground soymeal were used. The chemical composition of naked oat was determined before the experiment on the animals was begun. The following were determined in the samples:

- content of crude protein, ether extract, crude ash, and crude fibre according to AOAC (2000);
- content of mineral nutrients Ca and Na by atomic absorption spectroscopy

TABLE 1. Composition of mixtures for gilts and pregnant and lactating sows

Feed	Gilts and sows before 90th day of pregnancy			Lactation		
	D_1	D_2	K	D_1	D_2	K
	%					
Naked oats meal	40.00	20.00	–	40.00	20.00	–
Wheat meal	–	20.00	40.00	–	20.00	40.00
Barley meal	48.50	48.45	48.40	40.10	40.05	40.00
Soybean meal	9.00	9.00	9.00	17.00	17.00	17.00
Dicalcium phosphate	0.90	0.90	0.90	1.00	1.00	1.00
Fodder chalk	1.30	1.30	1.30	1.30	1.30	1.30
Premixture L-lysine 50%	–	0.05	0.10	0.20	0.25	0.30
NaCl	0.30	0.30	0.30	0.40	0.40	0.40
per 1 kg of mixture						
MJ	13.03	12.89	12.75	13.01	12.87	12.72
Crude protein (g)	138.03	137.18	136.32	160.71	159.85	159.00
Crude fat (g)	39.98	30.17	20.32	40.10	30.24	20.39
Crude fibre (g)	85.40	62.37	41.51	77.77	64.52	42.57
Lysine (g)	5.98	5.97	5.96	8.58	8.57	8.56
Methionine + cystine (g)	5.06	4.95	4.84	5.62	5.51	5.40
Ca	7.69	7.73	7.78	8.43	8.48	8.53
P	5.73	5.64	5.55	6.27	6.18	6.09
Na	1.37	1.36	1.35	1.79	1.78	1.77

Source: Own elaboration.

(ASA) and total phosphorus according to Fiske and Subbarow (1925);
- protein amino acid content by ion-exchange chromatography in an automatic amino acid analyser;
- fatty acid composition by gas chromatography using a chromatograph (Varion GC3800).

Sexual activity was observed in the gilts during the second and third oestrus. The standing reflex was tested twice a day, in the morning and afternoon, and its duration was determined by timing the positive reaction to a boar, to touch and to mounting. The intensity of oestrus symptoms was evaluated according to the scale developed by Stasiak (1996):
- 3 points – the gilts are very calm and manifest oestrus very clearly, reacting to the boar and to the mounting attempt for about 30–35 s;
- 2 points – the gilts are calm and manifest oestrus clearly, reacting to the boar and to the mounting attempt for about 20–25 s;
- 1 point – the gilts manifest oestrus faintly, reacting to the boar and to the mounting attempt for about 5 s.

Half of the gilts, selected in equal numbers from the control group and the experimental groups, were slaughtered between fifth and 10th day of the luteal phase of the second or third oestrous cycle. The number of corpora lutea was determined in these gilts. The reproductive organs were evaluated as well, taking into account the weight of the uterus, the length of the uterine horns and the weight of the ovaries. The remaining gilts (90) were mated during the second (45 gilts) and third (45 gilts) oestrus. Then the age at first farrowing, the weaning-to-conception interval between the first and second and the second and third litters, and the length of the farrowing interval between the first and second and the second and third litters were calculated.

The results were analysed statistically using one-way analysis of variance. Differences between means were tested by Duncan's test.

RESULTS AND DISCUSSION

The size of the reproductive organs and the number of corpora lutea during the second and third oestrus of the first reproductive cycle in the gilts are presented in Table 2. The highest uterine weight was noted in the gilts from the experimental groups. In both periods the weight of the uterus ranged from 372.8 (D_2, mated during the second oestrus) to 398.4 g (D_1, mated during the third oestrus). The weight of the ovaries was within the normal range, but significant differences were noted between the experimental and control groups. The length of the uterine horns ranged from 56.2 to 65.3 cm and was characterized by substantial standard deviations. The greatest number of corpora lutea was noted in the gilts in the D_1 groups (from 15.0 to 15.6). The highest sexual activity during oestrus (2.4–2.7 points) in the first, second and third reproductive cycles was observed in the sows that received naked oats in their feed ration (Table 3).

The earlier age at which the gilts fed mixtures with naked oats attained reproductive maturity affected their age at first farrowing. Gilts mated during the second oestrus of the first reproductive cycle had litters earliest, on average at the age of 314.7 days (group D_1), compared to 335.3 days in the case of gilts bred in the third oestrus of the first reproductive cycle (group D_1). The most beneficial farrowing interval in each reproductive cycle was noted in the groups of sows receiving mixtures containing naked oats. Sows mated during the second oestrus of the first reproductive cycle had somewhat longer farrowing intervals. The length of the farrowing interval did not exceed 181 days in any of the groups of sows. The shortest farrowing interval (second and third litter), lasting 169.7 days, was noted in the group D_1 sows mated in the third oestrus of the first reproductive cycle. The longest farrowing interval, lasting 180.7 days (first and second litter), was observed in the control group of sows mated for the first time during the second oestrus of the first reproductive cycle.

Analysis of the state of the reproductive organs and of potential fertility in each group shows a slight positive effect of oats on uterine weight, the length of the uterine horns, the weight of the ovaries and the number of corpora lutea. Similar correlations were observed by Stasiak et al. (2000). The weight and

TABLE 2. Size of reproductive organs and number of corpora lutea in the luteal phase of the second and third oestrus cycle

Oestrus number	Experimental groups	*n*	Uterus weight (g)		Right ovary weight (g)		Left ovary weight (g)		Length of right uterine horn (cm)		Length of left uterine horn (cm)		Number of corpora lutea on both ovaries	
			$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
II	D_1	15	379.5	±46.79	4.5	±0.25	4.7	±0.24	59.4	±6.72	62.3	±7.14	15.0	±2.67
	D_2	15	372.8	±51.12	4.6^{A}	±0.24	4.6	±0.19	57.9	±7.41	59.3	±7.72	14.7	±2.82
	K	15	367.5	±56.53	4.3^{B}	±0.29	4.5	±0.31	56.2	±7.94	58.5	±8.39	14.1	±3.01
III	D_1	15	398.4	±51.34	4.7^{A}	±0.27	4.8^{a}	±0.30	63.5	±6.95	65.3	±7.20	15.6	±2.53
	D_2	15	390.7	±52.86	4.6	±0.28	4.7	±0.29	59.8	±7.41	61.6	±7.83	15.1	±2.61
	K	15	384.4	±56.09	4.4^{B}	±0.31	4.5^{b}	±0.32	58.1	±8.10	59.5	±8.42	14.4	±2.95

A, B – significant differences within groups of gilts in the second or third oestrus at $P \leq 0.01$.
a, b – significant differences within groups of gilts in the second or third oestrus at $P \leq 0.05$.
Source: Own elaboration.

TABLE 3. Sexual activity and selected reproductive indicators of primiparous sows mated during the second or third oestrus

Oestrus number	Experimental groups	Sexual activity before pregnancy (pts)						Weaning-to-conception interval (days) between				Age at first parturition (days)		Farrowing interval (days)			
		1		2		3		1st and 2nd litter		2nd and 3rd litter				1st and 2nd litter		2nd and 3rd litter	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
II	D_1	2.5	±0.22	2.6	±0.33	2.6	±0.37	18.6	±5.31	16.3	±4.12	314.7	±10.12	174.8	±12.38	172.6	±13.03
	D_2	2.4	±0.19	2.4	±0.20	2.5	±0.23	19.5	±6.43	18.4	±5.21	318.8	±12.05	175.8	±13.18	174.8	±14.10
	K	2.2	±0.33	2.2	±0.31	2.3	±0.15	24.6	±7.53	21.0	±6.31	324.0	±13.89	180.7	±10.76	177.2	±12.68
III	D_1	2.6	±0.34	2.6	±0.33	2.7	±0.40	15.5	±4.91	13.1	±3.76	335.3	±11.22	171.8	±13.64	169.7	±13.92
	D_2	2.4	±0.20	2.5	±0.21	2.6	±0.38	17.2	±5.10	14.1	±3.41	340.0	±13.51	173.4	±13.70	170.6	±13.84
	K	2.3	±0.21	2.4	±0.20	2.4	±0.29	22.4	±6.86	18.4	±5.23	345.3	±14.05	178.6	±12.91	174.7	±13.20

Source: Own elaboration.

dimensions of the reproductive organs determine the optimal number of embryos for them (Klocek 1997). Martin-Rillo et al. (2001) demonstrated a positive correlation between the length of the vagina and the number of piglets born. This correlation was not confirmed by Casimiro and Kirkwood (2002). According to Bennett and Leymaster (1989), the length of the uterus is highly correlated with the embryo survival rate, and thus with the actual fertility rate of the sows. Similarly, Vianna et al. (2004) stated that the number of embryos that can develop in the uterus depends on the length of the uterine horns. Kiss and Bilkei (2000) found that a well-developed reproductive tract increases the possibility of successful embryo implantation and thus better embryonic development. They recommend carrying out selection directed towards the volume of the uterus in maternal lines. Many authors draw attention to the variation in the size and weight of the reproductive organs of gilts reared in different feeding and housing conditions (Szostak and Sarzyńska 2006). In the present study the weight of the ovaries was greater in gilts fed mixtures containing naked oats. The left ovary weighed slightly more than the right, which confirms previous findings of other author concerning the greater functionality of the left ovary (Stasiak 1996).

The weight and function of the ovaries is linked to the number of eggs released per oestrus. In our experiment estimation of this trait was based on the number of corpora lutea counted post mortem on the ovaries. The higher number of corpora lutea in the experimental groups is an indicator of the suitability of naked oats in the diet of gilts. The effect of including fat or starch in the diet of sows before breeding is unclear. Kauffold et al. (2004) demonstrated a beneficial effect of a high-calorie diet on sow fertility. Grandhi (1988), on the other hand, found that adding animal fat had no effect on the number of eggs released. In a study by Koczanowski et al. (2008) sows fed a mixture containing soya oil had a higher embryo survival rate than the control.

Sows fed naked oats exhibited greater sexual activity in successive oestrous cycles. Studies by Cronin et al. (1982) and Stasiak (1996) have shown that gilts arousing greater interest in boars have a higher conception rate and greater fertility. Stasiak and Kamyk (2003) reported a higher number of piglets born per litter in gilts exhibiting full readiness for mating in comparison with those with a weak standing reflex. The desired length of the farrowing interval, which depends on the physiological properties of the body of the sow and on how the piglets are reared, is between 160 and 180 days (Bocian et al. 2010).

CONCLUSIONS

A significant effect of complete mixed rations with the participation of naked oats on some elements of the reproductive system of sows was found. The highest uterine weight was noted in the gilts from the experimental groups, its mass ranged from 372.8 (D_2, mated during the second oestrus) to 398.4 g (D_1, mated during the third oestrus).

Significant differences between control and experimental groups on ovarian weight in favor of the experimental groups were observed.

The highest sexual activity during oestrus (2.4–2.7 points) in the first, second and third reproductive cycles was observed in the sows that received naked oats in their feed ration.

The results obtained in the present study were within this range, and there was a tendency towards shorter farrowing intervals in sows fed mixtures containing naked oats.

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Streszczenie: *Wpływ owsa nagiego na stan narządów rozrodczych loszek i wybrane wskaźniki reprodukcji loch*. Doświadczenie zostało przeprowadzone na 180 loszkach rasy polskiej białej zwisłouchej. Zwierzęta zostały podzielone na trzy grupy – dwie eksperymentalne i jedną kontrolną, 60 osobników w każdej. Loszki karmiono mieszankami pełnodawkowymi w ilościach zgodnych z zaleceniami podanymi w „Normach żywienia trzody chlewnej" (wydanych w 1993 roku przez Instytut Fizjologii i Żywienia Zwierząt Polskiej Akademii Nauk). Mieszanka pełnodawkowa dla grup eksperymentalnych zawierała 40% (D_1) i 20% (D_2) owsa nagiego odmiany Akt. U loszek aktywność seksualna była obserwowana w drugiej i trzeciej rui. Odruch tolerancji badano dwa razy dziennie, rano i po południu, mierząc czas pozytywnej reakcji na knura. Połowa loszek, wybranych w równych ilościach z grupy kontrolnej i z grup doświadczalnych została poddana ubojowi między piątym a dziesiątym dniem fazy lutealnej drugiego lub trzeciego cyklu rujowego. U ubitych loszek określono ilość ciałek żółtych. Narządy rozrodcze były oceniane pod względem: ciężaru macicy, długości rogów macicy i masy jajników. Pozostałe (90) loszki były kojarzone w czasie drugiej (45 loszek) i trzeciej (45 loszek) rui. W pracy obliczono: wiek pierwszego oproszenia, okres międzymiotu między pierwszym i drugim, drugim i trzecim miotem, a także długość okresu międzymiotu między pierwszym i drugim, drugim i trzecim miotem. Największą masę macicy zanotowano u loszek z grup eksperymentalnych. W dwóch okresach jej masa mieściła się w przedziale od 372,8 (D_2, w okresie drugiej rui) do 398,4 g (D_1, w okresie trzeciej rui). Masa jajników była w normie, chociaż zaobserwowano istotne różnice między grupami doświadczalnymi i kontrolną. Największą aktywność seksualną podczas rui (2,4–2,7 punktów) w pierwszym, drugim i trzecim cyklu rozrodczym obserwowano u loch, które otrzymały w dawkach pokarmowych owies nagi. Loszki kojarzone w okresie drugiej rui wydawały mioty najwcześniej, średnio w wieku od 314,7 (grupa D_1) do 335,3 dni w przypadku loszek w trzeciej rui (grupa D_1). Najdłuższy okres międzymiotu miały loszki z grupy żywionej mieszanką z dodatkiem owsa nagiego.

Słowa kluczowe: owies nagi, loszki, aktywność seksualna, narządy rozrodcze, wskaźniki rozrodu

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Influence of application of (1,3)-(1,6)-β-D-glucans and mannans on production results of sows and piglets

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Abstract: *Influence of the application of (1,3)-(1,6)-β-D-glucans and mannans on production results of sows and piglets.* The aim of the study was the assessment the production results and health status of sows and their offspring modulated by supplementation of (1,3)-(1,6)-β-D-glucans and mannans. The experiment was carried out in farrowing and nursing sector. It was performed using crossbreed (Polish Large White × Polish Landrace) pigs. The additive of (1,3)-(1,6)-β-D-glucans (G) or (1,3)-(1,6)-β-D-glucans and mannans (GM) was introduced to the feed for sows from the 85th day of gestation and for their offspring from the 22nd (after weaning) to the 90th (moving to the fattening sector) day of life. Production results were estimated basis on: changes of body weight of sows during lactation and their offspring during the experimental time, average feed intake, feed conversion ratio, mortality. To estimate health status blood samples from sows and their offspring were taken to determine the total protein level and its fraction in serum. The obtained results indicate that the additive of G or GM to lactation sows and weaned piglets can usefully influence on catabolism process in sows, growth performance and health status in piglets.

Key words: sows, piglets, (1,3)-(1,6)-β-D-glucans, mannans, growth performance, health status

INTRODUCTION

The European ban of antibiotic growth promoters from January 2006 opened new era in feed additives industry. The total withdrawal of antibiotic promoters from pig diets caused looking for the best alternatives to them. Promising group of such additives which connect immunoprotective and immunostimulating effect are mannanooligosaccharides (MOS) and (1,3)-(1,6)-β-D-glucans isolated from the brewer's yeast *Saccharomyces cerevisiae.* Mannans can stimulate the growth and activity of beneficial bacteria and regulate the process of fermentation in the gastrointestinal tract (Cummings and Macfarlane 2002). (1,3)-(1,6)-β-D-glucans creating absorption capacity and stimulates the immune system – especially macrophages (Kogan 2000, Majtán et al. 2005). Thanks to its properties numerous studies proved their beneficial influence on health status and production performance in growing pigs (Pettigrew 2000, Miguel et al. 2003, Li et al. 2006, Szuba-Trznadel et al. 2014).

Some researchers did not observe similar effects in adult sows (Veum et al. 1995). However, from a live yeast are recommended for pregnant and nursing sows. The profitable influence on milk value and piglets rearing was evidenced by Fuchs et al. (2007), Kim et al. (2010), Wealleanes and Litten-Brown (2010).

The aim of the study was assessment of production results and health status of sows and their offspring modulated by supplementation of (1,3)-(1,6)-β-D-glucans and mannans in the experimental doses in swine production conditions.

MATERIAL AND METHODS

The investigation was carried out on crossbreeds Polish Large White × Polish Landrace pigs at a commercial farm in Poland. Experiment started at 85th day of gestation when pregnant sows obtained lactation feed (LF). All feed mixtures for sows and weaners were prepared within the standards accepted for these animal groups (NRC 1998) – Table 1.

Experimental animals were divided in 3 groups: C – control (standard feed used at farm), G (standard feed with additive of (1,3)-(1,6)-β-D-glucans), GM (standard feed with additive of (1,3)-(1,6)-β--D-glucans and mannans). The additives used in the experiment were isolated from the brewer's yeast *Saccharomyces cerevisiae* (the purity of the additives was beyond 76% and was confirmed by producer – Biorigin Company). The concentration of (1,3)-(1,6)-β-D-glucans and mannans in all experimental feed mixtures was at the same level, there was added 1 kg of pure additives, containing 0.05% (1,3)-(1,6)-β-D-glucans in G group and 0.05% (1,3)-(1,6)--β-D-glucans and 0.05% mannans in GM group.

There was chosen 90 pregnant sows, according to analogue rules (age, the same proportion of primi- and multiparous at every group), after moving to the farrowing sector experimental procedures were carried out on 30 sows and their litters in 3 experimental groups.

Animals were subject to routine-care and veterinary treatment performed on the farm. On the second day after parturition litters were standardized according to size and piglets' body weight, the smallest and weakest piglets according to farm technology were culled. In a farrowing unit, piglets were housed with their dams in triple slatted farrowing pens. Iron was given to piglets as an intramuscular injection two times: on the 2nd and 14th day of life. During suckling period piglets had not access to solid feed what is a standard routine at farm. After weaning (after 21st day of life) piglets were moved to nursery building and kept in pens in groups of 20. They were fed in groups Prestarter up to the 45th day of life and from this day to the end of staying in nursery unit they obtained Starter feed.

Sows after moving to farrowing unit (112th day of gestation) were kept in experimental groups and their offspring stayed in those group to the end of investigations. The daily feed mixture dosage for sows was increased gradually up to the 7th day after parturition, next they were fed *ad libitum* to the 19th day and 2 last days of lactation the daily feed dosage was limited to the level of 4.5 kg.

During the experimental time there were collected following production parameters: body weight of sows on the

TABLE 1. Chemical composition and nutritive value of standard feed mixture used at farm

LF feed mixture (in % of mixture): Wheat 36.55, Barley 18.0, Triticale 15.0, Wheat bran 9.0, Fish meal 72% 2.0, Soybean meal 46% 10.0, Rape seed 4.0, Rape oil 2.0, Calcium formate 0.2, Chalk 1.0, 1-Ca phosphate 0.8, Salt 0.5, Lysine HCl 0.28, DL-methionine 0.05, L-threonine 0.12, Premix* 0.5[a]			
Prestarter feed mixture (in % of mixture): Wheat 63.18, HP-300 Hamlet protein from soybean 10.0, Fish meal 72% 7.0, Whey powder 10.0, Soybean meal 46% 2.5, Rape oil 4.0, Calcium formate 0.7, 1-Ca phosphate 0.55, Salt 0.2, Aroma 0.03, Lysine HCl 0.54, DL-methionine 0.42, L-threonine 0.24, L-tryptophan 0.08, Zinc oxide 0.03, Sodium butyrate 0.03, Premix* 0.5[b]			
Starter feed mixture (in % of mixture): Wheat 25.76, Maize 30.0, Barley 18.0, HP-300 Hamlet soya oil meal 7.0, Fish meal 72% 5.0, Whey powder 5.0, Soybean meal 46% 4.0, Rape oil 2.0, Chalk 0.3, 1-Ca phosphate 0.55, Salt 0.3, Lysine HCl 0.64, DL-methionine 0.24, L-threonine 0.24, L-tryptophan 0.04, Sodium butyrate 0.03, Nordocid (Acidifier) 0.4, Premix* 0.5[c]			
Nutritive value (1 kg of mixture contains):	LF	Prestarter	Starter
Metabolic energy (MJ)	13.8	14.23	13.81
Crude protein (g)	167.5	203.00	183.30
Lysine (g)	9.80	14.60	13.80
Methionine (g)	3.20	5.60	5.60
Methionine + Cystine (g)	6.20	8.70	8.30
Threonine (g)	6.90	9.10	8.80
Tryptophan (g)	2.00	2.40	2.10
Ca (g)	8.90	8.00	8.10
P-total (g)	6.50	7.30	7.10
Na (g)	2.20	2.40	2.20

[a] Composition of mineral and vitamin mixture – Premix 0.5% – Vitamin A 2 400 000 iu; Vitamin D_3 400 000 iu; Vitamin E 16 000 mg; Vitamin B_1 200 mg; Vitamin B_2 800 mg; Biotin 60 000 mcg; Vitamin B_6 600 mg; Vitamin B_{12} 4 000 mcg; Vitamin K 400 mg; Niacin 4 018 mg; Folic acid 1000 mg; Pantothenic acid 3 000 mg; Choline 40 000 mg; Mn 10 000 mg; Zn 20 000 mg; Co 100 mg; Se 60 mg; Cu 4 500 mg; Fe 25 000 mg; J 200 mg; Betaine 17 000 mg; + Antioxidant.

[b] Composition of mineral and vitamin mixture – Premix 0.5% – Vitamin A 3 000 000 iu; Vitamin D_3 400 000 iu; Vitamin E 10 000 mg; Vitamin B_1 500 mg; Vitamin B_2 1 000 mg; Biotin 12 000 mcg; Vitamin B_6 1 000 mg; Vitamin B_{12} 6 000 mcg; Vitamin K 500 mg; Niacin 6 008 mg; Folic acid 100 mg; Pantothenic acid 3 000 mg; Choline 21 000 mg; Mn 8 000 mg; Zn 30 000 mg; Co 100 mg; Se 60 mg; Cu 4 500 mg; Fe 25 000 mg; J 200 mg; Betaine 12 000 mg; + Antioxidant.

[c] Composition of mineral and vitamin mixture – Premix 0.5% – Vitamin A 2 400 000 iu; Vitamin D_3 400 000 iu; Vitamin E 8 000 mg; Vitamin B_1 400 mg; Vitamin B_2 1 000 mg; Biotin 6 000 mcg; Vitamin B_6 600 mg; Vitamin B_{12} 6 000 mcg; Vitamin K 400 mg; Niacin 5 009 mg; Folic acid 100 mg; Pantothenic acid 3 000 mg; Choline 21 000 mg; Mn 8 000 mg; Zn 28 000 mg; Co 100 mg; Se 60 mg; Cu 4 500 mg; Fe 20 000 mg; J 200 mg; Betaine 12 000 mg; + Antioxidant.

2nd and 21st (weaning) day after parturition; body weight of piglets on the 2nd, 21st (weaning), 45th (feed changed) and 90th (moving to fattening unit – the end of experiment) day of life; there were calculated daily gains, feed intake by sows and weaners; losses; in the end there were calculated daily gains and feed conversion ratio. To estimate health status blood samples were collected from 7 randomly

selected sows in frame of 1 group on the 2nd and 21st day after parturition and from 7 offspring chosen according to the same rule in frame of 1 group per pen on the 21st, 45th and 90th day of life. Blood samples were collected from the *vena jugularis externa* into serum tubes for total protein and its fraction estimation. The level of total protein was estimated using BCA test, and its fractions: albumins, α-, β- and γ-globulins using the filter paper electrophoresis method.

Collected data were statistically estimated in experimental groups. Treatment comparisons were made by analysis of variance procedures for a completely randomized design using Statistica 12.5 statistical package (StatSoft.Inc., Tulsa, OK). Differences between means were determined by the post-hoc Duncan test at 0.05 and 0.01 level.

RESULTS

Body weight changed of sows and feed intake during lactation are presented in Table 2. The initial body weight was similar in all groups. Control sows loosed 17% of body weight during lactation, while the experimental ones circa 12% and this difference was significantly important at $P \leq 0.05$. The average feed intake of experimental sows was higher approximately 8% during this time.

Table 3 presents production results of piglets and weaners. The initial number of piglets after culling was higher approximately 4.7% in experimental group (G and GM) compared to control one. The average losses during suckling period in whole piglets' population was 10.6% (mean value of piglets losses from 2nd to 21st day counted for all groups together). The lowest mortality was observed in G group (8.9%), the highest was in C (13.1%). After weaning comparable losses were noted in G and GM groups and they were 1.96 and 0.99%, respectively. The highest value was observed in C group – 4.3% at this time. Up to 90th day of life only one weaner died in C group.

At weaning the average body weight was 6.18 kg and was similar in all groups. After the first part of nursing period when there was changed feed from Prestarter into Starter the heaviest piglets were in GM treatment and this result was statistically differ compared to animals from C

TABLE 2. Body weight and feed intake of lactating sows

Specification	Statistics	Treatments		
		C	G	GM
Body weight of sows on 2nd day after farrowing (kg/head)	$\bar{x}$	252	258	249
	SD	18.6	20.7	16.4
Body weight of sows on weaning day (21st day of piglet life) (kg/head)	$\bar{x}$	209	226	218
	SD	27.1	17.8	14.3
Losses of body weight during lactation (kg/sow)	$\bar{}$	42.8 a	32.0 b	30.6 b
	SD	10.9	5.0	3.9
Feed intake of mixture for sows during lactation (kg/sow/day)	$\bar{x}$	6.80	7.28	7.30
	SD	0.95	0.28	0.25

a, b – $P \leq 0.05$.

TABLE 3. Rearing results of piglets during experimental time

Specification	Statistics	Treatments		
		C	G	GM
Number of animals (head)				
On the 2nd day		107	112	112
On the 21st day		93	102	101
On the 45th day		89	100	100
On the 90th day		88	100	100
Losses of piglets (%)				
From 2nd to 21st day	$\bar{x}$	14	10	11
	SD	13.08	8.93	9.82
From 21st to 45th day	$\bar{x}$	4	2	1
	SD	4.30	1.96	0.99
From 45th to 90th day	$\bar{x}$	1	–	–
	SD	1.12	–	–
From 21st to 90th day	$\bar{x}$	5	2	1
	SD	5.38	1.96	0.99
Body weight (kg)				
On the 2nd day	$\bar{x}$	1.55	1.62	1.66
	SD	0.14	0.09	0.09
On the 21st day	$\bar{x}$	6.05	6.30	6.20
	SD	1.28	0.82	0.97
On the 45th day	$\bar{x}$	13.54 a	13.92 a	14.83 b
	SD	0.56	0.64	0.57
On the 90th day	$\bar{x}$	37.16 Aa	40.12 b	41.64 B
	SD	2.13	1.58	1.3
Daily gain (g)				
From 2nd to 21st day	$\bar{x}$	236.7	246.2	239.4
	SD	66.7	42.0	52.9
From 21st to 45th day	$\bar{x}$	312.1	321.5	355.3
	SD	37.0	53.7	22.6
From 45th to 90th day	$\bar{x}$	524.9 A	582.2 B	595.9 B
	SD	36.0	21.9	20.4
From 21st to 90th day	$\bar{x}$	450.87 A	491.54 B	512.17 B
	SD	15.04	27.67	10.28
Feed conversion ratio (kg/kg)				
From 21st to 45th day	$\bar{x}$	1.58	1.58	1.42
	SD	0.18	0.18	0.09
From 45th to 90th day	$\bar{x}$	2.32 A	2.01 B	2.10 B
	SD	0.16	0.08	0.07

a, b – $P \leq 0.05$; A, B – $P \leq 0.01$.

and G treatments ($p \leq 0.05$). In the end of experiment, on the 90th day of life still the heaviest were animals from the GM treatment and this result was statistically differ to control animals ($P \leq 0.01$), additionally there were noticed statistical differences between C and G animals ($P \leq 0.05$). There were observed differences confirmed statistically at $P \leq 0.01$ between both experimental treatments and control one in daily gains and feed conversion ratio. The highest daily gains (from 46th to 90th day of piglets life) were observed in experimental groups (G and GM) and they were higher from control animals 10.9 and 13.5%, respectively what was statistically confirmed at $P \leq 0.01$. At this time, the highest feed conversion ratio was in control animals. The differences compared to G and GM animals were 13.4 and 9.48%, respectively, and it was statistically important at $P \leq 0.01$.

Table 4 presents the mean values of total protein and its fraction in sows' serum on the 2nd and 21st day of lactation. The average concentration of total protein was at the upper line of the physiological reference (Winnicka 20014). The lowest concentration of total protein was observed in control sows during the whole observation period and it was statistically, differ to experimental animals

TABLE 4. Total protein and its fraction of sows serum on 2nd and 21st day after farrowing (g/l)

Specification	Statistics	Treatments		
		C	G	GM
2nd day after farrowing				
Total protein	$\bar{x}$	78.00 a	84.50 b	79.00 a
	SD	1.12	1.012	5.76
Albumins	$\bar{x}$	36.85	36.47	33.94
	SD	2.07	1.55	2.23
α-Globulin	$\bar{x}$	13.97	16.48	14.48
	SD	0.50	2.04	2.11
β-Globulin	$\bar{x}$	11.59 a	14.20 b	12.89
	SD	0.52	1.51	1.49
γ-Globulin	$\bar{x}$	15.59	17.35	17.68
	SD	1.18	3.65	4.56
21st day of lactation				
Total protein	$\bar{x}$	86.51 a	89.00 b	90.00 b
	SD	5.43	3.79	3.06
Albumins	$\bar{x}$	39.06 a	37.53 a	34.53 b
	SD	1.50	2.16	2.62
α-Globulin	$\bar{x}$	14.03	14.73	14.87
	SD	0.85	2.83	0.54
β-Globulin	$\bar{x}$	16.60	15.82	13.16
	SD	1.15	3.04	1.93
γ-Globulin	$\bar{x}$	20.44 a	20.80 a	27.34 b
	SD	2.50	4.07	4.00

a, b – $P \leq 0.05$.

(G and GM treatments) on 21st day of lactation and only to G treatment at 2nd day. There was no distinct differentials in total protein fractions between sows. The highest concentration of albumin and γ-globulin at 21st day of lactation was observed in GM treatment.

Table 5 presents the average total protein content and its fraction in experimental offspring population. The

TABLE 5. Total protein and its fraction of offspring serum on 21st, 45th and 90th day of life (g/l)

Specification	Statistics	Treatments		
		C	G	GM
21st day of life				
Total protein	$\bar{x}$	68.59 Aa	66.40 Ab	62.50 B
	SD	1.64	2.19	0.0
Albumins	$\bar{x}$	38.05 A	32.36 B	30.83 B
	SD	0.91	2.15	1.49
α-Globulin	$\bar{x}$	11.74	13.88	12.73
	SD	1.13	2.09	2.31
β-Globulin	$\bar{x}$	11.29 A	10.92 A	9.82 B
	SD	0.48	0.65	0.36
γ-Globulin	$\bar{x}$	7.41	9.13	8.98
	SD	0.48	1.89	0.89
45th day of life				
Total protein	$\bar{x}$	60.50 a	67.00 b	66.00 b
	SD	1.20	4.47	2.85
Albumins	$\bar{x}$	25.83	27.77	26.18
	SD	2.08	2.80	1.15
α-Globulin	$\bar{x}$	14.87 a	17.68	18.85 b
	SD	1.90	2.62	2.17
β-Globulin	$\bar{x}$	10.56	11.83	11.85
	SD	1.05	1.53	2.10
γ-Globulin	$\bar{x}$	9.23	9.71	9.10
	SD	2.53	1.71	1.14
90th day of life				
Total protein	$\bar{x}$	78.50	66.50	75.00
	SD	8.94	8.94	6.85
Albumins	$\bar{x}$	34.16	32.37	32.24
	SD	5.01	3.12	2.99
α-Globulin	$\bar{x}$	16.62	13.84	14.56
	SD	5.37	3.33	2.41
β-Globulin	$\bar{x}$	14.80 a	9.49 b	12.39
	SD	2.24	2.89	1.77
γ-Globulin	$\bar{x}$	14.91	10.79	15.80
	SD	3.67	1.85	5.10

a, b – $P \leq 0.05$; A, B – $P \leq 0.01$.

obtained results were on the borderline of the reference range in all groups (Winnicka 20014). These values were changeable during the experimental period. Albumin fraction content was the highest in C treatment on 21st and 90th and the lowest on 45th day of life. The lowest concentration of γ-globulin was measured in C group at weaning day. During the next 3 weeks this fraction increased in C treatment 24.6%, in G and GM – 6.4 and 1.3%, respectively. On the 90th day of life the lowest concentration of γ-globulin fraction was measured in G treatment, next in C and the highest was in GM treatment.

DISCUSSION

It is a challenge and interesting thing to find out the right level suggested share of yeast derivatives in feed to pigs (Broadway et al. 2015). It is known that glucans and mannans influence on immunity system increasing immunoglobulin synthesis (especially SIgA). Additionally, β-glucan from yeast cell wall is a biological response modifier, which can enhance activity macrophages and neutrophils (Bohn and BeMiller 1995, Broadway et al. 2015). From the other side, they counteract development of pathogenic bacteria disturb them in localization on mucosal membrane of intestines (Li et al. 2006, Jang et al. 2013). Looking into literature there are in used different levels of these additives. Usually it is recommended higher level of such products e.g. Dritz et al. (1995) used 0.1%, Rozeboom et al. (2005) 0.2 and 0.3%, while in presented study it was 0.05%. Lower doses of such additives used Nochta et al. (2009). They were observed influence on piglets (weaned at 28th day of life) immunity status even 0.01% additive of pure mannans and it is recommended by concentration of mannans in feed. After 2 weeks of feeding 0.01% mannans supplementation enhanced some advantage in specific and non-specific immune responses. There was observed higher concentration of total protein fractions, especially γ-globuline in G and GM sows serum on the 2nd day after parturition, what can testify about their immunological system stimulation comparing to C group sows. The same tendency was observed at weaning day. Additionally, there was observed higher variety in experimental animals compared to control ones. Presented results are slightly higher compared to these noted by Potočnjak et al. (2012), but they can confirmed high health status of experimental animals. Changes in γ-globuline concentration in weaners through the nursing period look interestingly. However, they are slightly higher comparing to noted by Rząsa et al. (2007) and Potočnjak et al. (2012), dynamics of their changes confirm less activity of G and GM weaners immune system, what can be considered as a positive influence of experimental additives. Up to 45th day of life there was observed distinct increase of γ-globuline fraction in control animals and nearly constant in other experimental groups. Thanks to this lower immunological stimulation probably there were observed better production results in both experimental groups measured by body weight. Additives decrease also stress connected with change of feed, relocation of animals and other environmental factors (Pereira et al. 2012).

Some researchers (van der Peet-Schwering et al. 2007, Shen et al. 2009) demonstrated that the addition of yeast or yeast and cell wall product containing mannan oligosaccharides to weaning pig diets increases feed intake and efficiency, the others (Kim et al. 2008, 2010) had not the same observations in sow diets. In presented study was not observed statistically significant increasing of feed intake in experimental sows' group during lactation time, opposite to earlier study (Szuba-Trznadel et al. 2014) where addition 200 ppm (1,3)-(1,6)-β-D-glucans in diet of animals significantly increase feed intake in comparison to control group. In case of weaners better feed conversion ratio and higher daily weight gains was determined in treatments received additives in diet. Similar results associated with daily gains are reported in literature. Veum et al. (1995), Chau et al. (2009) Nochta et al. (2009) did not observed increasing of daily gains when they used yeast additive to piglets feed. Most authors showed the opposite results (Kim et al. 2010, Shen et al. 2011, Živković et al. 2011). Supplementation of yeast or pure mannans used in nutrition of gestating and lactating sows influenced some production results: observed greater feed intake in lactating sows, noted more born piglets that were heavier at birth and at weaning, moreover better intake of Prestarter feed during suckling period. Interesting explanation for such differences gives Kornegay et al. (1995). This author reported that the growth promoting effect of yeast may be dependent on feed ingredients. He observed increasing of average daily gain in pigs fed a feed enriched by yeast culture, when a diet contained soybean hulls and there was no such effect when they were replaced by corn – soybean meal.

It is worth to underline the positive effect of yeast derivatives on sows' body weight changes during lactation period. Lower body weight losses by experimental sows could be explained by the shorter period of negative energy balance and better appetite and feed intake. Additionally, there was observed lower variation within those groups demonstrated by lower standard deviation.

Our data showed that additives of G or GM to offspring diets significantly improved body weight and feed conversion ratio after the 45th day of life. Our previous study also demonstrated differentiation of body weight on the 45th day of life in pigs fed with a mixture with a yeast preparation (Fuchs et al. 2007). These results are similar with the observations of Dritz et al. (1995) and Decuypere et al. (1998) performed on the same age group. Very important from production point of view is observed tendency to the lower number of losses and culling during the whole rearing period in the groups obtained experimental additives. The same relationship was also demonstrated by Kim et al. (2000).

Obtained results showed that yeast derivatives can directly or indirectly influence animal performance and could be used as tools for improving the efficiency of pigs production. Observed lower immunological stimulation in weaners can influence higher production results because of higher homeostasis stability.

CONCLUSIONS

The performance results obtained and presented in this report, including body weight, feed conversion and the number of animals eliminated during the rearing period, can be explained by the addition of (1,3)-(1,6)-β-D-glucans and mannans – especially in group when the feed additives were added to the diet together.

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Streszczenie: *Wpływ zastosowania (1,3)-(1,6)-β-d-glukanów i mannanów na wyniki produkcyjne loch i prosiąt.* Celem przeprowadzonych badań była ocena wyników produkcyjnych i stanu zdrowia loch, a także ich potomstwa po dodaniu (1,3)-(1,6)-β-D-glukanów i mannanów do ich paszy. Eksperyment przeprowadzono na 90 lochach i 331 prosiętach (rasy wbp × pbz). Doświadczenie rozpoczęto w 85. dniu ciąży loch (w momencie rozpoczęcia stosowania mieszanki dla loch karmiących) i kontynuowano go przez okres odchowu prosiąt (do 90. dnia ich życia). Zwierzęta podzielono na trzy grupy. Grupy doświadczalne otrzymywały w mieszankach: β-glukan (G) lub β-glukan z mannanami (GM). Wyniki produkcyjne oszacowano na podstawie: zmian masy ciała macior w trakcie laktacji i ich potomstwa w okresie odchowu, średniego pobrania paszy, zużycia paszy oraz śmiertelności zwierząt. Aby oszacować stan zdrowia zwierząt od macior i ich potomstwa pobrano próbki krwi, w których oznaczono proteinogram (białko całkowite oraz jego frakcje: albuminy, α-globuliny, β-globuliny i γ-globuliny).

Uzyskane wyniki wskazują, że dodatek G lub GM stosowany w mieszankach dla loch karmiących i prosiąt może korzystnie wpływać na procesy katabolityczne u loch oraz wzrost i zdrowotność u prosiąt.

Słowa kluczowe: lochy, prosięta, glukany, mannany, tempo wzrostu, zdrowotność

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Nutritional value variability of different poultry species meat in the organic production system

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Abstract: *Nutritional value variability of different poultry species meat in the organic production system.* The aim of the present study was to determine the real nutritional composition for selected quality attributes of breast muscles of different organically-farmed bird species. The study covered four species of poultry – the Broad Breasted White turkey (group T), broiler chickens (group C), Muscovy ducks (group D), and Zatorska geese (group G). The animals came from an organic farm in Sukowska Wola (Poland). The number of birds raised on the farm was 80 per each species of poultry. The birds were raised in accordance with the organic farming standard for Poland sited in the Council Regulation (EC) No 834/2007. During the course of the present experiment, the species was found to have a significant ($P \leq 0.01$; $P \leq 0.05$) effect on the dressing percentage and the muscle and giblet content of the carcasses, which was in agreement with findings of other researches. The muscle and giblet contents served as reference values. The average body weight before slaughter was, by species: turkeys (T – 11.69 kg), chickens (C – 2.45 kg), ducks (D – 2.60 kg), and geese (G – 6.85 kg). The bird species kept in the ecological farm have largely influenced the quality of the pectoral muscle of the poultry. It can be seen that the turkey and chick muscles contained slightly more protein, while less collagen, compared to the group of ducks and geese.

Key words: organic system, poultry, quality of meat

INTRODUCTION

Across developed countries, there has been a noticeably rising interest in optimal consumer nutrition with a focus on animal well-being, as well as in high animal welfare standards that result in high quality products. Rising consumer expectations are increasingly drawing attention to the issue of meat quality. Among consumers, mainly centered in large cities, there is a growing interest in organically-grown products. In Poland, the intensive farming system is the most prevalent in poultry production. Alternative systems include free-range and organic farming. This creates opportunities for increased environmental awareness, biodiversity conservation, and the development of environmentally friendly production methods. Organic farming is a system of agricultural production that utilizes natural processes occurring within a farming environment. However, this requires the introduction of regulations that control all aspects of organic production, from the economics, through production technology, to animal rearing. The Act on organic farming of 28 June 2007 forms the legal basis on

this issue in Poland. The Act states that "organic stock farming should respect high animal welfare standards and meet animals' species-specific behavioral needs and animal-health management should be based on disease prevention. In this respect, particular attention should be paid to housing conditions, husbandry practices and stocking densities. Moreover, the choice of breeds should take account of their capacity to adapt to local conditions". According to the literature, meat from e.g. slow-growing chickens is characterized by higher protein content and low fat content (desirable by consumers), while prolonged rearing periods impact the concentration of chemical compounds in breast and leg muscles, which results in the more desirable odor and taste of the meat, and, as such, its better sensory attributes. Organic farming as a sector aims to ensure high animal welfare, operation devoid of negative environmental impact, and high quality products for the consumer (Vaarst and Alrøe 2002). Longer rearing periods, access to green fodder, on-farm produced feed, and high standards of animal welfare in the organic system all generate high production costs and significantly higher market prices than intensive systems (Naspetti et al. 2014). However, consumers are willing to pay the higher price for organically-grown products (Crandall et al. 2009). Prolonged rearing periods impact the concentration of chemical compounds in breast and leg muscles, which results in the more desirable odor and taste of the meat, and, as such, its better sensory attributes (Fujimura et al. 1994). In organic farming, animals are fed on-farm produced feeds or feeds purchased from other organic farms. The feed must contain no genetically-modified ingredients, synthetic amino acids or coccidiostats. It is recommended that the ratio of the open-air run area per bird should be no less than: 4 m^2 for broiler chickens, 4.5 m^2 for ducks, 10 m^2 for turkeys, and 15 m^2 for geese (Castellini et al. 2007, Pomykała 2010). At least once per year, every organic farm is subject to detailed inspection by a certification body accredited by the Polish Ministry of Agriculture and Rural Development for organic farming certification, in accordance with the PN-EN 45011 standard. These bodies are authorized to carry out inspections and issue/revoke organic farming certificates (Domagalska and Buczkowska 2015).

The aim of the present study was to determine the real nutritional composition for selected quality attributes of breast muscles of different organically-farmed bird species.

MATERIAL AND METHODS

The study covered representatives of poultry – the Broad Breasted White turkey (group T), broiler chickens (group C), Muscovy ducks (Group D), and Zatorska geese (group G). The animals came from an organic farm in Sukowska Wola (Poland). The certification body for the farm is "Ekogwarancja PTRE" PLC, whose registered office is in Lublin, conformity certificate number 434. The farm is a family business with a multi-faceted production scheme that includes poultry production. The number of birds raised on the farm was 80 per each species of poultry. The birds were raised in accordance with the organic farming standard for Poland (Council

Regulation (EC) 834/2007, Domagalska and Buczkowska 2015), which specifies that no more than 10 birds or 21 kg of live poultry can be raised per square meter of poultry house. The poultry house had a floor area of 90 m^2. The birds were fed grain-based feed from on-farm production (triticale – 35%, oats – 15%, rye – 10%, and wheat bran – 40%). In addition, birds had access to a 10,000 m^2 open-air run. The area of the open-air run conformed to the Polish regulation standard for organically reared birds. Within the open-air run area, the birds had access to green fodder which had the following botanical composition: perennial ryegrass (45%), creeping red fescue (30%), meadow grass (15%), nettle leaf (5%), and common yarrow (5%). The open-air run allowed to birds to indulge in basic instinctual behaviours such as scratching and dustbathing. The run area was dry, properly sunlit and had a permeable ground cover.

Six birds of each species (with a sex ratio of 1 : 1) were collected for the study. The age of the birds was: 18 weeks – chickens, 22 weeks – turkeys, ducks and geese. Body weights were recorded on slaughter day. The selected birds were fasted for 12 h, with constant access to water, and then slaughtered. Carcasses were chilled with the air method at a temperature of 4°C for 24 h. Then, dissection was performed following the methodology described by Ziołecki and Doruchowski (1989). Dressing percentage was calculated, i.e. content of muscles and content of giblets (gizzard, liver and heart) in respect of the body weight before slaughter. The collected breast muscles were weighed, individually marked, protected, and left for further analyses.

Chemical composition of meat

The dissected breast muscles were subjected to the chemical and physicochemical analysis. The proximate chemical composition of breast muscles were determined with standard methods: protein content – with the Kjeldahl's method using a conversion factor of 6.25 (acc. to PN-75/A-04018); and fat content – with the Soxhlet's method (acc. to AOAC, 2005).

Physical properties of meat

The pH value of meat samples was assayed according to PN-ISO 2917:2001 with a CP-411 pH-meter (Elmetron, Zabrze, Poland), using a combined glass-calomel electrode. The electrode was calibrated against buffers of pH 4.0 and 7.0.

The breast muscle was disintegrated twice in a meat grinder with a hole diameter of 3 mm and thoroughly mixed to assure homogeneity of the sample. In thus prepared sample, pH value was measured.

Water holding capacity (WHC) was determined according to Grau and Hamm (1953), three replications of muscle were tested and the average value was taken as the result.

Colour parameters (L*, a*, b*) were analysed with a Minolta CR-410 chroma meter using ground meat (wide-area illumination / 0° viewing angle). Each measurement was carried out in five replications, taking their average value as the result. Parameter L* (colour brightness) can assume values from 0 to 100. Parameters a* – redness and b* – yellowness are trichromaticity coordinates. They can assume positive and negative

values; +a* corresponds to red, –a* to green, +b* to yellow, and –b* to blue.

To determine cooking loss, breast muscles were weighed to the nearest 0.01 g (m_1), and heated in a water bath at 90°C for about 30 min (until the internal temperature reached 75 ±2°C in the geometric centre). Cooked meat was cooled at room temperature for about 1 h and moved to a cold store room (4–6°C) for 24 h, after which it was weighed again (g). Cooking loss (%) was calculated using the formula:

$$\text{cooking loss} = [(m_1 - m_2) : m_1] \times 100$$

The data obtained were analyzed statistically using a one-way analysis of variance (least squares) using SPSS 23.0 software (SPSS, Chicago, IL, USA). Differences were found significant at $P \leq 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

Dressing percentage of meat

Production performance is affected by several factors, the most significant of which are these genetic in nature, though environmental factors also do play a role (Fanatico et al. 2008). During the course of the present experiment, the genotype was found to have a significant (^{a,b} $P \leq 0.01$; ^{A,B} $P \leq .05$) effect on the dressing percentage and the muscle and giblet content of the carcasses, which was in agreement with findings of other researches. The muscle and giblet contents served as reference values. The average body weight before slaughter was, by species: turkeys (T – 11.69 kg), chickens (C – 2.45 kg), ducks (D – 2.6 kg), and geese (G – 6.85 kg). A study by Habig et al. (2017) demonstrated the body weight of 18-week-old turkeys to be lower. On the other hand, Horsted et al. (2010) reported body weight gains for New Hampshire chickens concurrent with the present study, namely 2.1–2.16 kg at 110 days of age. Finally, Castrománe et al. (2013) demonstrated the weight of organically-farmed slow-growing chickens to be 2 kg, and that of chickens from intensive farming to be 2.5 kg. Hrncar et al. (2014) found that eight-week-old ducks from caged production systems weighed 2.94 kg, and showed a different value for ducks reared in deep litter indoor housing (2.77 kg). The lower body weight of birds may be the result of their physical activity. The differences from the results of the present study may stem from the choice of genotype, nutrition and rearing method. Another research group showed that 17-week-old Zatorska geese had a body weight of 5.65 kg (Kapkowska et al. 2011).

The literature indicates a marked variance in slaughter value between different species of poultry. The analysis of the data provided in Table 1 shows that the genotype of organically-reared birds had a significant impact on dressing percentage. Group T had the highest dressing percentage, whereas group C performed the worst in this regard. Farghly and Mahrose (2017) obtained a lower dressing percentage of turkeys from an intensive farming system. This discrepancy may result from different rearing periods, as organically-reared turkeys were slaughtered six weeks later that the industrially-farmed birds. Kapkowska et al. (2011) demonstrated lower dressing percentages in younger geese as

TABLE 1. Results of slaughter analysis of poultry, least square mean values (%)

Group	Dressing percentage	Muscles		Giblets			Abdominal fat
		breast	legs	stomach	liver	heart	
T	84.22^{A}	23.75^{A}	15.13^{A}	2.85^{B}	1.08^{B}	0.34^{B}	0.78^{A}
C	70.20^{B}	14.47^{Ba}	13.46^{B}	2.61^{B}	2.26^{A}	0.39^{B}	0^{B}
D	75.54^{BAC}	17.49^{B}	8.04^{BA}	3.05^{B}	1.75^{AB}	0.87^{A}	0.52^{C}
G	78.45^{BAD}	9.49^{Bb}	8.29^{BA}	4.20^{A}	1.67^{AB}	0.60^{AB}	2.83^{BAD}
SEM	0.599	1.196	0.362	0.162	0.104	0.024	0.200

Means in a columns with different capital or lowercase letters are significantly different at ^{a,b} $P \leq 0.01$ or ^{A,B} $P \leq 0.05$, respectively.

well. However, Jankowski et al. (2017) reported a dressing percentage of 80.6% in 16-week-old turkeys. In EU Member States, the dressing percentage of broiler chickens averages 70–71%. The chickens examined under the present study showed satisfactory dressing percentage when compared to literature data. Chen et al. (2013), for instance, noted a 69.5% dressing percentage in their study, while research by Bilal et al. (2013) showed values of 64.71–66.63%, thus indicating that the farming system does have an impact on the dressing percentage of chickens. In case of geese, Boz et al. (2017) calculated lower dressing percentage and significantly higher yields of breast and leg muscles. This difference may be due to large amounts of abdominal fat having been removed along with the viscera, which could have impacted the dressing percentage and muscle yield.

For a number of years, the yield of breast meat has been noted as a factor of importance. In traditionally-reared, fast-growing chickens, the yield of breast meat may reach 30–31% and is significantly higher when compared to slow-growing chickens raised in alternative systems. In slow-growing chickens, the yield may range from 18 to 22% (Fanatico et al. 2009). Research conducted by Połtowicz and Doktor (2011) indicates that among the hybrids examined, Ross 308 rooster × Green-legged Partridge hen hybrids offered the highest yield of breast muscles (22.38% on average), whereas crossbreeding with Sussex hens produced the lowest results (17.99%). The present study indicates that geese have lower breast meat yields, while the reverse holds true for turkeys. Low leg muscle and breast muscle yields in geese carcasses results from the lack of the intensive selection for larger muscle mass that broiler chickens and turkeys were subjected to. Owens et al. (2006), Fanatico et al. (2009), Wang et al. (2009), and Połtowicz and Doktor (2011) have demonstrated that access to open-air runs has a positive effect on leg muscle yield, thus correlating this variable with physical activity of the birds. Owens et al. (2006) suggest that this may be caused by the higher levels of exercise in birds with grass-covered open-air runs. In terms of muscle yields, group D birds (ducks) from the organic farm had significantly lower leg muscle

yields than those obtained by Hrncar et al. (2014). This can be attributed to the additional physical exercise provided by the open-air runs, the long bouts of walking and the conduct of natural behaviors. Similar breast and leg muscle yields were reported by Mikulski et al. (2011).

Birds from the organic system had a higher proportion of heart muscle in the carcass compared to the results obtained by other researchers (Chen et al. 2013, Hrncar et al. 2014, Farghly and Mahrose 2017, Kjærup et al. 2017). Polak (2005) also found significant ($P \leq 0.05$) increases in the heart weight of extensively-farmed birds. The author ascribed this effect to the increased cardiac activity caused by increased physical exercise taken by chickens on open-air runs.

A significantly ($P \leq 0.01$) higher liver and gizzard weight ($P \leq 0.01$) was observed in water birds. Group G was characterized by the highest gizzard weight, which results from the specificity of the digestive tract of these birds and the feed. Many researchers suggest that the higher gizzard weight may be caused by the consumption of insects, green fodder, sand from consumed green fodder may also contribute to the increased gizzard weight (Dou et al. 2009). Amerah et al. (2007) emphasize that the muscle mass of the gizzard strongly correlates with the type of feed. In turn, Lentle et al. (2013) strongly emphasize that including pellet feeds in the diet results in a reduction of the gizzard wall.

The present study showed a lower proportion of abdominal fat in the examined bird species when compared to other studies (Mikulski et al. 2011, Chen et al. 2013, Farghly and Mahrose 2017). According to Wężyk et al. (1998), the relative content of depot fat can range from 5 to 6% of the carcass weight, and its relative percentage content in the carcass varies depending on multiple factors (feeding, sex, age, farming system). A number of researchers emphasize that alternative bird farming systems result in lower levels of abdominal fat in the poultry (Castellini et al. 2002a, b, Wang et al. 2009), as confirmed in the present study (birds were found to have low fat contents, and the chicks had no detectable fat at all). According to Lewis et al. (1997), Castellini et al. (2002a), and Mahammad et al. (2017) increased exercise of poultry specimens using open-air runs contributes to the reduced fat content. In contrast, research by Gornowicz (2009) and Połtowicz and Doktor (2011) indicates that open-air run access adversely affects the fat content, increasing its relative content in the carcass.

Chemical composition of the breast muscle

The quality of meat is a product of numerous nutritional, biological and technological parameters. Quality is additionally determined by the chemical composition and physicochemical properties of the meat, as well as by the species and breed of the bird, individual traits, sex and age. The muscle tissue typically consists of about 75% water, 20% protein, 3% fat, and 2% soluble non-proteinaceous substances. The genotype of the organically-farmed birds tended to have a significant effect on their breast meat quality. As indicated, the turkey and chicken breast meat contained slightly more protein and less collagen when compared to ducks and geese (Table 2) – both beneficial qualities from a health

TABLE 2. Chemical composition of poultry meat (g/100 g), (♀ + ♂)

Group	Breast muscles			
	water	protein	fat	collagen
T	72.25^{ABa}	24.46^{Aa}	2.04^{B}	1.07^{B}
C	74.16^{A}	23.91^{Ab}	1.40^{bB}	0.95^{B}
D	71.16^{bB}	21.97^{Bd}	2.19^{aB}	1.74^{A}
G	69.68^{B}	22.77^{Bc}	5.11^{A}	1.69^{A}
SEM	0.267	0.18	0.225	0.120

Means in a columns with different capital or lowercase letters are significantly different at ^{a,b} $P \leq 0.01$ or ^{A,B} $P \leq 0.05$, respectively.

standpoint. According to Smolińska et al. (2009), meat of gallinaceous species is characterized by higher protein content than waterfowl meat. As confirmed by the present study, chicken and turkey meat were characterized by higher protein content in comparison with duck and geese meat (Table 2). There is a known correlation between protein content and fat content (Keeton and Eddy 2004). It was therefore expected that, when compared against waterfowl meat, the lower fat content of gallinaceous poultry meat (due to higher levels of exercise) would result in higher protein content.

Qiao et al. (2001) report that raw meat poultry has a water content of 75%. According to Słowiński and Mroczek (1997), the average water content of chicken breast muscle is 71.7–74.9%. In contrast, Kauffman (2001), as well as Keeton and Eddy (2004) claim that chicken meat contains 60–75% water, but this indicator varies greatly from muscle to muscle. The water content of meat is important, as it determines its storability as microorganisms grow rapidly in heavily-hydrated environments (Küçükyılmaz et al. 2012). Mean results of physical analysis of breast meat differed greatly across the groups. The present experiment showed that geese meat had the lowest (69.68%), while chicken meat had the highest water content (74.16%). Kralik and Kralik (2017) found that intensively-farmed chickens had a higher content of water in breast muscle, namely 75.28–76.01%. On the other hand, Küçükyılmaz et al. (2012) found that meat from slow-growing, organically-reared chickens had a higher water content (72.9%) compared to chickens raised in conventional systems (74.1%). Oblakova et al. (2016) analyzed the water content of turkey breast meat and showed its value to be higher for intensively-farmed turkeys (72.72 vs. 73.77%). Similarly, breast meat from ducks was found to have a higher water content compared to organically-reared birds (Wang et al. 2016). The results of the water content of goose breast meat (69.68%) obtained in our study were similar to those obtained by Boz et al. (2017) – 70.7%.

Breast meat, particularly chicken breast meat, is generally considered to be low fat (Parkhurst and Mountney 1988, Fanatico et al. 2007). Leg muscles, on the other hand, contain more fat than breast

muscles (Castellini 2002a) – however, fat enhances the taste and improves the sensory attributes, juiciness and tenderness of the meat. It is important to note that lower fat content, despite being a desirable trait for consumers, diminishes the sensory attributes of meat, though it positively influences its dietary value (Łukasiewicz et al. 2009), while also improving the flavor of the meat of, for example, slow-growing chickens (Culioli et al. 1990). Fat is necessary for maintaining proper body function, protects against heat loss, and is essential for the absorption of fat-soluble vitamins. In the present experiment, the average fat content in breast muscles was: 2.04% in turkeys, 1.40% in chickens, 2.19% in ducks, and 5.11% in geese. The fat content in chickens was significantly lower than that reported by other researchers (Küçükyılmaz et al. 2012, Chen et al. 2013, Kralik and Kralik 2017). This may be due to the higher degrees of physical exercise in the examined birds and the lower content of abdominal fat, resulting in reduced adiposis around muscles and organs. This trend is also evident in turkeys (Sarica 2011, Oblakova et al. 2016). However, the breast muscle has a higher fat content in waterfowl in general and in geese in particular.

Physicochemical analysis of breast muscles

Water holding capacity (WHC), pH and the rate of cooking loss all have an effect on the technological value of meat (and, as such, also bear indirectly on the quality of the processed products). Value of pH of the meat is related to its water holding capacity. Water holding capacity decreases at lower pH values, while the water holding capacity of muscle proteins increases with pH (Połtowicz 2000). The pH of the breast meat was found to be in the 5.33–5.38 range and was not influenced by the genotype. Castromán et al. (2013) also achieved lower pH values for meat from organically-farmed birds when compared with conventional farming. This trend is corroborated by other studies (Kapkowska et al. 2011, Sarica et al. 2011, Chen et al. 2013, Oblakova et al. 2016, Wang et al. 2016). The present study has shown that the genotype has a significant effect on the cooking loss and WHC of breast muscles. The highest cooking losses were observed in group C, while the least ones in group T. Both values were statistically significant ($P < 0.01$). Group C had the highest WHC value (9.46 cm^2/g), followed by group G (8.53 cm^2/g), group T (5.79 cm^2/g), and finally group D (2.90 cm^2/g) – Table 3. The extent of muscle mass loss or cooking loss has a significant effect on the sensory attributes, particularly palatability and juiciness. According to Bielański (2004), mass loss (cooking loss) measured as the amount of weight loss after thermal processing, are significantly higher in meat with low WHC, resulting in a less juicy finished product. In addition, lower water holding capacity of meat results in a diminished processing value and lower quality of the resultant meat product. Changes in water holding capacity are closely linked to the rate of the post-slaughter processes and the rate of the pH decrease. The tenderness of meat is partly related to glycolysis and pH decrease, but it is also dependent on proteolytic enzyme activity and other factors. The tenderness and the WHC of meat are among the

TABLE 3. Physicochemical properties of poultry meat (♀ + ♂)

Group	Breast muscles		
	pH_{24}	cooking loss (g/100 g)	WHC (cm^2/g)
T	5.38	6.13^{B}	5.79^{b}
C	5.38	13.49^{A}	9.46^{aA}
D	5.37	11.60^{A}	2.90^{aB}
G	5.33	11.52^{A}	8.53^{aA}
SEM	0.034	0.700	0.900

Means in a columns with different capital or lowercase letters are significantly different at ^{a,b} $P \leq 0.01$ or ^{A,B} $P \leq 0.05$, respectively.

most important determinants of sensory quality of meat as perceived by consumers. It has also been demonstrated that a higher fat content of meat (15%) reduces the mechanical extraction of water from the muscle fibers during heating and chewing – thus improving juiciness and tenderness. While the water content itself does not always correlate with juiciness, meat with a high water holding capacity is more juicy than meat that retains water poorly. Therefore, poultry leg meat is perceived to be more juicy than poultry breast under organoleptic examination – a result of the higher fat content and water holding capacity. According to the results of the present study, chicken meat had the superior WHC values, while turkey meat performed the best in terms of cooking loss. Chen et al. (2013) demonstrated lowered cooking loss in meat from birds with access to open-air runs, i.e. 17.5% for intensively-farmed chickens and 17% for free-range chickens slaughtered at 70 days of age (Chen et al. 2013). The results obtained in our study (13.49) show lower mass loss values, a trait desirable to consumers. Hashim et al. (2013) calculated a cooking loss at 33.9%, which diverges greatly from the results of the present study. This may stem from the texture of the meat. Meat from birds reared in intensive farming systems is delicate and tends to release water more readily. Lee et al. (2013) also demonstrated a cooking loss of over 30% in duck meat (31.52 and 31.8). Kirmizibayrak et al. (2011) achieved similar results for geese. The results in regards to WHC differ slightly between the present study and other research. Lee et al. (2013) obtained insignificantly higher results in ducks. Sarica et al. (2011) found no effect of the farming system on the WHC index. WHC results from Kapkowska et al. (2011) and Kirmizibayrak et al. (2011) were higher than these obtained in the present study. A possible cause is the lower muscle water content within the examined groups, which could have contributed to lower WHC values.

Colour parameters of poultry meat (breast muscles)

Consumers assess the freshness, and even the quality of meat, by means of visual inspection. Acidic conditions (pH 5.9–6.2) stabilize favourable colour properties in meat. Less acidic conditions (pH 6.3–6.4) stimulates deoxygenation of

oxymioglobin back to deoxymyoglobin, resulting the meat turning a dark red tint typical of DFD meat. The brighter colour results from the surface of open-structured meat reflecting more light as compared with the tight structure of the DFD meat. A statistical analysis of the colour measurement results showed differences in the L* values of breast muscles for the examined groups (Table 4). The colour of the meat depends on the heme pigment concentration, the pH value, and other factors. The pH value is often correlated with colour changes in poultry meat, particularly in the breast muscle. Breast muscles of ducks had the highest value of red saturation (the a* parameter), followed by geese. Both values were statistically significant. The b* parameter indicates the ratio of yellow and blue coloration. The results obtained for this parameter indicate different levels of yellow saturation of the examined poultry, at a statistically significant level. According to Kirkpinar et al. (2001), lower values of L* and higher values of a* and b* indicate more favourable colour parameters for broiler chicken meat. The colour of fresh meat carcasses is an important commercial feature and a primary mean of evaluation for consumers. The presence of heme pigments and the resulting hue of poultry meat is a product of numerous factors and depends primarily on the bird species, sex, age, feeding, muscle type, degree of exercise during life, and the degree of exsanguination (Fanatico et al. 2007). The colour is also affected by the fat content, the muscle tissue structure and active acidity (pH) of the meat (Połtowicz 2003). The colour of the meat indicates its suitability for culinary purposes (Połtowicz 2003) and derives from myoglobin content. The level of myoglobin in meat fluctuates significantly, and with it – the intensity of the red hue. Castromán et al. (2013) found that chickens from organic farming had brighter breast muscles (L* 57.7) as compared with conventionally-farmed chickens (L* 53.3). In contrast, Chen et al. (2013) found that meat from chickens with open-air run access was darker by

TABLE 4. Color parameters of poultry meat (♀ + ♂)

Group	Breast muscles		
	L*	a*	b*
T	48.33^{A}	7.29^{A}	4.58^{B}
C	48.97^{A}	4.76^{B}	3.93^{B}
D	26.06^{B}	19.25^{Ba}	4.06^{B}
G	34.26^{BA}	17.40^{BAb}	8.02^{A}
SEM	0.609	0.489	0.539

Means in a columns with different capital or lowercase letters are significantly different at ^{a,b} $P \leq 0.01$ or ^{A,B} $P \leq 0.05$, respectively.

L* – the axis of lightness L* is perpendicular to the hue plane and cuts it through at the site of crossing with axis a* and b*. The L* values range from 0 (black) to 100 (white), and between them there are all hues of grey; a * – represents colors from green (–a) to red (+a); b * – represents colors from blue (–b) to yellow (+b).

1.2 points of brightness. Küçükylmaz et al. (2012) reported brighter breast muscles (higher L* spectrum values) in birds reared in the organic system when compared to the conventional system. In case of turkeys, the results of the present study differ from these obtained by Sarica et al. (2011). The examined turkeys had darker meat than these produced in the intensive farming system. The results obtained for brightness differ from findings of other authors (Kirmizibayrak et al. 2011, Lee et at. 2013). The lower (darker) L* brightness axis values may be due to the greater concentration of myoglobin in the muscle, which results in a higher value on the a* axis and shift to higher red saturation. The results of the present analysis differ from those presented by other researchers. Castromán et al. (2013) show higher red colour saturation when compared with the present experiment. Yellow spectrum saturation (b*) was found to be lower. Numerous authors report higher values of this parameter (Kirmizibayrak et al. 2011, Castromán et al. 2013, Chen et al. 2013, Oblakova et al. 2016). This may be the result of low muscle fat content and the high concentration of myoglobin in the meat.

CONCLUSION

Summing up the results of the production and the quality of the meat, the following conclusions can be drawn: Ecoproducts are a valuable source of animal protein. In addition, slower-growing poultry characterized by the close involvement of the chest muscles and the fast-growing legs may be intended for the production of whole carcasses for consumation.

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Streszczenie: *Zmienność wartości odżywczej mięsa różnych gatunków drobiu utrzymywanych w ekologicznym systemie produkcji.* Celem badania było określenie rzeczywistego składu odżywczego dla wybranych cech jakości mięśni piersiowych różnych gatunków ptaków hodowanych w gospodarstwie ekologicznym. Badaniem objęto czterech przedstawicieli drobiu, tj.: indyk biały szerokopierśny (grupa T), kurczęta mięsne (grupa C), kaczki piżmowe (grupa D) i gęsi zatorskie (grupa G). Zwierzęta pochodziły z gospodarstwa ekologicznego w miejscowości Sukowska Wola. Liczebność każdego gatunku drobiu w gospodarstwie wynosiła 80 sztuk. Ptaki utrzymywano zgodnie z polską normą dla gospodarstw ekologicznych według Rozporządzenia Rady (WE) 834/2007. Zaobserwowano istotny wpływ ($P \leq 0,01$; $P \leq 0,05$) genotypu na wydajność rzeźną oraz udział mięśni i podrobów w tuszce ptaków. Średnia masa ciała przed ubojem poszczególnych gatunków wynosiła odpowiednio dla indyków (T – 11,69 kg), kurcząt (C – 2,45 kg), kaczek (D – 2,6 kg) i gęsi (G – 6,85 kg). Genotyp ptaków utrzymywanych w gospodarstwie ekologicznym przeważnie wpłynął w istotny sposób na jakość mięśni piersiowych drobiu. Można zauważyć, że w porównaniu z grupą kaczek i gęsi mięśnie piersiowe indyków i kurcząt zawierały nieco więcej białka, ale mniej kolagenu.

Słowa kluczowe: ekologiczny system chowu, drób, jakość mięsa

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Induced gynogenesis as potential tool for detection of receive deleterious mutations carriers in salmonids fishery

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Abstract: *Induced gynogenesis as potential tool for detection of receive deleterious mutations carriers in salmonids fishery.* Eight rainbow trout females were tripped and the eggs were subjected to induced gynogenesis procedure. Approximately half of three female's progeny showed type of malformations occasionally observed in rainbow trout stock kept in Rutki Station. The case study describes the potential usefulness of gynogenesis as breeding test to reveal carriers of harmful recessive alleles.

Key words: induced gynogenesis, unconventional gene mapping, breeding test

INTRODUCTION

The induced gynogenesis as a part of so called chromosomal engineering, i.e. influencing on the level of ploidy and origin of chromosomes, is an old field of studies. Hertwig (1911) run a course of experiments fertilizing frog's eggs with semen subjected to increasing doses of UV radiation in order to establish the correlation between the mortality and UV dose. When the mortality reached 100% level, he kept continuing his trials still increasing the UV dose. Unexpectedly he started to obtain normal embryos. The conclusions were as follows: as long as the destroyed by UV paternal chromosomes were present, they caused abnormal (fatal) development of zygotes. Only after total destruction they did not influence the development anymore and zygotes could continue the normal mitotic single divisions with presence of maternal, i.e. intact chromosomes. The totally inactivated spermatozoons started second meiotic divisions and expelling the second polar bodies from egg cells, but did not contribute genetic material which was destroyed. Finally gynogenetic haploid embryos were obtained and some of them even reached the adult stage.

Kawamura (1939a, b, c) fertilized eggs with inactivated semen additionally applying thermal shocks preventing expelling the second polar bodies. Thus he obtained gynogenetic diploid frogs.

The experiments mentioned above were followed up in aquaculture field. Usage of normal (not inactivated) semen together with thermal shocks lead to obtain triploid spine stickleback fish *Gasterosteus atus* (Svarup 1959). That pilot approach was continued with consumption fish. Golovinskaya (1968) got gynogenetic diploid carps *Cyprinus*

carpio. Purdom (1970, 1976, 1993) laid the foundation of applying gynogenesis in flatfish culture. Chourrout (1980) first succeeded in producing gynogenetic rainbow trout *Oncorhynchus mykiss* and four years later the same author published paper (Chourrout 1984) showing the advisability of replacing thermal shocks with hydrostatic pressure. This method has been found to be very effective improving both the percentage of arrested anaphases – particularly those of first mitosis – delayed shock and general survival of treated eggs. Many other experiments carried out gathered knowledge of details in induced gynogenesis procedure applied to different fish species – moment of starting the shock after fertilization, it's strength and duration time – for revue see Ihssen et al. (1990). Peruzzi and Chatarin (2000) produced ginogenetic European sea bass *Dicentrarchus labrax* L. comparing effects of temperature and pressure shocks.

In Salmonid Reaserch Laboratory Rutki (experimental station of Institute of Inland Fishery in Olsztyn) the selection program of rainbow trout was started in early 1990s (Dobosz et al. 1992, Życzyński et al. 1995). Broodstock consisting of different imported lines after crossing was divided into families evaluated after family selection performed in each generation.

The initial aim of the study was a comparative evaluation of gynogenetic progeny of broodstock females, originating from different strains. This strains have been previously evaluated on progeny obtained in traditional biparental way (Dobosz et al. 1992).

MATERIAL AND METHODS

Eight females from different strains crosses were stripped and eggs were mixed with milt of randomly chosen male. The milt was inactivated according to procedure described by Goryczko et al. (1991). Fertilization was done in 10°C. Heat thermal shock (28°C) lasting 20 min was applied 20 min after adding inactivated semen to eggs. The insemination was done in 10°C. After finishing the shock, the eggs were transferred to eight trays and placed in incubator. The hard roe was seen to in the routine way for rainbow trout and passed eyed, hatching and swim up stages. Having started to feed actively the alevins were transferred into eight tanks with capacity of 1 m^2 each. Mortality during growing period was not registered.

RESULTS AND DISCUSSION

The fish were screened at the parr stage. It was noted that in three out of eight tanks many malformed fish were found (in roughly equal proportion to normal shaped ones). Among the progeny of remaining five females only normal fish were observed. Karyological analysis of malformed fish samples revealed some additional chromosome arms (NF value amounted to 106), but for the sake of low quality of metaphase spreads the data are omitted.

The first suggestion to explain the presence of malformed fish seemed to be obvious and follows the experience of Hertwig (1911) mentioned above. Non-adequate (not sufficient) inactivation of semen caused aneuploidy of fish

what could be confirmed by presence of extra chromosomes arms in metaphase spreads. Eggs of all eight females were treated with the same sample of semen, so malformed fry should be found in all tanks, not just in three of them.

It has to be mentioned that such malformations were occurring in Rutki Station. Almost each year few specimens of this kind were noted. These fish – flattened with curved spine (Fig. 1) – grow much slower comparing to normal ones and are unable to swim against water current, spending most of their time lying flat on tank's bottom. But for the first time such a big number of malformed fish was found. The presence of so numerous abnormal fish and lack of properly recorded data concerning mortality in all experimental tanks ruined the designed scenario of whole work. Instead of carrying out comparative analysis of progeny groups performance we focused on malformed fish, trying to explain situation encountered.

The proposed explanation of this event is as follows. The malformation is caused by recessive mutation and it's presence was revealed by gynogenesis procedure.

Purdom (1970) concluded the induced gynogenesis can be an useful method to obtain totally inbred lines – much quicker comparing to standard mating of closely related animals. Just to remind – attaining the 100% coefficient of inbreeding – total homozygosity by mating full siblings takes twenty generations (Falconer and McKay 2009). In case of gynogenesis like in self-fertilization rapid increase of inbreeding is expected.

Anyway, the gynogenesis described here (which is based on retention of second polar body and the final product – diploid zygote) consists of double set of sister chromatids – no second anaphase. Sister chromatids are identical by definition provided crossing over has not occurred. In case of recombination, exchanged fragments from both

FIGURE 1. Malformed progeny obtained after gynogenesis (photo by author)

homologous chromosomes can contain different alleles from both female's parents. If mother is heterozygous in *loci* mapped on chromatin fragments subjected to recombination, chromosome after first anaphase will contain different sister chromatid's in fragments of interest (after c/o). Thus gynogenetical progeny remains heterozygous in these regions.

These phenomena allows to run so called unconventional gene mapping. The idea of unconventional mapping is straightforward. Heterozygous female is stripped and the eggs after mixing with inactivated semen are subjected to gynogenetic shock to restore diploids by retention of second polar body. The genotypes of gynogenetic fry are screen. The proportion of heterozygous fish in *loci* studied shows the distance between these *loci* and centromere of chromosomes where they are located on (Thorgard et al. 1983). These authors, however, in case of rainbow trout, during meiotic division always just one and no more chiasma can be observed on bivalents. This means total interference, probably due to very small sizes of fish chromosomes – not enough place for multiply recombination. The chiasma itself is located approximately in the middle of chromosome's arm, between the centromere and telomere, so during every meiotic division in each bivalent always one and not more crossing over takes place.

As the result in gynogenetic fish about half of the genome is homozygous (*loci* located between centromeres and chiasmas) and the other half (*loci* between chiasmas and telomeres) remain heterozygous, if their mothers were heterozygous. As the place of the chiasma is more or less constant, gynogenetic fish have coefficient of inbreeding approximately equal to 50% and this value cannot be risen by repeating induction of gynogenesis. Contrary to coefficient of inbreeding obtained by mating of siblings it does not increase in subsequent gynogenetic generations. Thus Purdom's idea of getting homozygous lines by gynogenesis is not realistic in case of rainbow trout.

However, the case described herewith suggests another application of gynogenesis, to our best knowledge not noticed, and explored in fish breeding so far. It is quick and reliable test for carriers of harmful recessive genes as they are revealed in homozygous state at gynogenetic progeny. Of course it must be reminded once again that the test stands only for loci not involved in obligatory crossing over, i.e. situated in regions between chiasmas and centromeres. But in spite of these limits half of the genome is thus scanned and checked what can prevent spreading harmful alleles to next generation.

So if the heterozygous *locus* is mapped on the region not involved in the crossing over, after gynogenesis two types of homozygous (recessive and dominant) will be obtained in equal proportion of 1 : 1 (Fig. 2). If the heterozygous *locus* of interest is subjected to crossing over, only dominant phenotypes will be obtained 100% heterozygous. Thus the presence of recessive allele is not detected. As it has been mentioned, in the case described herewith, the mortality of fry in all eight tanks has not been recorded. However the approximate proportion of malformed fish to normal judged as

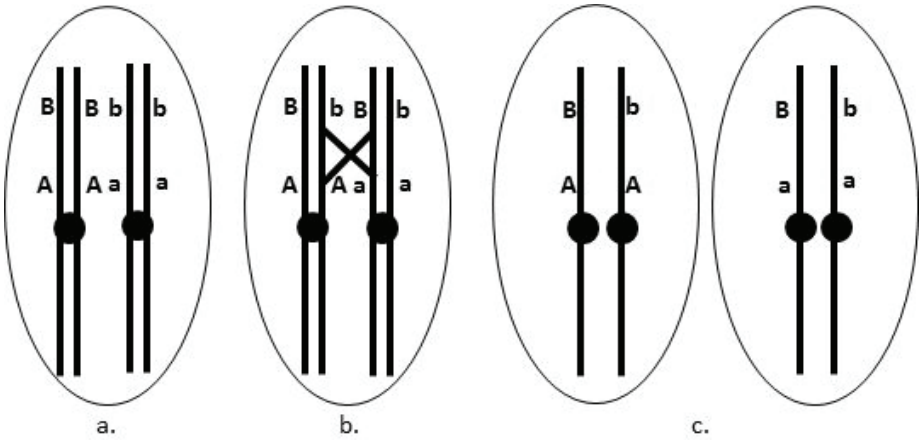

FIGURE 2. Scheme of meiotic during gynogenesis: a – one bivalent (tetrad) with two heterozygous *loci* (AB//ab) 2n, 4C; b – the same bivalent after crossing over 2n, 4C. On each homologue in the region between centromere and chiasma two identical copies of gene remain (AA//aa). In region between chiasma and telomere – two different copies of gene *B* (Bb//Bb); c – two egg cells after gynogenesis. No contribution of semen chromosomes. Retention of second polar body restores diploidy 2n, 2C. In regions between centromere and chiasma homozygosity (A/A; a/a) and in region between chiasma and telomere, heterozygosity (B/b; B/b). Half of individuals express recessive phenotype resulting from a/a configuration (own elaboration)

1 : 1, can be safety treated as reliable, even without the exact data.

CONCLUSIONS

It is recommended to test by gynogenesis broodstock females used in long-term selection program as well as in production stocks. So far this advantage of induced gynogenesis, following the results described here was applied in Rutki Station, and the families potentially carrying described mutation were culled out of selection program. Thus the induced gynogenesis (second polar body retention) in case of rainbow trout breeding, offers the possibility of testing 50% of genome for presence of recessive alleles (including deleterious ones), in one go. Dispite the fact, that the other half of the genome is not checked, it is still a big advantage in good evaluation of breeding animals. To our best knowledge this aspect of gynogenesis is deserves both wide popularization and application as it has been already done in Rutki Station.

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Streszczenie: *Indukowana ginogeneza jako potencjalne narzędzie detekcji nosicielstwa szkodliwych nośników mutacji w akwakulturze łososiowatych*. Potomstwo trzech ginogenetycznie rozmnożonych samic pstrąga tęczowego wykazało deformacje pokrojowe, które sporadycznie były widywane w stadzie produkcyjnym i hodowlanym pstrąga w Pracowni Hodowli Ryb Łososiowatych Rutki (IRŚ). Zabieg ginogenezy prowokowanej okazał się tym samym narzędziem do wykrywania nosicielstwa recesywnych alleli. Skuteczność tego zabiegu ogranicza się jednak do *loci* mapowanych w rejonach między centromerami a telomerami chromosomów.

Słowa kluczowe: indukowana ginogeneza, niekonwencjonalne mapowanie, genetyczne, test hodowlany

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