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Contents

- BRZOZOWSKI M., GŁOGOWSKI R., GRZESZCZAK-PYTLAK A. Reproductive efficiency of mink females, selected for weaned litter size after first season of reproduction 5
- CHALONY L., GRODZKI H., ŁADOŃ Ł. Assessment of usefulness of Ovultral for the improvement of dairy cows fertility effects 9
- CHRZANOWSKI Sz., MAŚKO M. The evaluation of the scores obtained by mares at stationary performance tests at (Polish) Training Stations in 2007–2011 17
- CHRZANOWSKI Sz., MAŚKO M., OLEK-SIAK S. The evaluation of the results of field performance tests for mares conducted in 2002–2011 23
- DAMAZIAK K., MICHALCZUK M., SIEN-NICKA A. Effect of turkeys genotype on results on their slaughter performance 29
- DZIERŻANOWSKA-GÓRYŃ D., GRZESZCZAK-PYTLAK A., GÓRAL K. The effect of herbal formula on the amount of water intake by the chinchilla (*Chinchilla laniger*) 39

- GAJEWSKA J., RIEDEL J., BUCKA J., ŻABIK A., MICHALCZUK M. Influence of prebiotics and butyric acid on the composition of intestinal microflora of broiler chickens 47
- HONKO S., MADRAS-MAJEWSKA B., ZAJDEL B., ŚCIĘGOSZ J. A comparison of honey production in bee colonies with instrumentally inseminated and naturally mated queens 55
- JARMUŁ-PIETRASZCZYK J., TUMIALIS D., IGRAS J., KLIMASZEWSKI K., KAMIONEK M., MAZURKIEWICZ A. The occurrence of entomopathogenic fungi in two catchment basins of the Zagożdżonka and Zwoleńka Rivers in Masovian Province, Poland 59
- KUCZYŃSKA B., PUPPEL K., KLIŚ P., GOŁĘBIEWSKI M., KORDYASZ M., WÓJCIK A., NAŁĘCZ-TARWACKA T. Changes in concentration of saturated fatty acid in milk of high performance Polish Holstein-Friesian cows during the lactation 65
- ŁOZICKI A. Effect of beef from different fattening systems and fish meat on the selected indicators of diet utilization and health state of rats as model animals 73
- MICHALCZUK M., ŁUKASIEWICZ M., NIEMIEC J., PIETRZAK D., WALAS E. Effect of genetic stock on production results and growth rate of chickens 81
- MICHALCZUK M., ŁUKASIEWICZ M., NIEMIEC J., WNUK A., MROCZEK-SOSNOWSKA N. Effect of L-carnitine on performance and dressing percentage of broiler chickens 89
- MORALES VILLAVICENCIO A., NIŻNIKOWSKI R. Preliminary characterization of the structure of race, sex, and color of the Polish population of alpacas 101
- PRZYSUCHA T., GRODZKI H., GOŁĘBIEWSKI M., ŚLÓSZARZ J., PIOTROWSKI T. Analysis of body measurements and pelvic area index of Limousine cows 107
- RADZIK-RANTA A., KUŹNICKA E., RANT W. The fatty acid composition of *longissimus dorsi* muscle of Polish Lowland ram lambs fattening under overhead shelter versus those in a barn 113
- RADZIK-RANTA A., ROZBICKA-WIECZOREK A., RANT W., CZAUDERNA M., KUCZYŃSKA B. The content of *cis-9, trans-11* CLA isomer determined by two methods in ewe's milk fat 119
- SAWOSZ F., PINEDA L., HOTOWY A., JAWORSKI S., PRASEK M. Influence of Ag nanoparticles, ATP and biocomplex of Ag nanoparticles with ATP on morphology of chicken embryo pectoral muscles 127
- WIERZBICKI M., SAWOSZ E., GRODZIK M. Caveolin-1 localization in chicken embryo chorioallantoic membrane treated with diamond and graphite nanoparticles 133
- WÓJCIK A., NAŁĘCZ-TARWACKA T., GOŁĘBIEWSKI M. Effect of housing system and season of birth on the calves rearing results 139

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Reproductive efficiency of mink females, selected for weaned litter size after first season of reproduction

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Abstract: *Reproductive efficiency of mink females, selected for weaned litter size after first season of reproduction.* The aim of this study was to evaluate the reproduction results of mink females, selected for the number of kits weaned in their first reproductive season. The analysis of the reproductive efficiency in two following years indicates that such criterion of mink females selection was apparently ineffective. Slightly increased litter size in the second year was followed by significant decrease in the third cycle of reproduction.

Key words: mink, litter size at weaning, reproductive efficiency.

INTRODUCTION

The profitability of mink production is strongly affected by reproduction efficiency. It grows when the number of weaned kits from the female increases, therefore the control and management of the reproduction are essential in the development of the fur animal sector.

The number of born kits does not determine the survival rate at 6th week of life. The mortality of mink kits in the first few months of life can be significant, and the farm practical observations show that smaller litters have higher weaning rate than those of larger size. (Hansen and Berg 2008, Koivula et al. 2008, Lafevre and Murphy 2008, Rozempolska-Rucińska et al. 2000).

The aim of this study was to evaluate the effectiveness of the mink selection criterion based on the number of weaned kits from the primiparous females by means of the analysis of the reproduction in the following years.

MATERIAL AND METHODS

The breeding records of 90 mink females were analyzed in three consecutive years on the farm located in north-central Poland.

The major criterion of selection for primiparous females was the number of kits weaned. Four or more young minks had to be separated from a female to include her in the breeding group in the next year.

The following reproduction indices were analyzed:

- litter size at birth,
- the rate of mortality in the 42nd day of life,
- the number of weaned kits.

Results in tables are presented as means \pm standard deviation. The significance of differences was evaluated with Duncan test, using Statistica ver. 20 software (StatSoft, Poland).

RESULTS AND DISCUSSION

Table 1 presents the general reproduction results of the analyzed reproductive seasons.

TABLE 1. The reproduction results of the mink females in the analyzed group

Index	Season		
	I	II	III
Litter size at birth	634	686	624
Stillborn	34	47	57
Kits that died before 42nd day	15	30	54
Weaned kits	585	30	54

The average litter size in primiparous females was 7.2 (with the highest number of 12), where 0.51 were stillborn kits. The average number of weaned kits were 6.3 (11) with the average mortality of 0.36.

Similar weaning results were reported by Rozempolska-Rucińska et al. 2000, Socha and Markiewicz 2001, Rozempolska-Rucińska et al. 2002 and Święcicka 2006.

It has to be noted that the average reproduction results for the whole population of primiparous minks ($n = 335$) were lower than those in the group that passed the selection criterion ($n = 90$), so for the informational reasons they were included in Table 2.

TABLE 2. The comparison of the reproduction indices of all primiparous mink and the selected, included in the analysis

Index	Total*	Selected**
Litter size at birth	6.31 ±2.34	7.04 ±1.79
Stillborn	0.71 ±1.23	0.38 ±0.80
Kits that died before 42nd day	0.43 ±1.07	0.17 ±0.50
Weaned kits	5.17 ±2.62	6.50 ±1.66

*All primiparous mink.

** Primiparous included in the analysis.

Table 3 presents the reproduction results for positively selected primiparous females in the next seasons.

TABLE 3. The results of the reproduction indices of selected females in three consecutive seasons

Index	Season		
	I	II	III
Litter size at birth	7.04 ±1.9	7.62 ±1.67 ^a	6.93 ±1.84 ^a
Stillborn	0.38 ±0.8	0.52 ±0.67	0.63 ±1.43
Kits that died before 42nd day	0.17 ±0.5 ^{A,b}	0.33 ±0.82 ^{b,c}	0.60 ±1.01 ^{A,c}
Weaned kits	6.50 ±1.66 ^d	6.77 ±1.81 ^B	5.70 ±2.47 ^{B,d}

^{A, B} – values in rows with the same letters differ significantly at $p < 0.01$.

^{a, b, c, d} – values in rows with the same letters differ significantly at $p < 0.05$.

The previously published results show that the highest results of mink female reproduction are typically noted in the second year of reproduction (Biernacka and Kubacki 1982, Socha and Markiewicz 2001, Rozempolska-Rucińska et al. 2002, Socha and Kołodziejczyk 2006). Indeed, the comparison of the first and second season revealed insignificant differences in reproduction. Interestingly, the significant decrease the efficiency of reproduction which appeared in the third reproduction season looks somehow unexpected. It may result from a low rate of heritability of traits related to reproduction. Kuźniewicz and Filistowicz (1999) reported that heritability index mink litter size at birth is 0.20, and at weaning – 0.24. The authors also indicate that the reproductive performance depends largely on environmental factors (nutrition, facilities, weather conditions) and possibly environmental factors may be

the cause of the high proportion of dead kits during rearing in the third reproduction season.

CONCLUSIONS

It can be concluded that the weaned litter size in the first year of reproduction appeared ineffective selection criterion and did not provide consistency of the reproduction in the following years. The explanation of decrease studied index during third reproduction season require further studies.

REFERENCES

- BIERNACKA H., KUBACKI S., 1982: Wpływ wieku i krotności krycia na plenność samic nerek standard HDI. 9: 14–16.
- HANSEN B.K., BERG P., 2008: Reduced litter size and percent kits alive is a consequence of selecting for high body weight. *Scientifur* 32: 15.
- KOIVULA M., STRANDEN I., MANTYSAARI E.A., 2008: Genetic parameters for litter size and grading traits in Finnish mink population. *Scientifur* 32: 53–58.
- KUŹNIEWICZ J., FILISTOWICZ A., 1999: Chów i hodowla zwierząt futerkowych. Wydawnictwo AR, Wrocław.
- LAFEVRE P., MURPHY B.D., 2008: Physiological constraints on litter size in mink. *Scientifur* 32: 13–14.
- ROZEMPOLSKA-RUCIŃSKA I., JEŻEWSKA G., TARKOWSKI J., ZIĘBA G., 2002: Charakterystyka poziomu cech funkcjonalnych nerek pastelowych. *Przegląd Hodowlany* 64: 11–12.
- ROZEMPOLSKA-RUCIŃSKA I., JEŻEWSKA G., ZIĘBA G., 2000: Parametry genetyczne cech rozrodu, masy ciała i okrywy włosowej nerek pastelowych. *Przegląd Hodowlany* 58: 15–16.
- SOCHA S., KOŁODZIEJCZYK D., 2006: Analiza czynników wpływających na plenność nerek standardowych i palomino. Wydawnictwo Uniwersytetu Marii Curie-Skłodowskiej, Lublin, *Annales* Vol. XXIV, 56: 403–408.
- SOCHA S., MARKIEWICZ D., 2001: Analiza czynników wpływających na plenność nerek. *Medycyna Weterynaryjna* 11: 840–843.
- ŚWIĘCICKA N., 2006: Wpływ wieku samic oraz terminu wykotu na wyniki rozrodu u wybranych odmian nerek. *Komunikaty Naukowe Polskiego Towarzystwa Zootecznego w Bydgoszczy* 71: 18–21.

Streszczenie: Wyniki rozrodu nerek selekcyjnowanych na wielkość miotu przy odsadzeniu po pierwszym sezonie rozplodowym. Celem pracy było określenie wskaźników plenności samic norczych selekcyjnowanych na wielkość miotu przy odsadzeniu w pierwszym sezonie rozplodowym. Analiza uzyskanych wyników wskazuje, że zastosowane kryterium selekcyjne nie przyniosło spodziewanego efektu. Wyniki plenności w drugim roku użytkowania okazały się lepsze, jednak nie była to różnica statystycznie istotna. W trzecim sezonie nastąpiło statystycznie istotne pogorszenie wyników odchowu.

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Assessment of usefulness of Ovulitral for the improvement of dairy cows fertility effects

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Abstract: *Assessment of usefulness of Ovulitral for the improvement of dairy cows fertility effects.* Fertility of high producing dairy cows is today a big issue for commercial farm in Europe and all around the world. This study was made on Polish Holstein Friesian cows of Agricultural Experimental Farm Wilanów-Obory (Poland) to evaluate the improvement of fertility permitted by the use of a specific supplementation (Ovulitral) given to the cows just before insemination. This specific dietary supplement is composed of trace elements, vitamins and amino-acids selected to enhance hormonal dialogue and reduce oxidation stress and hence improve fertility of cows. This experimentation has been made from October 2010 to July 2011, the 146 cows of the trial were assigned to a control/test group depending on their characteristics: lactation rank, days from calving, milk yield at peak. These cows were inseminated on natural heat by inseminators and the test group received 100 ml of Ovulitral at the insemination. Pregnancy of cows was validated by scan at 35 days after insemination. Success of insemination is about 48% on the control group versus 65% on Ovulitral group ($p < 0.05$). That improvement constitutes a significant difference. The improvement of fertility is higher for the cows in 1st and 2nd lactation. It is also interesting to point out that there is no difference of improvement of fertility between the cows inseminated before or after 80 days on the two groups on trial. These results confirm results obtained with Ovulitral on commercial farms in France.

Key words: dairy cows, fertility, dietary supplementation.

INTRODUCTION

New, more and more precious evaluation methods of breeding value and cattle selection together with an improvement of feedstuffs quality, rations composing as well as housing conditions caused a distinct increase of cows' milk yield in the recent years. In the first place it concerns the farms applying the new feeding and housing technologies and using the achievements of the world's and national genetics. In many medium and big cow herds the average lactation yield exceeds 10,000 kg demonstrating a growing tendency in subsequent years (Ocena i hodowla bydła mlecznego 2012). Unfortunately, this beneficial tendency of milk yield increase accompany some negative occurrences like cows' longevity shortening and reproduction effects worsening (Coleman et al. 1985, Bousquet et al. 2004, Krzyżewski et al. 2004). In order to break this tendency, in 2007 a new index "Production-Functionality" was introduced to breeding value evaluation. In the index milk performance traits in 50%, and not in whole as previously, influence its value. In the remaining 50% the index is influenced by functional traits, including the low inherited fertility. This low inherited fertility traits cause a low effec-

tiveness of selection in this range. Therefore farmers are to be inclined to use feeding supplements to the feedstuffs for cows in order to improve the effectiveness of the fertility rate.

In the presented research the influence of the preparation Ovultral on dairy cows fertility was examined. This special dietary supplementation was created in order to improve the fertility by stimulation of hormonal expression and oxidative stress reduction.

An improvement of fertility was proven on comparative experimentation concerning this supplementation made in several farms in France (Chalony and Michel 2009).

To validate the effectiveness of Ovultral and eliminate the influence of environmental conditions of particular barn, it was decided to conduct the research on a big herd of high yielded cows kept in the equal conditions at the Experimental Station Wilanów-Obory.

Ovultral is an unique natural feeding addition for dairy cows used just prior the insemination in order to improve the fertility rate and embryo nidation. The superior goal of use of the preparation is oxidative stress reduction by providing the suitable level of vitamin E and methionine. The preparation contains also vitamins A, B1, D3 as well as pro-vitamin β -carotene also influencing the fertility (Girard 1998, LeBlanc et al. 2004, Bulvestre 2007). Moreover it contains amino-acids such as lysine, arginine and tryptophan that are present in GnRH (Gonadotropin Releasing Hormone) (Shaver and Bal 2000, Hugentobler et al. 2007). The optimal distribution of Ovultral is 100 ml per cow.

MATERIAL AND METHODS

This experimentation was conducted on the dairy farm of Agricultural Experimental Farm Wilanów-Obory (Poland) from October 2010 to July 2011. In this farm, average milk production is about 10,000 kg per cow per year for the 360 cows milked. Cows are kept in 3 free stall barns and divided into 3 groups depending on lactation rank, milk yield and days in lactation. Cows are milked twice daily; milk yield is measured everyday by milking machine. Cows have a total mixed ration distributed daily; composition is described in crude matter for each feed. The ration consisted of the following farm feedstuffs: corn silage (26 kg), lucerne and grass silage (15 kg), siled maize corns (7 kg) and straw (1 kg). These feedstuffs were supplemented (appropriately to the yield) by a concentrate consisting of: soya, rape and wheat meals, fat component, salt and mineral-vitamin compound. The TMR is stable along the period of experimentation, so there is no bias linked to the composition of ration. Results of reproduction are improvable on this herd, the average is about 2.3 straws per pregnancy in 2010, and bulls do the service for cows at more than 4 failed insemination. Average calving-to-calving interval is about 430 days. Inseminations are made by inseminators of the farm after heat detection. Inseminations are usually made after 60 days after calving and average interval between calving and first insemination is about 90 days.

The research was carried out on 146 cows divided into 3 groups created according to the subsequent lactation criterion (the first, second and third). Within

each lactation group 2 subgroups were allocated: cows inseminated until the 80th day after calving and cows inseminated later than on the 80th day after calving. In each group one half constituted the experimental cows receiving Ovultral, and the second half cows not receiving it.

The repartition was made by the manager of the farm for each cow coming on heat and apt to the reproduction. On test group, the cows were given the quantity of 100 ml of Ovultral; Ovultral was distributed in the 4 hours before the insemination. Cows were inseminated randomly with semen of several bulls. There is no bias between semen used on the cows.

Pregnancy diagnosis was performed by ultrasound at 35 days after insemination by the vet of the farm without knowing from which group are the cows (blind test). Data were analyzed using logistic regression with Minitab (MINITAB 2000). Otherwise, Chi-square test was used to evaluate quality of grouping depending on qualitative factors (lactation rank, interval of lactation). T-test was also used to evaluate difference of milk yield at peak and at AI.

The repartition of the 146 cows was well made so that there is no difference between the two groups, as it is established in the Table 1. Cows in trial are primiparous for 35.6%, 37.0% for the 2nd lactation and 27.4% for the 3rd lactation. There is no significant difference between control and experimental groups on this criterion. The average (control and experimental group) milk yield of the cows is about 35 kg at the peak for primiparous and 48 kg for cows in 2nd lactation and 48.4 kg for cows in the 3rd. There is no significant difference of milk production

TABLE 1. Characteristics of cows in experimentation

Specification	Number of cows		Milk yield		Percentage of cows inseminated before 80 days (%)		Percentage of cows inseminated after 80 days (%)	
	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group
1 st lactation	26	26	35.1 ± 4.7 a	35.1 ± 4.9 a	62	62	38	38
2 nd lactation	28	26	48.5 ± 6.7 b	47.2 ± 7.2 b	43	42	57	58
3 rd lactation	19	21	50.3 ± 8.5 b	46.5 ± 6.7 b	42	48	58	52
×	Chi-2 test: P = 0.91		T-test:		Chi-2 test:		T-test: NS	

a, b, c – statistical significance at $P \leq 0.05$.

between control and test group. There is no significant difference between control and test group concerning interval between calving and insemination. The figures are proving that the grouping is well balanced between control and test group. Cows in experimentation were inseminated from October 2010 to July 2011; scans for control of gestation were done on the same period. The average date of insemination is similar in the two groups for each lactation rank.

RESULTS AND DISCUSSION

The logistic regression analysis of results is very interesting to class the factor by rank of influence (Table 2). The two preponderant criteria are milk yield at the AI and Ovulitral distribution. These factors have a significant effect with a *p*-value very closed to 5%. Ovulitral effect is positive for the fertility rate in first AI. In contrary, milk yield has a negative effect on fertility rate. Interval from insemination to calving and lactation rank are not significant in this regression analysis, but these factors are also linked to milk yield.

As the grouping is well balanced, it is possible to analyze the results individu-

ally on these criteria. First significant factor is milk yield; fertility rate decreases with milk yield at AI that confirms conclusion of several studies (Mulligan et al. 2007, Balendran et al. 2008). Pregnancy in 1st AI decreases of about 5 percentage points for 5 kg of milk. Hence, the regression indicates a fertility of 70% for cows producing 25 kg per day at the AI, and at 50 kg per day the fertility is about 45%.

The second significant criterion is the Ovulitral distribution (Fig. 1). There are 48% of pregnancies after the 1st insemination on the control group, this result is coherent comparing to results of fertility obtained in fields on Polish Holstein Friesian cows. Results on the Ovulitral group is higher than control group and reach 64.4% of success. This significant improvement of fertility is interesting, with a gap of more than 15 percentage points between the two groups. This evolution is in concordance with results obtained in other trial designed on multiple farms (Piron 2005, Chalony and Michel 2009).

Grouping made for the trial has been proven to be not biased between control and Ovulitral group, so that average can be interpreted directly. But cows on trial have some different characteristics, and results depending on these criteria are also interesting to be studied, even if it is not the major factors.

Lactation rank is a non-significant criterion in the logistic regression, but it is correlated to the milk yield of the cows and usually has a depreciative effect on fertility (Balendran et al. 2008). It is interesting to point out that milk yield is also linked to parity of cows, i.e. first lactation cows have a lowest milk yield.

TABLE 2. Results of logistic regression

Factor	Regression coefficient	<i>P</i> -value
Ovulitral	0.65	0.058
Milk yield at AI	-0.049	0.053
AI before 80 days after calving	0.278	0.432
Lactation rank	-0.01	0.979
Constant	1.658	0.052

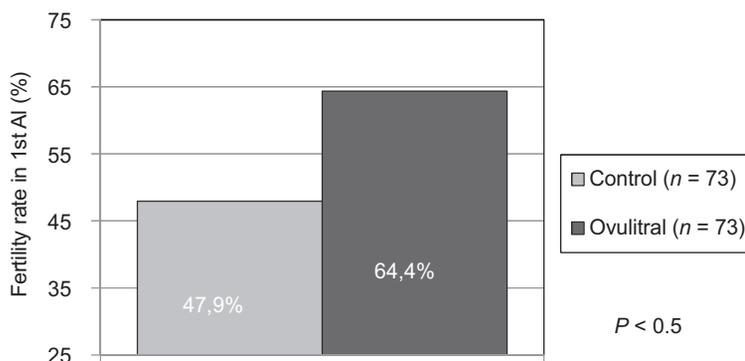


FIGURE 1. Results of cows fertility

Improvement of fertility rate on 1st and 2nd lactation is very important on Ovultral group – respectively 31% and 23% (Fig. 2). The difference between control and Ovultral groups is superior to 20 percentage points. Otherwise, on 3rd lactation the result is not interesting.

The interval between calving and insemination is known in bibliography to be important on fertility rate, this criteria is linked to the milk yield (Baldi et al. 2000, Caldwell 2003, Ponsart et al. 2007). On this experimentation, fertility on control group is about 50% on cows

inseminated before 80 days and also on cows after 80 days (Fig. 3). Pregnancy rate after first insemination in control group, amounting to about 50%, is commonly achieved in majority of herds in Poland (Krzyżewski et al. 2004, Olechnowicz and Jaśkowski 2011). It indicates, that the herd, in which the research was conducted, is a good reference point for Ovultral effectiveness evaluation. In Ovultral group, fertility is about 68% before 80 days after calving, corresponding to an improvement of 18 percentage points of fertility. On cows inseminated

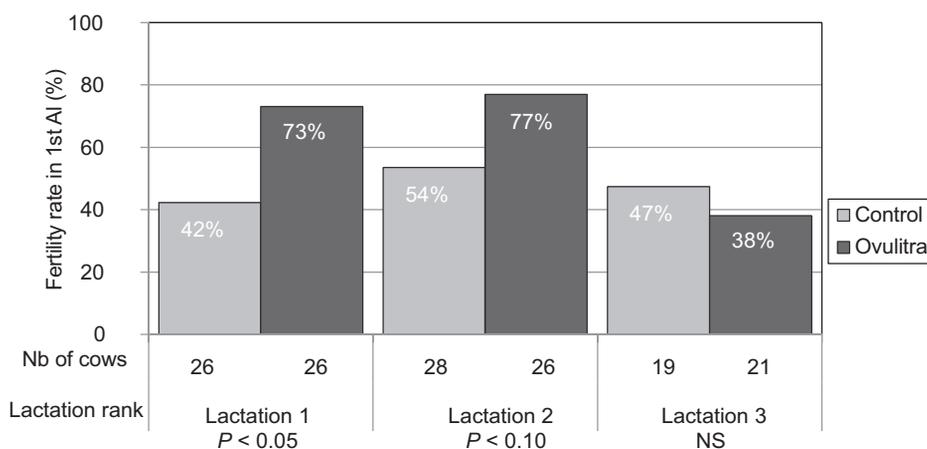


FIGURE 2. Effect of Ovultral supplementation depending on lactation rank

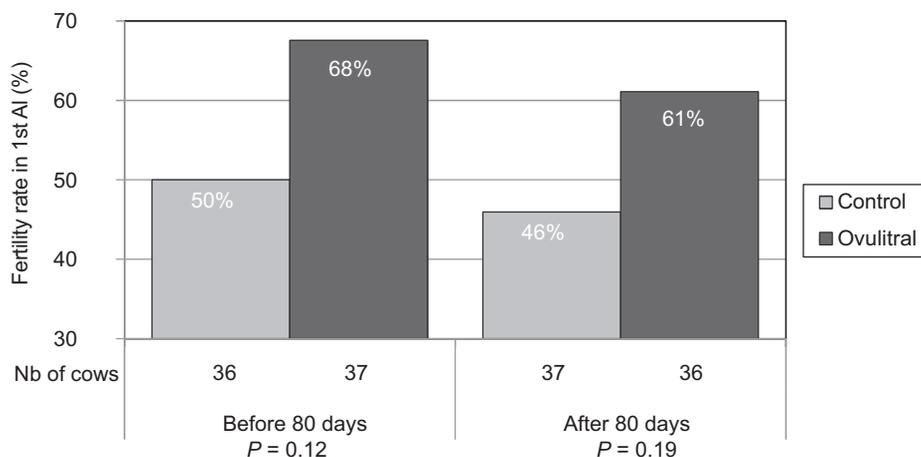


FIGURE 3. Effect of Ovultral supplementation depending on time elapsed from calving to insemination

after 80 days, the fertility is at 61% on Ovultral group, that is a difference of 15 points compared to control group. To resume, there is a huge improvement of fertility on Ovultral compared to control group.

The date of insemination should have an effect on the fertility, depending on composition of alimentation and luminosity of barns (Roche et al. 2000, Olechnowicz and Jaśkowski 2011). In fact fertility is really stable along the period test within the two groups. Fertility on control group is stable about 40 to 50% of fertility. And on the test group the fertility is about 60 to 70%.

CONCLUSIONS

The results of the experiment indicate on beneficial influence of Ovultral application at about 4 hours before insemination on cows fertilization effectiveness, especially in the 1st and 2nd lactation. In groups receiving that preparation,

comparing to the control group, fertility effectiveness after first insemination significantly increased by 31 percentage points (from 42 to 73) in the 1st lactation and by 23 percentage points (from 54 to 77) in the second lactation. In the 3rd lactation not significant decrease of fertility effectiveness appeared. The obtained results show, that there is real possibility of reproduction effects improvement by use of preparations increasing and intensifying heat symptoms and consequently enhancing fertility effectiveness.

REFERENCES

- BALDI, A., SAVOINI G., PINOTTI L., MONFARDINI E., CHELI F., DELLORTO V., 2000: Effects of vitamin E and different energy sources on vitamin E status, milk quality and reproduction in transition cows. *J. Vet. Med.* 47: 599–608.
- BALENDRANA., GORDON M., PRETHEEBAN T., SINGH R., PERERA R., RAJAMAHEDRAN R., 2008: Decreased fertility with increasing parity in lactat-

- ing dairy cows. *Can. J. Anim. Sci.* 88 (3): 425–428.
- BOUSQUET D., BOUCHARD E., DUTREMBLAY D., 2004: Decreasing Fertility in Dairy Cows: Myth or Reality? Proceedings of the WBC Congress, Québec, Canada: 6 pp.
- BULVESTRE M.D., 2007: Influence of beta-carotene on reproduction performances on dairy cows. *Vet. Thesis, France*: 124 pp.
- CALDWELL V., 2003: La reproduction sans censure: la vision d'un vétérinaire de champ. Symposium sur les bovins laitiers, Canada: 20 pp.
- CHALONY L., MICHEL A., 2009: Une solution pour améliorer la fertilité. *Bull. Techn. Insém. Artif.* 132: 24–29.
- COLEMAN D.A., THAYNE W.V., DAILEY R.A., 1985: Factor affecting reproductive performance of dairy cows. *J. Dairy Sci.* 68: 1793–1803.
- GIRARD C.L., 1998: B-complex vitamins for dairy cows: a new approach. *Can. J. Anim. Sci.* 78 (Suppl. 1): 71–90.
- HUGENTOBLER S.A., DISKIN M.G., LEESE H.J., HUMPHERSON P.G., WATSON T., SREENAN J.M., MORRIS D.G., 2007: Amino-acids in oviduct and uterine fluid and blood plasma during the estrous cycle in the bovine. *Mol. Reprod. Dev. Apr.* 74 (4): 445–454.
- KRZYŻEWSKI J., STRZAŁKOWSKA N., REKLEWSKI Z., DYMNICKI E., RYNIOWICZ Z., 2004: Wpływ długości okresów międzyciążowych u krów rasy hf na wydajność, skład chemiczny mleka oraz wybrane wskaźniki rozrodu. *Med. Weter.* 60 (1): 76–79.
- LeBLANC S.J., HERDT T.H., WEYMOUR W.M., DUFFIELD T.F., LESLIE K.E., 2004: Peripartum serum vitamin E, retinol, and beta-carotene in dairy cattle and their associations with disease. *J. Dairy Sci.* 87: 609–619.
- MINITAB, 2000: MINITAB Reference Manual Release 13.32. MINITAB Inc., State College, PA.
- MULLIGAN F.J., O'GRADY L., GATH V.P., RICE D.A., DOHERTY M.L., 2007: Nutrition and fertility in dairy cows. *Irish Vet. J.* 60, 5: 12–19.
- Ocena i hodowla bydła mlecznego. Dane za rok 2011. *Polska Fed. Hod. Bydła i Prod. Ml.*, 2012: 46–100.
- OLECHNOWICZ J., JAŚKOWSKI J.M., 2011: relation between clinical lameness and reproductive performance in dairy cows. *Med. Weter.* 67 (1): 5–9.
- PIRON A., 2005: Estimation of use of a new nutritional supplement on fertility of dairy cows – student report. *Ecole Supérieure d'Agriculture d'Angers*: 37 pp.
- PONSART C., FRAPPA B., Le MEZEC P., FRERET S., SEEGER S., PACCARD P., HUMBLLOT P., 2007: A wide range of tools to improve reproduction in dairy cows. *Renc. Rech. Rumin.*, France: 1 pp.
- ROCHE J.F., MACKEY D., DISKIN M.D., 2000: Reproductive management of postpartum cow. *Anim. Repr. Sci.* 60–61: 703–712.
- SHAVER R.D., BAL M.A., 2000: Effect of dietary thiamin supplementation on milk production by dairy cows. *J. Dairy Sci.* 83: 2335–2340.

Streszczenie: *Ocena przydatności Ovulitralu do poprawy efektów rozrodu krów mlecznych.* Płodność wysokowydajnych krów mlecznych jest dziś dużym problemem gospodarstw w Europie i na całym świecie. Niniejsze doświadczenie zostało przeprowadzone na krowach odmiany polskiej holsztyńsko-fryzyjskiej Rolniczego Zakładu Doświadczalnego Wilanów-Obory w celu oceny poprawy płodności przy zastosowaniu specjalnego dodatku paszowego o nazwie Ovulitral podanego krowom tuż przed inseminacją. Ten specyficzny preparat został opracowany z użyciem elementów śladowych, witamin i aminokwasów wyselekcjonowanych w celu poprawy ekspresji hormonalnej i zmniejszenia stresu oksydacyjnego, a w konsekwencji poprawy płodności krów. Doświadczenie przeprowadzono w okresie od października 2010 do lipca 2011 roku. 146 krów doświadczalnych przydzielono do grupy kontrolnej i testowej w zależności od ich charakterystyki,

na którą składały się wydajność laktacyjna, liczba dni po wycieleniu, szczytowa wydajność mleka. Po wykryciu rui, krowy były inseminowane, a grupa doświadczalna otrzymywała dawkę 100 ml preparatu Ovulitral przy inseminacji. Ciąża była oceniana ultrasonograficznie w 35. dniu po inseminacji. Zapłodnialność wynosiła 48% w grupie kontrolnej i 65% w grupie krów otrzymujących preparat Ovulitral ($p < 0,05$). Poprawa płodności była wyższa u krów w pierwszej i drugiej laktacji. Interesujące jest również to, że nie stwierdzono różnicy między krowami inseminowanymi przed i po 80. dniu od wycielenia. Uzyskane wyniki potwierdzają wyniki doświadczenia przeprowadzonego na farmach we Francji.

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The evaluation of the scores obtained by mares at stationary performance tests at (Polish) Training Stations in 2007–2011

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Abstract: *The evaluation of the scores obtained by mares at stationary performance tests at (Polish) Training Stations in 2007–2011.* An analysis of results achieved by mares at stationary performance test was carried out. The research material included 901 halfbred mares entered for the Stationary Performance Test in 2007–2011. The effect of the mare's breed on the evaluation scores in free jumping, movement evaluation in walk, trot and canter, rideability, training aptitude and the total score, which was the ultimate evaluation score. The effect of matings on the total score was evaluated. The models included the effects of the year and of the Training Station. Calculations were performed with the SPSS Statistical Package ver. 19.0 – 2012, GML procedure. On the basis of the conducted analyses it was concluded, that the mean values of the scores for most evaluated features in Polish Halfbred and Wielkopolski mares by imported sires were significantly higher in comparison to the results of mares deriving from two Polish parents.

Key words: mares, evaluation of utility value/utility evaluation, stationary performance tests.

INTRODUCTION

In the Polish breeding programmes the following methods of utility evaluation of mares are applied: field performance test, stationary performance tests, Polish Championships for Young Horses (MPMK), equestrian sports, and racing for halfbred horses. In the stationary tests, mares undergo a 60-day training

at the Training Station and enter into the performance test. The Head of the Training Station evaluates the horse's character, temperament, training aptitude, the test rider evaluates rideability and the Committee evaluates free jumping and movement in walk, trot and canter. The sum of scores for each trait is the final score in the performance test. According to Uphaus et al. (1994) introduction of performance value assessment for mares in Germany resulted in increasing of the breeding progress by 25 percent in one generation. In Poland, selection is based on the phenotypic values. According to Viklund et al. (2011), due to low heridity of performance traits of horses, the breeding value estimated by means of multi-trait BLUP method should be used as the selection criterion.

MATERIALS AND METHODS

The aim of the present work is to evaluate the scores of halfbred mares participating in stationary performance tests in 2007–2011 with respect to their breeds and cross-breed matings (the sire's breed – the dam's breed).

The research material constituted of the results of 901 mares, which underwent the tests at six Training Stations in

2007–2011. The results were retrieved from the Polish Association of Horse Breeders' database. The biggest number of mares were tested at the following Training Stations: Biały Bór Training Station – 37,9%, Bogusławice Training Station – 31%, Doruchów Training Station – 11%, Włocławek – 11%, Ciecuchów – 9% and Racot – 2%.

The numbers of mares in each breed was as follows: Polish Halfbred: 433 heads – 48 percent; Wielkopolski: 281 heads – 31 percent; Małopolski: 187 heads – 21 percent.

All calculations were performed using the statistical package SPSS ver. 19.0 – 2012, the GLM procedure.

In order to determine the effect of the breed on the results obtained by the mares in movement in walk, trot and canter (see Table 2), rideability, free jumping, training aptitude (see Table 1) and the total score for the entire test, the data set was divided into three groups: 1. Małopolski horse (młp), 2. Polish Halfbred (sp) 3. Wielkopolski horse (wlkp).

TABLE 1. The effect of the breed of the mare on the scores in free jumping, training aptitude and rideability in the stationary performance test (SPT)

Breed	<i>n</i>	Training aptitude		Free jumping		Rideability	
		LSM	SE	LSM	SE	LSM	SE
Małopolski	187	7.0 A	0.10	6.8 A	0.09	6.5 A	0.15
Polish Halfbred	433	7.4 B	0.06	7.0 B	0.06	7.0 B	0.09
Wielkopolski	281	7.3 C	0.07	6.8 A	0.07	7.0 B	0.10
Total	901	7.3	0.06	6.9	0.05	6.8	0.08

The differences between mean values marked with the same letters of alphabet are statistically insignificant.

For each of the traits the three factor analysis of variance was performed by means of the SPSS statistical package:

$$Y_{kmno} = \mu + Rkl_k + Year_m + TS_n + e_{kmno} \quad (1)$$

where:

μ – mean value,

Y_{kmno} – the score for the evaluated trait,

Rkl_k – the effect of the mare's breed ($k = 1, 2, 3$),

$Year_m$ – the effect of the year of the test ($m = 1, 2, \dots, 5$),

TS_n – the effect of the Training Station ($n = 1, 2, \dots, 6$),

e_{kmno} – random error.

Due to the distribution not being normal, analysis of variance was not performed for the scores in character and temperament.

The assymetry of the distributions might have been influenced by overrating or levelling of the scores. Similar tendencies for various traits were observed by Kownacki et al. (1993) and Kaproń (2006). According to Lewczuk (1999) the most substantial drawback of performance tests was subjective evaluation of traits.

In order to determine the effect of the mares' origin on the total score (the sum of points for all the evaluated traits) the research material was divided into 14 groups (see Table 3) depending on the matings of the parents' breeds. By means

TABLE 2. The effect of the breed of the mare on the scores in walk, trot and canter and the total score in the stationary performance test (SPT)

Breed	n	Walk		Trot		Canter		Total	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE
Małopolski	187	6.5 A	0.08	6.3 A	0.09	6.4 A	0.08	54.7 A	0.52
Polish Halfbred	433	6.7 B	0.05	6.7 B	0.06	6.7 B	0.05	57.4 B	0.32
Wielkopolski	281	6.7 B	0.06	6.5 C	0.06	6.6 A	0.06	56.5 C	0.36
Total	901	6.6	0.05	6.5	0.05	6.6	0.05	56.2	0.30

The differences between mean values marked with the same letters of alphabet are statistically insignificant.

TABELE 3. The effect of the types of matings on the total score at the stationary performance test (SPT)

Type of mating	n	LSM	SE
imp – imp	22	59.3 A	1.00
imp – sp	170	58.7 A	0.41
imp – wlkp	132	57.9 A	0.46
imp – Th	13	57.5 AB	1.31
sp – sp	99	56.3 B	0.53
sp – Th	23	56.2 B	1.01
imp – młp	59	56.2 B	0.69
młp – sp	21	55.7 B	1.06
sp – wlkp	99	55.7 B	0.52
młp – wlkp	35	55.2 B	0.82
wlkp – wlkp	50	55 B	0.70
młp – młp	131	54.6 B	0.56
Th – wlkp	20	54.2 B	1.04
młp – Th	17	53.5 B	1.17
Total	891	56.1	0.31

The differences between mean values marked with the same letters of alphabet are statistically insignificant.

– imp – imported parcut; sp – Polish Halfbred; wlkp – Wielkopolski; Th – Thoroughbred; młp – Małopolski.

of the SPSS statistical package, triple factor analysis of variance was carried out according to the following model:

$$Y_{kmno} = \mu + K_k + Year_m + TS_n + e_{kmno} \quad (2)$$

where:

μ – mean value,

Y_{kmno} – the score for the evaluated trait,
 K_k – effect of the matings ($k = 1, 2, \dots, 4$),

$Year_m$ – effect of the year of the test ($m = 1, 2, \dots, 5$),

TS_n – effect of the Training Station ($n = 1, 2, \dots, 6$),

e_{kmno} – random error.

RESULTS AND DISCUSSION

Training aptitude – defines to a large extent the horse's aptitude for work under saddle. While giving a score for training aptitude, director/head of the training station takes into consideration all the traits evaluated at the Training Station. Pearson correlation coefficients between the scores for training aptitude and the scores for the remaining traits were statistically significant. They varied from 0.2 with the scores for movement in trot to 0.7 with the total score of the test. The differences between the means of this trait for the evaluated breeds were statistically significant. On this basis, it may be concluded that Polish Halfbred mares showed the highest and Małopolski mares the lowest training aptitude. The low results of the Małopolski mares can be explained by small proportion of imported breeding stock in their pedigrees. The data in Table 3 suggest that the mares with one imported parent scored statistically significantly higher for the total score of the performance test.

Free jumping – similar to training aptitude evaluation the Polish Halfbred mares had statistically higher means for this trait in comparison to Wielkopolski and Małopolski mares. The differences between the means on Małopolski and Wielkopolski mares were statistically insignificant.

Movement evaluation – the mean scores for walk, trot and canter were the highest in Polish Halfbred mares, 235 out of which were had one imported parent. The lowest mean scores for movement were that of Małopolski mares, where only 33 out of 187 had one parent of foreign breeding.

Rideability – is a very substantial element of the evaluation of a saddle horse which is included in the breeding programmes of halfbred horses. It refers to the predisposition of the horse for recreational horse riding and for equestrian sports for less experienced riders. (Becker et al. 2011). The Małopolski mares had statistically significantly lower means of the trait score in comparison to Polish Halfbred mares and Wielkopolski mares.

Final score – the differences among the means of the evaluated breeds were statistically significant.

In the world breeding of halfbred horses it is common to use high quality breeding material from various stud books (Koenen et al. 2004). This method is also used in the Polish breeding of Halfbred and Wielkopolski horses and to a smaller extent in Małopolski breed.

Out of 901 mares included in this research, only 514 were by Polish-bred sires. Among the 387 mares by imported sires, the greatest number were by Holstein sires – 112, and were sired by KWPN stallions – 66.

Table 3 presents the means of the final score for the Training Station test for mares deriving from various matings. The most numerous groups of the evaluated mares, were that deriving from Polish-bred dams of Polish Halfbred and Wielkopolski breeds with sires of foreign origin. Using the imported breeding stock significantly improved the performance value of Polish breeds. The mean total scores for Polish Halfbred and Wielkopolski mares deriving from one imported parent were statistically significantly higher than the mean total scores of mares deriving from other matings.

Low mean scores of mares deriving from Małopolski, Polish Halfbred and Wielkopolski parents in comparison to the mares with one imported parent are evidence of low effectiveness of the breeding work conducted.

On the basis of the evaluation of the effect of matings on the mares' stationary performance tests scores it might be concluded, that the improvement of saddle traits of these breeds proceeds mainly through the use of imported breeding stock.

As a summary of the evaluation of the results achieved by the mares in stationary performance tests it should be concluded that Polish Halfbred and Wielkopolski mares deriving from imported sires have the greatest predispositions for work under saddle.

CONCLUSIONS

Significantly higher mean values in most of the evaluated traits in Polish Halfbred mares and Wielkopolski mares by imported sires in comparison to the mares by Polish sires are evidence of low effectiveness of the conducted breeding work.

The research conducted indicates, that improvement of saddle traits in halfbred horses proceeds mainly through use of imported breeding stock.

REFERENCES

- BECKER A.C., STOCK K.F., DISTL O., 2011: Genetic correlations between free movement and movement under rider in performance tests of German warmblood horses. *Livestock Science* 142, 245–252.
- KAPROŃ M., 2006: Wykorzystanie parametrów genetycznych w modyfikacji systemów prób dzielności ogierów półkrwi. *Prace i Materiały Zootechniczne Jastrzębiec, Zeszyt Specjalny* 16, 69–78.
- KOENEN E.P.C., ALDRIDGE L.I., PHILIPSSON J., 2004: An overview of breeding objectives for warmblood sport horses. *Livestock Production Science* 88, 77–84.
- KOWNACKI M., LIPIŃSKA Z., KOZACZYŃSKI K., 1993: Selekcja ogierów w Zakładach Treningowych na podstawie wyników oceny użytkowości. *Rocz. Nauk. Zoot. Vol. 20, 2*, 31–38.
- LEWCZUK D., 1999: Genetyczne uwarunkowania zdolności skokowej koni półkrwi. *Prace i Materiały Zootechniczne Jastrzębiec, Zeszyt* 55, 17–30.
- UPHAUS H.I., KALM E., 1994: Feld und Stationsprüfung für Stuten. II. Nutzung im Rahmen eines Zuchtprogrammes. *Zuchtskunde* 66, 4, 268–280.
- VIKLUND A., NASHOLM A., STRANDBERG E., PHILIPSSON J., 2011: Genetic trends for performance of Swedish Warmblood Horses. *Livestock Science* 141, 113–122.
- Streszczenie:** Ocena wyników klaczy uzyskanych w stacjonarnych próbach dzielności w zakładach treningowych w latach 2007–2011. Celem niniejszej pracy była ocena wyników klaczy ras półkrwi uczestniczących w stacyjnych próbach dzielności z uwzględnieniem rasy oraz kojarzeń (rasa matki – rasa ojca). Materiał badawczy stanowiły wyniki 901 klaczy przechodzących próby w sześciu zakładach treningowych w latach 2007–2011. Wszystkie obliczenia wykonano za pomocą pakietu statystycznego SPSS 19.0 rok 2012, procedura GLM. Oceniono wpływ rasy na wyniki uzyskane przez klacze w skokach luzem, ruchu w stępie, kłusie i galopie, jeźdźności, przydatności do treningu oraz sumy punktów za całość próby. Oceniono również wpływ pochodzenia klaczy na wyniki końcowe wyrażone w sumie punktów za oceniane cechy. W podsumowaniu wyników oceny klaczy w stacyjnych próbach dzielności stwierdzono, że największe predyspozycje do

użytkowania wierzchowego mają klacze szlacheckiej półkrwi i wielkopolskie pochodzące po reproduktorach importowanych.

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The evaluation of the results of field performance tests for mares conducted in 2002–2011

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Abstract: *The evaluation of the results of field performance tests for mares conducted in 2002–2011.* The analysis of results of mares at field performance tests was carried out. The research material consisted of 651 mares, which underwent the field performance test (FPT) in 2002–2011. The effect of the breed on the results in evaluation of free jumping, movement in walk, trot and canter, rideability and on the total score of the test was investigated. The effect of matings on the total score of the test was determined. The models included the effect of the year on the test, and the effect of the District Horse Breeder's Association, where the tests were organized. All calculations were performed using the statistical package SPSS ver. 19.0 – 2012, the GLM procedure. On the basis of the analyses carried out, it was concluded that mares of solely Polish origin had statistically significantly lower scores in comparison to the imported mares. The analysis of matings showed that Polish Halfbred mares and Wielkopolski mares which had one imported parent achieved statistically significantly higher mean values in all the evaluated traits in comparison to the mares descending from parents of Polish breeding.

Key words: mares, evaluation of performance value, field performance tests.

INTRODUCTION

In Polish breeding programmes of halfbred horses the assessment of the mares' performance value is not obligatory. The effect of the dam on the progeny is as significant as that of the sire, therefore the

results of the performance tests should be included in selection for breeding (Nowicka-Posłuszna 2001, 2006). For halfbred mares field performance test are conducted. The advantages of this assessment form include: low costs, the possibility to evaluate a big number of horses, the fact that mares do not undergo selection prior to the evaluation. The training and preparation for the test are carried out by the owner of the horse. The main disadvantage of field performance tests is a great diversity of environmental conditions (training, nutrition etc.) which can have a great effect on the test result. In the field performance tests (FPT), the evaluation of free jumping, movement in walk, trot and canter is carried out by the Judging Commission appointed by the Breeder's Association which keeps the Stud Book. Rideability is evaluated by the test rider. Each trait is evaluated with 1 to 10 points, and the final result of the test is the sum of scores for each trait.

MATERIAL AND METHODS

The aim of the present work was to evaluate the results of halfbred mares participating in field performance tests in

2002–2011 taking into consideration the breed and matings (sire's breed – dam's breed).

The research material were the results of field performance tests of 651 mares evaluated in 2002–2011 included in the Polish Association of Horse Breeders' database. The popularity of this assessment method was diversified. The greatest number of mares were evaluated at field performance test in the District Horse Breeders Associations (DHBA) for the regions of Kujawsko-Pomorskie 32.9%, Wielkopolskie 28.3% and Warmińsko-Mazurskie 0.7%, and in Śląsko-Opolskie 9.8%. In five DHBAs no such test was organized. The number of mares for each year of the period in question varied from 106 in 2006 to 45 in 2003. The most numerous breeds in evaluation were the Polish Halfbred mares – 47.2% and the Wielkopolski mares – 38.4%.

All calculations were performed using the statistical package SPSS ver. 19.0 – 2012, the GLM procedure.

In order to determine the effect of breed on the results of the mares in free jumping, movement in walk, trot and canter, rideability and the total score for the test, the research material was divided into five groups: 1) imported mares (imp) 2) other Polish-bred mares (Thoroughbred, warmblood type, Trakehner, other) 3) Małopolski (młp), 4) Polish Halfbred (sp), 5) Wielkopolski (wlkp). For each of the traits, the triple factor analysis of variance was carried out using the SPSS statistical package, according to the following model:

$$Y_{kmno} = \mu + MBr_k + Year_m + DHBA_n + e_{kmno} \quad (1)$$

where:

μ – mean value,

Y_{kmno} – score for the evaluated trait,

MBr_k – effect of the mare's breed ($k = 1, 2, \dots, 4$),

$Year_m$ – effect of the year of the test ($m = 1, 2, \dots, 10$),

$DHBA_n$ – effect of the District Horse Breeders Association ($n = 1, 2, \dots, 7$),

e_{kmno} – random error.

Estimation of interaction with the year or DHBA was impossible due to lack of data form some of the subgroups.

In order to evaluate the effect of the origin on the total scores at the FPT (evaluation of traits expressed as sum of points), the research material was divided into subgroups depending on the matings the mares derived from. Due to low number of the horses, it was impossible to distinguish among the matings of dam's breed – sire's breed. The following types of matings were evaluated: 1) imp – imp, 2) młp – imp, 3) młp – wlkp, 4) młp – sp, 5) młp – młp, 6) sp – imp, 7) sp – sp, 8) sp – wlkp, 9) wlkp – imp, 10) wlkp – wlkp, 11) Thoroughbred – wlkp, 12) Thoroughbred – imp, 13) Thoroughbred – sp.

Triple factor analysis of variance was performed, using SPSS statistical package, according to the following model:

$$Y_{kmno} = \mu + M_k + Year_m + DHBA_n + e_{kmno} \quad (2)$$

where:

μ – mean value,

Y_{kmno} – the score for the evaluated trait,

M_k – the effect of the matings ($k = 1, 2, \dots, 4$),

$Year_m$ – the effect of the year of the test ($m = 1, 2, \dots, 10$),

$DHBA_n$ – the effect of the District Horse Breeders Association ($n = 1, 2, \dots, 7$),
 e_{kmno} – random error.

In order to eliminate the effect of diversification of environmental conditions (training and preparation, the composition of the Judging Commission, the test riders etc.) on the results of particular breeds and matings, the effect of the year of the test and effect of the District Horse Breeders Association were included in both models. Due to low number of mares entered for the FPT in certain locations, they were grouped into the regions corresponding to District Horse

Breeders Associations. These factors were statistically significant.

RESULTS AND DISCUSSION

Tables 1 and 2 show the mean values of the smallest squares of the trait for each breed.

Free jumping – according to many authors good free jumping scores are a proof of the horse’s predisposition for the discipline of show jumping (Dubois et al. 2008, Posta et al. 2009, Becker et al. 2011). Imported mares obtained statistically significantly higher mean value

TABLE 1. The effect of the breed of the mare on the scores in free jumping and in particular gaits at the field performance test (FPT)

Breed	n	Free jumping		Walk		Trot		Canter	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE
Imported	41	8.1 A	0.19	7.7 A	0.16	7.8 A	0.15	7.8 A	0.15
Małopolski	36	7.5 B	0.10	7.3 AB	0.22	7.3 AB	0.21	7.3 AB	0.21
Polish Halfbred	307	7.4 B	0.28	6.8 B	0.08	7.1 B	0.08	6.9 B	0.07
Other Polish breeds	17	7.2 B	0.27	6.6 B	0.23	6.9 B	0.22	6.9 B	0.21
Wielkopolski	250	7.2 B	0.11	6.9 B	0.09	6.9 B	0.09	6.8 B	0.09
Total	651	7.5	0.1	7.1	0.08	7.2	0.08	7.1	0.07

The differences between mean values marked with the same letters of alphabet are statistically insignificant.

TABLE 2. The effect of the mare's breed on the scores in rideability and on total score at the field performance test (FPT)

Breed	Rideability		Total score – sum of points	
	LSM	SE	LSM	SE
Imported	7.7 A	0.20	38.7 A	0.60
Małopolski	6.9 B	0.28	34.8 B	0.91
Polish Halfbred	6.8 B	0.10	34.5 B	0.71
Other Polish breeds	7.1 AB	0.29	35.0 AB	0.30
Wielkopolski	6.7 B	0.12	34.4 B	0.35
Total	7.0	0.1	35.5	0.31

The differences between mean values marked with the same letters of alphabet are statistically insignificant.

for the evaluation of this trait, compared to local breeds. It should be emphasized, that one of the methods of improving saddle traits of Polish halfbreeds is importation of breeding stock of mares and stallions. 221 Polish Halfbred mares and 83 Wielkopolski mares derived from one imported parent. Despite considerable proportion of imported ancestors in the mares' pedigrees, they had significantly lower mean total scores in most of the evaluated traits in comparison to the mares of foreign breeding. This results show the distance that separates Polish breeding from the European.

Movement evaluation – the highest mean values for evaluation of movement in walk, trot and canter, were achieved by the imported mares. These results were significantly higher in comparison to the mean values of Polish Halfbred mares, other Polish-bred mares and Wielkopolski mares. The lack of significant differences between the imported and the Małopolski mares can be explained by the fact, that in comparison to Polish Halfbred and Wielkopolski mares, only a small number of horses were entered for the test. It might be suggested, that the only Małopolski mares entered for the test, were the ones with very high movement predispositions.

Rideability – is a substantial element of saddle traits evaluation, included in the breeding programmes for halfbred horses. It reflects the predisposition of the horse for recreational riding and for equestrian sports for less experienced riders (Becker et al. 2011). Małopolski, Polish Halfbred and Wielkopolski mares had statistically significantly lower mean scores for this trait in comparison to the imported mares.

Total score (the sum of scores for all the traits) – is used as the final evaluation of the performance value. The imported mares achieved statistically significantly higher mean score in comparison to Polish-bred horses, and Polish Halfbred horses had higher mean score than Wielkopolski mares.

One of the methods used for improving saddle traits of halfbred horses in breeding worldwide is the use of top quality breeding stock from different Stud Books (Koenen et al. 2004). As it has already been mentioned, this method is also used in Polish breeding for Polish Halfbreds, Wielkopolski and – to a smaller extent – in Małopolski horses. Table 3 shows the mean values of the total scores of the FPT for mares deriving from different matings. The most numerous were the mares deriving from Wielkopolski and Polish Halfbred matings with imported breeding stock. Use of the imported breeding stock improved significantly the performance value of Polish breeds. The mean total scores of Wielkopolski and Polish Halfbred mares descending from one imported parent were statistically significantly higher than the mean values of the mares deriving from two Polish-bred parents.

The low mean values of mares deriving from Małopolski, Polish Halfbred and Wielkopolski parents in comparison to the results achieved by mares with one imported parent show the improvement of saddle traits of these breeds proceeds mainly through the use of imported breeding stock.

To summarize the assessment of performance value evaluation of the mares from the breeds that underwent the test it is the data from Table 3 should be once

TABLE 3. The effect of types of matings, from which the mares were born, on the total score achieved at the field performance test (FPT)

Type of matings	<i>n</i>	Mean	Sd
wlkp – imp	130	36.2 A	0.41
sp – imp	114	35.9 A	0.38
Th – wlkp	29	35.4	0.68
imp – imp	16	35.4	0.87
młp – imp	12	35.0	0.97
sp – sp	38	34.8 B	0.57
młp – wlkp	17	34.6	0.88
młp – sp	17	34.5	0.87
sp – wlkp	63	34.2 B	0.5
Th – imp	13	34.2 B	0.96
wlkp – wlkp	102	33.9 B	0.43
Th – sp	8	33.6 B	1.2
młp – młp	25	33.3 B	0.82
Total	584	34.7	0.28

The differences between mean values marked with the same letters of alphabet are statistically insignificant.

– wlkp – Wielkopolski; imp – imported parent; sp – Polish Halfbred; Th – Thoroughbred; młp – małopolski.

again quoted. They show that 269 mares had one parent of foreign breeding. The highest mean total scores were achieved by mares which derived from matings of Wielkopolski – imported and Polish Halfbred – imported, while the lowest mean total scores by Małopolski – Małopolski, Wielkopolski – Wielkopolski and Polish Halfbred – Polish Halfbred matings. The results achieved by mares which derived from matings of two Polish-bred parents resulted in lowering mean total scores for Polish Halfbred and Wielkopolski mares. These results indicate that a considerable distance separates Polish breeding from European.

CONCLUSIONS

Significantly lower mean values of the majority of mares of Polish breeds in

comparison to the imported ones are evidence of the great distance that separates the level of Polish breeding from that of European breeding.

The results achieved allow us to draw the conclusion, that improvement of saddle traits in Polish halfbreeds proceeds mainly through use of imported breeding stock.

REFERENCES

- BECKER A.C., STOCK K.F., DISTL O., 2011: Genetic correlations between free movement and movement under rider in performance tests of German warmblood horses. *Livestock Science* 142, 245–252.
- DUBOIS C., MANFREDI E., RICARD A., 2008: Optimization of breeding schemes for sport horses. *Livestock Science* 118, 99–112.

- KOENEN E.P.C., ALDRIDGE L.I., PHIL-IPSSON J., 2004: An overview of breeding objectives for warmblood sport horses. *Livestock Production Science* 88, 77–84.
- NOWICKA-POSŁUSZNA A., 2001: Ocena wartości użytkowej klaczy – ważne kryterium selekcji hodowlanej. *Przegląd Hodowlany* 1, 19–21.
- NOWICKA-POSŁUSZNA A., 2006: Próby dzielności i wstępna ocena wartości użytkowej klaczy półkrwi w SK Racot. *Prace i Materiały Zootechniczne Jastrzębiec, Zeszyt Specjalny* 16, 131–138.
- POSTA J., KOMLOSI I., MIHOK S., 2009: Breeding value estimation in the Hungarian Sport Horse population. *The Veterinary Journal* 181, 19–23.

Streszczenie: Ocena wyników polowych prób wierzchowych klaczy przeprowadzanych w latach 2002–2011. Celem niniejszej pracy była ocena wyników klaczy ras półkrwi uczestniczących w polowych próbach wierzchowych (PPW) z uwzględnieniem rasy oraz kojarzeń (rasa matki – rasa ojca). Materiał badawczy stanowiły wyniki polowych prób dzielności 651 klaczy ocenianych

w latach 2002–2011 zamieszczone w bazie danych prowadzonej przez Polski Związek Hodowców Koni. Wszystkie obliczenia wykonano za pomocą pakietu statystycznego SPSS 19.0 rok 2012, procedura GLM. Określono wpływ rasy na wyniki uzyskane przez klacze w skokach luzem, ruchu w stępie, kłusie i galopie, jeźdźności oraz sumy punktów za całość polowej próby dzielności. Następnie oceniono wpływ pochodzenia klaczy na wyniki końcowe PPW wyrażone w sumie punktów za oceniane cechy. Najliczniejsze grupy ocenianych klaczy pochodziły z kojarzeń koni wielkopolskich oraz szlachejnych półkrwi z importami. Używanie importowanego materiału zarodowego istotnie poprawiało wartość użytkową klaczy ras rodzimych. Średnie sumy punktów klaczy wielkopolskich i szlachejnych półkrwi pochodzących po jednym z rodziców importowanych były statystycznie istotnie wyższe od średnich klaczy pochodzących z kojarzeń rodziców hodowli polskiej.

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Effect of turkeys genotype on results on their slaughter performance

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Abstract: *Effect of turkeys genotype on results on their slaughter performance.* Two groups of turkeys: slow-growing primitive turkeys (♂♂ $n = 80$ and ♀♀ $n = 80$) and fast-growing Big-6 turkeys (♂♂ $n = 80$ and ♀♀ $n = 80$) were reared in a semi-intensive system: turkey hens until 14th week whereas turkey toms until 22nd week of life. Twelve males and twelve females with body weight corresponding to the mean body weight in a group were selected from each group for slaughter and slaughter performance analysis. A significantly ($P \leq 0.01$) higher body weight and weight of all anatomical parts examined were found in both hens and males of Big-6 turkeys. An exception was the weight of abdominal fat ($P \leq 0.05$) and weight of gizzard (NS) in males. In turn, Big-6 females were characterized by a significantly ($P \leq 0.01$) higher percentage of: breast muscles (by 8.7), leg muscles (by 2.4) and abdominal fat (by 0.9), and significantly ($P \leq 0.01$) lower percentage of: wings, heart, liver and gizzard (by respectively: 0.2, 0.1, 0.3 and 1.9) in the body weight. In the case of Big-6 males, analyses demonstrated a significantly ($P \leq 0.01$) higher percentage of breast muscles (by 16.5) and leg muscles (by 2.5, $P \leq 0.05$), and lower ($P \leq 0.01$) percentage of wing, heart, liver and gizzard (by respectively: 0.3, 0.2, 0.7 and 1.5) in the body weight, compared to the primitive turkeys.

Key words: turkeys, genetic group, effects of selection.

INTRODUCTION

Turkeys are a species of in the case of which successful turned out to be intensive selection for growth rate and musculature. According to Sütő et al. (2001),

in the year 2000 compared to the year 1973, in the 16th week of rearing heavy hybrids of turkeys were characterized by a higher body weight values, i.e. by 72 and 74% in hens and toms, respectively. This was primarily due to an increased contribution of muscles in the body weight of the birds (Reed et al. 2008), skeletal muscles in particular (Brenře and Kolstad 2000). Such a great advance in turkey breeding in such a short time span has been attributed to, among other things, easiness of intravital evaluation of bird muscles, knowledge of genetic correlations between muscle weight and body sizes, and high heritability of those traits (Aslam et al. 2011). Narrow orientation of breeding works has, however, led to anatomical disproportions and changes in body functioning. As a consequence, undesirable effects of selection, including e.g.: excessive body adiposity, cardiac muscle diseases, disorders in the ossification process, have been increasingly often observed (Czarnecki 1984, Julian 1998).

For in-depth analysis of changes in proportions of turkey body conformation, that occurred as a result of man's interference into the biology of this species, it seems substantiated to compare genetically very distant populations. A comparative analysis of anatomical characteristics between commercial hybrids of turkeys, e.g. Big-6, with other genetic

groups not subjected to oriented breeding, may provide a picture of changes in body conformation of those birds, of which ones may be acknowledged as intended and others as accidental effects of selection for growth and musculature characteristics.

Taking the above into account, the objective of this study was to compare heavy Big-6 turkeys with light primitive turkeys in terms of body weight and percentage share of: breast muscles, leg muscles, wings, abdominal fat and internal organs including heart, liver and gizzard, in their body weight.

MATERIALS AND METHODS

The experimental material were two different genetic groups of turkeys of both sexes, 160 birds each ($\text{♂♂ } n = 80$ and $\text{♀♀ } n = 80$). The control group (C) were light slow-growing turkeys which owing to phenotypical affinity to wild turkeys may be referred to as primitive. Those turkeys meet requirements that enable classifying them to “Heritage Turkeys”, constituting a genetic reserve of regional breeds developed in majority in the USA. At the end of the XIXth century,

they have been included into the genetic resources protection programme (American Poultry Association 2001). An experimental group (E) were fast-growing Big-6 turkeys. Both populations differ considerably not only in terms of the anatomical conformation of body. Owing to longstanding breeding in complete isolation, those populations are additionally characterized by a significant genetic distance (Kamara et al. 2007).

Both groups of turkeys were reared under conditions of a semi-intensive system. Until the end of the 5th week of life, poults were kept on litter accordingly to the intensive rearing technology elaborated by Faruga and Jankowski (1996). Since the 6th week of life, the birds were allowed to use earlier prepared free ranges (pastures). Stock density at pastures was 1 bird per 12.5 m², hence it was threefold lower than the value stipulated by this species by Damme (2003). The birds were feeding on free ranges in the summer season (June – October), using the natural day length (ca. 14 h per day).

The turkeys were fed ad libitum with the same standard feed mixtures by De Haus “Zielona Zagroda” company in a four-phase system (Table 1). Since the 6th week of life, the turkeys were supple-

TABLE 1. Declared by the manufacturer nutritive value of basal diet applied in turkeys feeding depending on their age

Parameter	Mixture I-IV			
	Age of turkey			
	0–4 weeks	5–11 weeks	12–15 weeks	over 15 weeks
AEMN (MJ·kg ⁻¹)	11.1	11.7	11.9	12.1
Crude protein (%)	26.0	20.1	16.4	15.1
Crude ash (%)	7.3	5.7	4.4	3.8
Crude fat (%)	3.3	3.9	4.2	4.3
Crude fibre (%)	3.8	4.0	3.8	3.9

menting their diet with green vegetation growing on pasture, the chemical analysis of which (Table 2) was conducted according to AOAC methods (2005). Since the first day of life till the end of rearing the birds were administered a mineral-vitamin preparation Polfamix B, diluted in the following ratio: 2.5 g in 1 l of water.

Mean body weight (g) and mean weight (g) of all isolated internal organs, muscles and abdominal fat as well as their percentage in respect of body weight before slaughter were computed within a group and within sex. Variability of the traits examined was expressed with a standard error of the mean (SE).

Table 2. Results of chemical analysis of fresh vegetation growing on pastures for turkeys (%)

Dry matter	Crude ash	Total protein	Crude fat	Crude fibre	N-free extract
22.32	3.96	4.52	0.74	4.50	8.60

Turkey females were reared until 14th week of life, whereas males until 22nd week of life. At the end of rearing, body weight values of the birds were measured exact to 1 g. From each genetic group, 12 females and 12 males with body weight values similar to the mean body weight in a group for respective sex were selected for slaughter. They were slaughtered with the method of spinal cord and cervical blood vessels section, and bled out (bleeding time: 15–20 min). Afterwards, they were scalded in water with a temperature of 64–65°C (scalding time: ca. 10–15 sec), plucked and chilled in a cold store to a temperature of ca. 5°C. After 20 h of chilling, a simplified slaughter analysis was conducted. Superficial breast muscles (SBM) and deep breast muscles (DBM) as well as leg muscles (thigh and shank together) were isolated without skin. Wings were cut off at the shoulder joint. Gizzard, liver and heart were isolated as well. Fat was separated from abdominal cavity. The particular elements were weighed exact to 0.1 g. The slaughter analysis was carried out following the methodology described by Hahn and Spindler (2002).

Results were elaborated statistically with a one-way analysis of variance and Mann-Whitney test from the statistical package SPSS 14.0 procedure GLM (SPSS 2006).

RESULTS

Body weight values were found to differ significantly ($P \leq 0.01$) between genetic groups in both sexes. In the 14th week of life Big-6 females were heavier than primitive ones by 6203 g (Table 3), whereas in the 22nd week of life Big-6 males reached a higher by 16,113.6 g body weight than the primitive males (Table 4).

The Big-6 turkeys compared to the primitive birds were also characterized by a higher weight of the major parts of muscles, wings and internal organs (Tables 3 and 4). The difference in the weight of breast muscles in females accounted for 1587.6 g, of which the superficial muscles were heavier by 1279.8 g, and the deep muscles by 307.8 g. The weight of the breast muscles of Big-6 males, compared to the respective mus-

TABLE 3. Comparison of body weight (g), weight (g) and share in percent (%) in body weight of the various anatomical parts of the primitive (C) and Big-6 (E) turkeys female

Trait	Primitive (C) (n = 12)	Big-6 (E) (n = 12)	±SE	Significance of differences
BW (g)	2426.3	8629.3	64.86	**
SBM (g)	261.5	1541.3	19.10	**
SMB (%)	10.8	17.9	0.25	**
DBM (g)	66.9	374.7	12.30	**
DBM (%)	2.8	4.3	0.14	**
WBM (g)	328.4	1916.0	23.40	**
WBM (%)	13.5	22.2	0.27	**
LM (g)	382.3	1560.4	25.10	**
LM (%)	15.7	18.1	0.35	**
W (g)	224.5	781.5	13.04	**
W (%)	9.3	9.1	0.15	NS
H (g)	11.7	35.7	0.53	**
H (%)	0.5	0.4	0.02	**
L (g)	43.6	133.7	3.37	**
L (%)	1.8	1.5	0.05	**
G (g)	79.7	118.1	3.77	**
G (%)	3.3	1.4	0.11	**
AF (g)	10.2	107.9	6.17	**
AF (%)	0.4	1.3	0.08	**

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

– BW – body weight; SBM – superficial breast muscle; DBM – deep breast muscle; WBM – whole breast muscle; LM – leg muscle; W – wings; H – heart; L – liver; G – gizzard; AF – abdominal fat.

cles of primitive turkeys, was higher by 5772.8 g, and the weight of the particular parts, by 5062 g and by 755.8 g, respectively. The difference in the weight of leg muscles was considerable and accounted for 1178 g in females and for 2928.4 g in males. The weight of wings of Big-6 females was higher by 557 g, and that of males by 12983 g. In both sexes the higher weight of all discussed parts of muscles and wings was confirmed statistically ($P \leq 0.01$).

The weight of internal organs and abdominal fat was higher in both sexes of Big-6 turkeys compared to the primitive birds. In the case of the females, differences in weight accounted for: 24 g – heart, 90.1 g – liver, 38.4 g – gizzard, and 97.7 g – abdominal fat, whereas in the case of toms for: 46.3 g, 91 g, 5.5 g, and 39.9 g, respectively. The statistical analysis confirmed a highly significant difference regarding all internal organs and abdominal fat in females, and

TABLE 4. Comparison of body weight (g), weight (g) and share in percent (%) in body weight of the various anatomical parts of the primitive (C) and Big-6 (E) turkeys male

Trait	Primitive (C) (n = 12)	Big-6 (E) (n = 12)	±SE	Significance of differences
BW (g)	6282.7	22396.3	150.16	**
SBM (g)	608.8	5670.8	73.84	**
SMB (%)	9.7	25.3	0.58	**
DBM (g)	198.8	954.6	7.91	**
DBM (%)	3.2	4.3	0.05	**
WBM (g)	807.7	6580.5	69.15	**
WBM (%)	12.9	29.4	0.58	**
LM (g)	932.8	3861.2	50.59	**
LM (%)	14.8	17.3	0.64	*
W (g)	524.5	1822.8	20.37	**
W (%)	8.4	8.1	0.14	NS
H (g)	30.5	76.8	1.51	**
H (%)	0.5	0.3	0.01	**
L (g)	102.2	193.2	5.72	**
L (%)	1.6	0.9	0.05	**
G (g)	139.8	145.3	9.24	NS
G (%)	2.2	0.7	0.12	**
AF (g)	31.8	71.7	9.02	*
AF (%)	0.5	0.3	0.08	NS

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

– BW – body weight; SBM – superficial breast muscle; DBM – deep breast muscle; WBM – whole breast muscle; LM – leg muscle; W – wings; H – heart; L – liver; G – gizzard; AF – abdominal fat.

regarding heart and liver in males. In the group of Big-6 males, the weight of abdominal fat was significantly higher ($P \leq 0.05$), whereas no significant differences were found for this sex in respect of gizzard.

The Big-6 hybrids were also characterized by a higher percentage share of breast and leg muscle in total body weight. The whole breast muscles constituted 22.2% of body weight of Big-6 females, which is by 8.7% more than in the primitive females (13.5%). In the case

of superficial muscles this difference accounted for 7.1%, whereas in the case of deep muscles – for 1.5% (Table 3). The highest percentage of breast muscles in body weight was noted in Big-6 males (29.4%), with the value being higher by 16.5% than in the control males. The percentage of superficial breast muscles was lower in the primitive males by 15.6%, and that of the deep muscles – by 1.1% (Table 4). The percentage of leg muscles was significantly ($P \leq 0.01$) higher in Big-6 females (by 2.4%) and males

(by 2.5%, $P \leq 0.05$) (Tables 3 and 4). In contrast to the major muscle parts, the percentage of wings in body weight was higher in the primitive turkeys of both sexes (Tables 3 and 4). The difference accounted for 0.2% in females and for 0.3% in males, yet it was not confirmed statistically.

The percentage share of particular internal organs in total body weight was always higher in the primitive turkeys. In the case of females, the difference reached 0.1% for heart, 0.3% for liver and 1.9% for gizzard (Table 3), whereas in the case of males respective differences reached: 0.2, 0.7 and 1.5% (Table 4). All those differences were confirmed statistically ($P \leq 0.01$). A negligibly higher (by 0.2%), yet statistically insignificant, percentage of adipose fat in body weight was determined in the primitive males (Table 4). In turn, in the case of females, the statistical analysis demonstrated significantly higher ($P \leq 0.01$, by 0.9%) adiposity in Big-6 birds (Table 3).

DISCUSSION

According to Sütő et al. (2001), in no other animal did the selection lead to such a vast breeding advance as in turkeys. This may be confirmed by a study of İşgüzar (2003), in which turkey females originating after parents selected for growth rate, displayed in the 14th week of life a higher by 4531.9 g body weight than the slow-growing Bronze females. Lay et al. (2002) also achieved significant differences between Big-6 hybrids and Royal Palm¹ turkeys, when

analyzing their growth up to the 56th day of life. It may, thus, be speculated that a higher body weight of the Big-6 turkeys strongly transformed through selection, compared to that of the primitive turkeys, resulted above all from oriented breeding works (Tables 3 and 4).

Apart from body weight, the basic characteristics taken into consideration in breeding works with turkeys is the content of breast and leg muscles. As expected, the Big-6 hybrids were characterized by a higher weight and a higher percentage in body weight of the major muscle parts (Tables 3 and 4). Alike results were reported by Werner et al. (2008), when comparing Big-6 turkeys and slow-growing Kelly Super Mini birds. The efficacy of oriented breeding in muscle content in the body weight of turkeys was also demonstrated by Wilkiewicz-Wawro et al. (2003), who were comparing 2 lines of turkeys selected for musculature improvement and 2 lines selected for reproductive performance improvement.

The aforementioned results may corroborate the efficacy of turkeys selections in respect of characteristics covered by the breeding programme. It is likely, however, that this programme takes no account of the weight of internal organs and their contribution in total body weight. Presumably, this may chiefly be due to the difficulty in intravital evaluation of those characteristics. The available literature lacks exhaustive data on genetic correlations between the weight of internal organs and body weight and musculature of birds. Results of our own studies, in which heavy Big-6 turkeys demonstrated a higher body weight but a lower percentage share of heart, live

¹Royal Palm – black-and-white strain of Heritage Turkeys, enlisted into the American Poultry Association in 1971.

rand gizzard in the body weight (Tables 3 and 4) compared to primitive turkeys, correspond with findings of Nestor et al. (1995). Those authors reported that with an increasing body weight of turkeys, the weight of internal organs was also increasing, yet not proportionally. As a consequence, the percentage share of those organs in body weight is decreasing in the successive generations of birds. Likewise Sarica et al. (2009), who compared the total percentage contribution of internal organs in body weight, between slow-growing Bronze turkeys, fast-growing Hybrid turkeys and their hybrids, demonstrated the highest percentage of heart, live rand gizzard in Bronze turkeys (4.2% in toms and 4.7% in hens), followed by hybrids (3.0% in toms and 3.9% in hens), and the lowest one in Hybrid turkeys (2.9% in toms and 3.0% in hens).

Similar dependencies as in the case of internal organs were observed in respect of the wings weight and its percentage share in body weight. A lower percentage contribution of wings in body weight of Big-6 turkeys, despite their higher body weight, compared to the C group (Tables 3 and 4), indicates that the traits not considered in the selection for growth rate are also subject to changes, but not proportionally to the traits covered by the breeding programme. Taking no account of wing weight in breeding works is due to the fact that the rate of breeding advance regarding particular traits displays an inverse tendency to the number of traits selected simultaneously in one population. It may, therefore, be speculated that in the case of wing weight, the economic value of this characteristics is too low, compared to e.g. weight of ma-

nor muscle parts, to consider it in selection plans. It also remains unconfirmed whether a decrease in the percentage contribution of wings in body weight of successive generations of birds has any impact on body functioning, as is the case with internal organs.

Along with a higher growth rate, apart from the expected changes in proportions of the anatomical conformation of turkeys, including: an increased percentage contribution of breast and leg muscles in body weight, negative effects of selection are being observed which, undoubtedly, include a diminished percentage contribution of internal organs and wings in body weight, and increased adiposity of birds (Brenøe and Kolstad 2000). According to Sonaiya and Benyi (1983), the best adiposity indicator in birds is the percentage contribution of abdominal fat in body weight. The primitive females were characterized by a considerably lower abdominal fat content, compared to the Big-6 females (Table 3). A higher percentage share of abdominal fat in body weight was, however, not reported in the case of Big-6 males, compared to the primitive males (Table 4). Sarica et al. (2009) also achieved significantly higher adiposity in fast-growing Hybrid females than in the slow-growing Bronze hens, but they also reported a similar dependency in males. Exact reasons of differences between results of our study and findings of other authors regarding males adiposity are, however, unknown. Presumably, they might have resulted from different conditions of birds maintenance. In a study by Sarica et al. (2009), turkeys were fed a feed mixture with a higher energy value and were using significantly smaller free-

-ranges. The feeding and rearing system may to a great extent affect the utilization of the genetic potential of birds in respect of this trait.

At the end of the rearing, both Big-6 females and males achieved a higher body weight than the primitive turkeys. This group was characterized by a higher weight of breast muscles (the superficial and the deep ones), leg muscles, wings, internal organs (liver, heart and gizzard), and adipose fat. The Big-6 hybrids demonstrated a significantly higher ($P \leq 0.01$) percentage contribution of the analyzed muscle parts in body weight, but a significantly lower ($P \leq 0.01$) percentage of internal organs. The percentage contribution of wings in body weight was also higher in the group of primitive turkeys, yet the difference was statistically insignificant. The Big-6 females were characterized by higher adiposity compared to the primitive females, a slightly higher percentage share of adipose fat was reported in the body weight of primitive males.

CONCLUSIONS

The observed negative effects of selection, including a lower percentage contribution of internal organs in body weight and greater adiposity, especially in the case of females, make that health problems resulting from disproportional body conformation of heavy turkeys may aggravate in the future. It seems that the only solution assuring further improvement of functional traits in this species, may appear to be consideration of internal organs weight in the breeding works. This will, however, be feasible only on

condition of in-depth recognition of the principles of those traits heritability and correlations between body weight and musculature of turkeys and the weight and percentage contribution of the discussed organs in the total body weight.

REFERENCES

- American Poultry Association, 2001: The American Standard of Perfection. APA, Troy, NY.
- AOAC, 2005: Official Methods of Analysis of AOAC International 16th Edition. Association of Analytical Chemists, Arlington, VA, USA.
- ASLAM M.L., BASTIAANSEN J.W.M., CROOIJMANS R.P.M.A., DURCO B.J., VEREIJKEN A., GROENEN M.A.M., 2011: Genetic variances, heritabilities and maternal effects on body weight, breast meat yield, meat quality traits and the shape of the growth curve in turkey birds. *BMC Genet.* 12, 14.
- BRENĚ U.Y., KOLSTAD K., 2000: Body composition and development measured repeatedly by computer tomography during growth in two types turkeys. *Poultry Sci.* 79, 546–552.
- CZARNECKI C.M., 1984: Cardiomyopathy in turkeys. *Comparative Biochemistry and Physiology* 77, 591–598.
- DAMME K., 2003: Ekologiczna Produkcja Indyków. II Międzynarodowa Konferencja Naukowo-Techniczna. UWM, Olsztyn, 16–18.
- FARUGA A., JANKOWSKI J., 1996: Indyki – Hodowla i Użytkowanie. PWRiL, Warszawa.
- HAHN G., SPINDLER M., 2002: Method of dissection of Turkeys carcasses. *World's Poultry Sci. J.* 58, 179–197.
- İŞGÜZAR E., 2003: Growth, carcass traits and meat quality of Bronze and White turkeys in Isparta province of Turkey. *Arch. Tierz., Dummerstorf* 45, 471–481.

- JULIAN R.J., 1998: Rapid growth problems: ascites and skeletal deformities in broilers. *Poultry Sci.* 77, 1773–1780.
- KAMARA D., GYENAI K.B., GENG T., HAMMADE H., SMITH E.J., 2007: Microsatellite market-based genetic analysis of relatedness between commercial and Heritage turkeys (*Meleagris gallopavo*). *Poultry Sci.* 86, 46–49.
- LAY J.C., ROSE S.P., KIRBY L.J., 2002: Development of the digestive tract in fast and slow-growing strains of turkeys and chickens. *Br. Poultry Sci.* 43, 59–60.
- NESTOR K.E., SAFI M.Y., EMMERSON D.A., ANTHONY N.B., 1995: The influence of genetic changes in body weight, egg production, and body conformation on organ growth of turkeys. *Poultry Sci.* 74, 601–611.
- REED K.M., MENDOZA K.M., JUNEJAB., FAHRENKRUG S., VELLMAN S., CHIANG W., STRASBURG G.M., 2008: Characterization of expressed sequence tags from turkey skeletal muscle. *Anim. Gen.* 39, 635–644.
- SARICA M., OCAK N., KARACAY N., YAMAK U., KOP C., ALTOP A., 2009: Growth, slaughter and gastrointestinal tract traits of three turkey genotypes under barn and free-range housing systems. *Br. Poultry Sci.* 50, 487–494.
- SONAIYA E.B., BENYI K., 1983: Abdominal fat in 12 to 16 weeks old broiler birds as influenced by age, sex and strain. *Poultry Sci.* 62, 1993–1999.
- SPSS, 2006: SPSS, 14.0 for Windows user's guide, 2006. SPSS Inc., USA.
- SÜTŐ Z., HORN P., KUSTOSNĚ PÖCZE O., HERENDY V., GYENIS J., MIHÖK S., 2001: Effect of genetic and nutrition conditions on the meat production of turkeys. *Baromfiágazat* 1, 28–34.
- WERNER C., RIEGEL J.V., WICKE M., 2008: Slaughter performance of four different turkeys strains, with special focus on the muscle fiber structure and the meat quality of the breast muscle. *Poultry Sci.* 87, 1849–1859.
- WILKIEWICZ-WAWRO E., WAWRO K., LEWCZUK A., MICHALIK D., 2003: Correlation between the thickness of breast muscle and meatiness in turkeys. *Czech J. Anim. Sci.* 48 (5), 216–222.
- Streszczenie:** Wpływ genotypu indyków na wyniki wydajności poubojowej. Dwie grupy indyków: wolnorosnące prymitywne (♂♂ $n = 80$ i ♀♀ $n = 80$) i szybkoorosnące Big-6 (♂♂ $n = 80$ i ♀♀ $n = 80$) odchowywano w systemie półintensywnym. Indyckizki utrzymywano do 14. a indory do 22. tygodnia życia. Do uboju i analizy rzeźnej wybrano po 12 samic i 12 samców o masie ciała średniej dla płci w grupie. Stwierdzono istotnie wyższą ($P \leq 0,01$) masę ciała i wszystkich części ciała u obu płci indyków Big-6. Wyjątek stanowiły u indorów masa tłuszczu brzuszego ($P \leq 0,05$) i masa żołądka mięśniowego (NS). Indyckizki Big-6 wykazały istotnie wyższy ($P \leq 0,01$) udział procentowy w masie ciała mięśni piersiowych (o 8,7), mięśni nóg (o 2,4) i tłuszczu brzuszego (o 0,9), ale istotnie niższy ($P \leq 0,01$) skrzydeł, serca, wątroby i żołądka mięśniowego (odpowiednio o 0,2, 0,1, 0,3, 1,9). Indory Big-6 wykazały istotnie wyższy ($P \leq 0,01$) udział procentowy mięśni piersiowych (o 16,5) i mięśni nóg (o 2,5, $P \leq 0,05$), ale niższy ($P \leq 0,01$) skrzydeł, serca, wątroby i żołądka mięśniowego (odpowiednio o 0,3, 0,2, 0,7, 1,5) w porównaniu do indyków prymitywnych.

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The effect of herbal formula on the amount of water intake by the chinchilla (*Chinchilla laniger*)

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Abstract: *The effect of herbal formula on the amount of water intake by the chinchilla (*Chinchilla laniger*). The aim of this study was to determine the amount of water intake of the chinchilla in a day and to check whether addition of herbal formula with an intense aroma and sweet taste to the water will affect the amount of water intake. Based on our experience it can be concluded that there are large differences in daily water intake between individuals in the sample and individual differentiation is the only factor that influenced the occurrence of differences. Other factors such as gender or provision of hay, did not affect significantly (statistically significant) on water intake. Experience has shown that there are highly significant differences in the consumption of water by the same animals before and after addition of the herbal preparation with a sweet taste. However, the addition of compounds, or drugs in the water requires the prior observation of the animal and determine the quantity of water consumed by it, due to the large differences between individuals. The study, which would give the answer if we should continue researching whether the sex, weight, age or other factors can affect the intake of the water by the chinchilla.*

Key words: chinchilla, water uptake, herbal preparation, taste preference.

INTRODUCTION

Water is an essential component of the body. It is a very good solvent, allows the transport of nutrients, digestion by-products and biocatalysts. It is involved

in regulating body temperature. It is essential in digestion and defecation. Without sufficient water supply the growth and reproduction are impeded. Lack of water prevents the hydrolytic enzymes to take effect (Minakowski and Weidner 1998, Meyer et al. 2006). Animal Protection Law of August 21, 1997, requires each holder of the animals to ensure their access to water (Dz.U. z 1997 r. nr 111 poz. 724).

The sense of taste mediates between the inside and outside of the body. It has characteristics typical of the other senses, such as the quality and intensity. The role of flavour is to assess the desirability of the potential food consumption in relation to current physiological needs. It is the taste that ultimately determines whether a substance is introduced into the body or not (Scott 2009).

It is assumed that the human perception of taste is based on a combination of five flavours:

- sweet, related to the presence of simple carbohydrates;
- umami, generated by peptides and amino acids;
- salty, related to the presence of Na ions and sometimes other ions;
- sour, generated by the presence of acids;

- bitter, related to potential poisons, such as alkaloids (Breslin and Spector 2008).

Some species of mammals are not able to distinguish all of the above. But there are also species that can differentiate more of them. These flavours are usually generated by the polysaccharides, starch and fats (Breslin and Spector 2008). Most animals are actively looking for sweet foods. Naturally occurring substances with a sweet taste are sugars such as glucose, but also some amino acids such as glycine (Breslin and Spector 2008).

Experience of the salty taste is generated in rodents only by sodium or lithium ions (lithium). For herbivore rodents and some omnivore rodents it is extremely important to find salty foods because their bodies do not have the storage capacity of these ions. As you know, sodium is essential to life, and animals are losing it steadily as a result of different processes. The salty taste for them is a sodium detector for them in a sense (Breslin and Spector 2008).

The presence of the sour taste may be a warning signal, as it often means that the food went bad (Miyamoto et al. 2000, Breslin and Spector 2008). Ability to feel the bitter taste is also a warning system, because they are often combined with the presence of toxins in the food. For every person the experience of the sour flavour is very diverse. The experience of the same flavour may be different for every person (Breslin and Spector 2008).

In addition to these flavours there are others that are not yet fully understood, but there is no doubt that they are no less important. Rodents can taste the

polysaccharides and starch, which is different from an ordinary sweet flavour. Animals can sense the flavour of fat. We don't know however, whether this is based on touch and fragrance or the existence of fat. Metallic taste is known for a long time. This feeling may be due to a combination of different taste sensations (Breslin and Spector 2008).

The ability to perceive, recognize and respond to chemical signals is essential for the survival of animals. New-born mammals must be able to distinct between different flavours and choose those that will enable them to grow. With age, the number and morphology of taste buds changes. The taste buds reach maturity after 30 days after birth (Andres-Barquin and Conte 2007, Zhang et al. 2008). Thanks to these cells it is possible to distinguish the taste. Their function is to monitor the chemical environment of the mouth, and in particular, assessment of what food is tasty, which is toxic, and which has high nutritional value for the body. The signals generated by the taste buds are transmitted to higher centers in the brain (Roper 2009).

The aim of this study was to determine the amount of water absorbed by the chinchilla on a daily basis and to also check whether the addition of herbal formula with an intense aroma and flavour to the water will affect the intake of water.

MATERIALS AND METHODS

The experiment was conducted on 18 chinchillas (12 males and 6 females), aged from 2 to 6 years. None of the animals were in heat.

Chinchillas were kept in standard farming cages. Each cage was equipped with a pan, allowing the drinker and the dust bathing house. The room in which they were housed during the experiment is equipped with air conditioning, which provides a constant micro-climate conditions: temperature of 19°C, relative humidity 45%. The light are electronically controlled 12 : 12 (day : night).

Chinchillas were fed granulated feed at libidum. In addition, two times a week they were fed hay. Unlimited hay was provided for a period of 24 hours, and after that whatever was left was removed.

Water was supplied in plastic bottles. Water was supplied to the cage with a rubber hose with a baby bottle top. The water in the dispensers was replaced every day. The experiment was divided into two parts. The first part, lasting from 8th to 21st of December, was based on a determination of the amount of water collected per day by individual animals. During the experiment the daily consumption of water was measured 11 times. For the second part of the experiment 13 chinchillas were randomly selected and divided into two groups. First group comprised of 7 animals (6 males, 1 female), the other group comprised of six animals (4 males, 2 females). The first group continued to receive clean water to drink. The second group received the water formula consisting 1 ml of Restobal (by Farmwet) per animal per day.

Restobal, a herbal immunomodulator with an intense odour, used as an anti-stress formulation. Further preferably stimulates the immune system of animals. This preparation, in addition to herbs (*Withania somnifera*, *Ocimum sanctum*, *Phyllanthus emblica*, *Mangi-*

fera indica), contains in its composition molasses (molasses) – by-product of sugar production in the food, that sweet taste comes from sucrose.

The second part of the experience lasted from the 18th of February to 5th of March. At that time, 11 measurements of water consumption by animals were performed daily. Measurement methods in either case was the same (measured once a day at the same time).

In the experiment we tried to find out if the addition of herbal preparation to the water has an impact on the amount of water intake of the animals, and if water intake is dependent on their diet (hay application).

For statistical analysis of results the SPSS 17.0 program was used. Was used a multivariate analysis of variance, which demonstrated that the results differences between the groups were statistically significant. Has been checked if sex of the animals, bigger ration of hay and the herbal formula has an impact on the amount of water used. In addition to the multivariate analysis of variance also performed Wilcoxon's test (Olech and Wieczorek 2002), to determine changes in water intake in both parts of the experiment. Animals which received clean water and the ones which received in phase II of the experience water supplemented with herbal preparation were analysed separately.

RESULTS AND DISCUSSION

In the first part of the experiment our research has shown that the average consumption of water by the chinchilla was 33.36 ml per individual per day (Table 1).

TABLE 1. The average amount of water intake in both parts of the experiment

Part of the experiment	Number of animals	X ±SD	
		I	II
I – a whole group	18	33.36 ±19.32	
II – water	7	31.91 ±16.13	33.90 ±11.06
II – water with herbal supplement	6	30.76A ±21.73	37.00B ±20.21

– A, B $P \leq 0.01$;

– Means marked with different capital letters differ significantly.

In the second part of the experience the average water intake was 33.90 ml of water per individual per day (Table 1). These same animals were drinking an average of 31.91 ml of water per individual per day in the first part of the experiment. Comparison of water intake by the same animals in I and II part of the experiment are shown in Figure 1.

In the second part of the experiment an average consumption of water with the herbal preparation was 37.00 ml per individual per day (Table 1). These animals were drinking an average of 30.76 ml

of water in the first part of the experiment. Comparison of water intake in the first part of the experiment and herbal water in the second part of the experiment by the same animals are shown in Figure 2.

From the 18 animals tested, 8 had a daily water consumption according to previously published studies, that is in between 20–30 ml (Jarosz and Rżewska 1996). Three individuals drank less water per day (no. 1 – 16.09 ml, no. 8 – 18.82 ml, no. 10 – 19.55 ml), and 7 animals achieved results far exceeding those val-

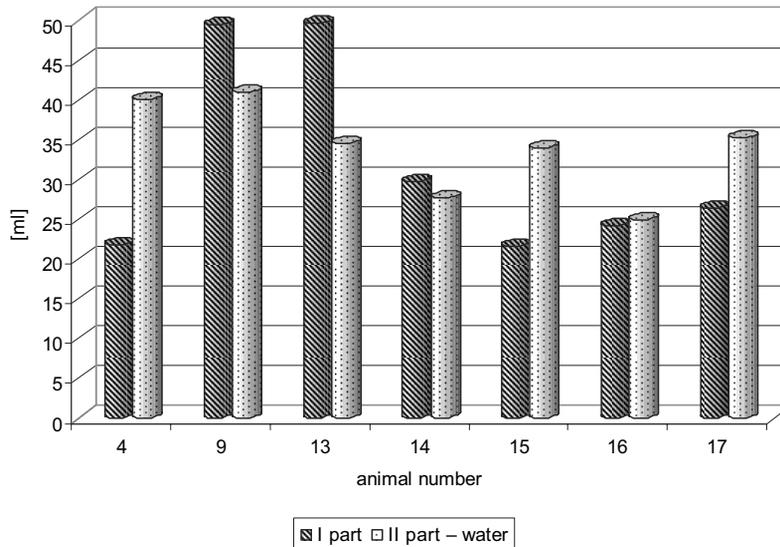


FIGURE 1. Mean water intake (ml) by individual animal in parts Ist and IInd of the experiment

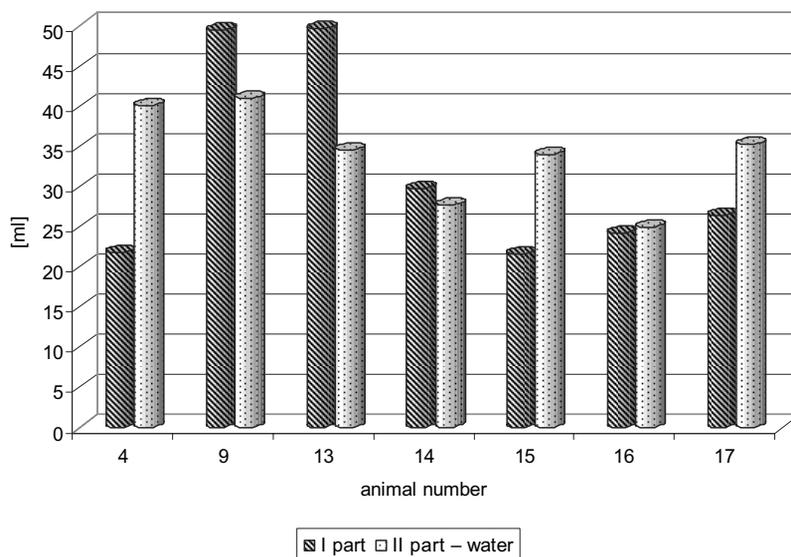


FIGURE 2. Mean water intake (ml) by individual animal in Ist part of the experiment and the mean intake of water with herbal supplement in IInd

ues. Animal number 3 had the highest average quantity of water consumed per day (69.09 ml) and it also had the biggest average intake (66.91 ml) in the second part of the experiment. Slightly different results were presented by Sakar et al. (2005) where chinchilla's average water intake ranged from 38.25 to 39.2 ml of water per day. Rak's research (2007) showed that an average water consumption per day in the control group was 21.67 ml per chinchilla.

Chinchillas originate from South America. It is known that rodents from this continent are adapted to live in conditions characterized by low water content in the environment. Bozinovic et al. (2007) conducted an experiment that was to confirm this. They examined the physiological responses of eight species of rodents captured and removed from the natural environment. All their habitats varied in degrees of humidity,

but all in all the humidity was relatively low. After a period of adaptation to new environment the rodents tested to verify the body's response to water deprivation. The results were compared on an interspecific and intraspecific level. As it turned out, there are significant differences in response to water deprivation between the different species. Animals responded with weight loss, but to different degree. Individual differentiation was observed. Influence of the habitat on change in water intake was not noted.

Individual differentiation in water intake noted in experiments means different demands for water in tested individuals. The smallest value observed is 2 ml, the largest 100 ml. Difference between these values is very large (98 ml).

Figure 1 shows the differences in an average water consumption during first and second part of the experiment. This differences are not statistically signifi-

cant. For specimens no. 4, 15, 16 and 17 water consumption has increased in the second part of the experiment, whereas for individuals 9, 13 and 14 it declined. Average water consumption in part I was smaller for the same individuals than in part II and amounted 31.91 and 33.90 ml, respectively. Based on observations it is difficult to determine what caused these differences. Animals were kept in the same conditions of temperature and humidity. The feed did not change. According to some sources (Gromadzka-Ostrowska 1998), chinchillas can stop drinking water even for a period of two weeks. It is probably possible, but only in their natural environment. There, rodents eat plant with very succulent tissue. One of the most consumed plants is *Puya berteroniana* (Cortes et al. 2002). In this experiment the lowest daily water consumption was 2 ml. This result demonstrates that in an artificial, man-made environment, fed with industrial feed, chinchillas must have constant access to fresh water.

In the experiment, there was no statistically significant difference in water intake after feeding hay to the animals. There is therefore no confirmation of the Buffenstein's experiment results (1984), which stated that basing the diet on feed with a high content of dietary fibre makes the demand for water higher than other diets.

Also, sex does not significantly affect the water consumption, which differs from the studies that were conducted on rats of both sexes. Studies have shown that female rats have a greater demand for water than males (McGivern et al. 1994).

Average water consumption after addition of the herbal formula was 37 ml

thus was higher than average water consumption by the same individuals in the first stage of the experiment (30.76 ml). After adding the formula consumption of water increased by 20%, which may indicate a preference to the taste. In the experiment on chinchillas conducted by Sakar et al. (2005), after administering a various doses of sulfadimidine (a bacteriostatic drug), water consumption decreased by 10 and 24%.

The result obtained in the Wilcoxon's rank sum test for two codependent sample groups showed that adding the herbal formula to water resulted in high, statistically significant difference between groups – 0.004 ($P < 0.01$). Comparing two groups, which in both parts of the experiment consumed only water with no additives had no statistically significant differences (Table 1).

In the experiment carried out on different strains of mice, which tried determine the effect of sweet flavour on the amount of water consumed, it turned out that taste preferences are largely dependent on the tribe. This may indicate that the degree to which animals are taste the flavour is linked to the gene pool (Sclafani 2006).

CONCLUSION

Based on our experience it can be concluded that there are large differences in daily water consumption between individual chinchillas in the sample. Differences between individuals are the only factor in different water consumption. Other factors such as gender or hay provision, did not have statistically significant influence on water intake.

It has been proved though, that there are significant differences in the water consumption by the same animals before and after adding the sweet herbal formula. However, the addition of formula or drugs to the water requires prior observation of the animal to determine the quantity of water consumed, due to the large differences between individuals.

Studies of this type would give us the answer to the question of whether water consumption of a chinchilla can be affected by that animal's sex, weight, age or other factors, and therefore should be continued.

REFERENCES

- ANDRES-BARQUIN P.J., CONTE C., 2007: Molecular basis of bitter taste. *Cell Biochemistry and Biophysics* 41, 99–112.
- BOZINOVIC F., CRUZ-NETO A.P., CORTES A., DIAZ G.B., OJEDA R.A., GIANNONI S.M., 2007: Physiological diversity in tolerance to water deprivation among species of South American desert rodents. *Journal of Arid Environments* 70, 427–442.
- BRESLIN P.A.S., SPECTOR A.C., 2008: Mammalian taste perception. *Current Biology* 18, 148–155.
- BUFFENSTEIN R., 1984: The effect of a high fibre diet on energy and water balance in two Namib desert rodents. *Journal of Comparative Physiology. B: Biochemical, Systemic, and Environmental Physiology* 2, 211–218.
- CORTES A., MIRANDA E., JIMENEZ J.E., 2002: Seasonal food habits of the endangered long-tailed chinchilla (*Ch. lanigera*): the effect of the precipitation. *Mammalian Biology* 67, 167–175.
- GROMADZKA-OSTROWSKA J., 1998: Studia nad fizjologią szynszyli ze szczególnym uwzględnieniem rozrodu i odporności. Zeszyty Naukowe Akademii Rolniczej im. H. Kołłątaja w Krakowie, Rozprawy nr 238.
- JAROSZ S., RZEWSKA E., 1996: Szynszyle, chów i hodowla. PWRiL, Warszawa.
- McGIVERN R.F., HENSCHEL D., HUTCHESON M., PANGBURN T., 1994: Sex difference in daily water consumption of rats: effect of housing and hormones. *Physiology & Behavior* 59, 653–658.
- MEYER U., STAHL W., FLACHOWSKY G., 2006: Investigations on the water intake of growing bulls. *Livestock Science* 103, 186–191.
- MINAKOWSKI W., WEIDNER S., 1998: *Biochemia kręgowców*. PWN, Warszawa.
- MIYAMOTO T., FUJIYAMA R., OKADA Y., SATO T., 2000: Acid and salt responses in mouse taste cells. *Progress in Neurobiology* 62, 135–157.
- OLECH W., WIECZOREK M., 2002: Zastosowanie metod statystyki w doświadczeniach zootechnicznych. Wydawnictwo SGGW, Warszawa.
- RAK K., 2007: Wpływ dodatku tłuszczu zwierzęcego i roślinnego na strawność paszy u szynszyli. Praca magisterska wykonana w Zakładzie Hodowli Zwierząt Futerkowych i Drobno Inwentarza SGGW w Warszawie, maszynopis.
- ROPER S.D., 2009: Parallel processing in mammalian taste buds. *Physiology & Behavior* 97, 604–608.
- SAKAR D., PREVENDAR CRNIĆ A., JANIĆ D., SAKAR T., 2005: Safety of peroral sulfadimidine sodium treatment in chinchillas (*Chinchilla lanigera*). *Veterinarski Arhiv* 75 (4), 283–291.
- SCLAFANI A., 2006: Fat and sugar flavor preference and acceptance in C57BL/6J and 129 mice: Experience attenuates strain differences. *Physiology & Behavior* 90, 602–611.
- SCOTT T.R., 2009: Taste, reward, and physiology. *Chemosensory Perception* 3, 3–15.
- Ustawa z dnia 21 sierpnia 1997 r. o ochronie zwierząt (Dz.U. z 1997 r. nr 111 poz. 724).

ZHANG G., ZHANG H., DENG S., QIN Y., WANG T., 2008: Quantitative study of taste bud distribution within the oral cavity of the postnatal mouse. *Archives of Oral Biology* 53, 583–589.

Streszczenie: *Wpływ dodatku preparatu ziołowego na ilość pobranej wody przez szynszyle.* Celem badań było określenie ilości pobieranej wody przez szynszyle w ciągu doby, oraz sprawdzenie czy dodatek preparatu ziołowego o intensywnym zapachu i słodkim smaku wpłynie na ilość pobranej wody. Podawanie preparatów bądź lekarstw do wody wymaga wcześniejszej obserwacji zwierzęcia i ustalenia ilości pobieranej przez niego wody, ze względu na duże różnice między osobnikami. Smak decyduje ostatecznie czy dana substancja zostanie wprowadzona do organizmu, czy też nie. Doświadczenie wykazało, że istnieją bar-

dzo duże statystycznie istotne różnice w spożyciu wody u tych samych zwierząt przed i po dodaniu preparatu ziołowego o słodkim smaku. Istnieją duże różnice w dobowym pobraniu wody między poszczególnymi osobnikami w próbie, a zróżnicowanie osobnicze to jedyny czynnik, który miał wpływ na występowanie tych różnic.

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Influence of prebiotics and butyric acid on the composition of intestinal microflora of broiler chickens

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Abstract: *Influence of prebiotics and butyric acid on the composition of intestinal microflora of broiler chickens.* The main problem addressed in this study was to determine the influence of prebiotic preparations DSM Nutritional Products (Biofit Booster and Alphamune) containing hemicelluloses in the form of extracts from the cell wall of *Saccharomyces cerevisiae* yeast and butyric acid (preparation Adimix) on the quantitative and qualitative status of intestinal microflora of broiler chickens. The study demonstrated a highly beneficial effect of the Alphamune food additive, containing glucans and mannans as an extract from *Saccharomyces cerevisiae* yeasts cell wall (index *colilacto*: 0.21).

Key words: intestinal microflora, prebiotics, butyric acid, broiler chickens.

INTRODUCTION

After the removal of antibiotic growth promoters from diets for poultry and pigs, commonly used are their substitutes, i.e. probiotics, prebiotics and organic acids, adapted for intestinal lactic acid bacteria (LAB). Most frequently use is made of plant preparations in the form of herbs and spices (Wężyk and Urbańczyk 2002, Michalczuk et al. 2008), and of enzymatic preparations: amylase, protease, xylanase, and phytase (Gornowicz and Koślacz 1998). Health and production performance of broilers chicken is in-

fluenced by the composition of bacterial microflora of the gastrointestinal tract. Therefore, preparations that do not interfere with the state called homeostasis of the normal microflora, present in the form of indigenous intestinal colonizers, should be used to this end (Gajewska et al. 2003, 2009, 2010; Józefiak et al. 2002; Seskevičienė 2005a, 2005b). The primary task is to stimulate growth promoters, while the main microflora inhibits the growth of other groups of microorganisms (Michalczuk et al. 2008). A very good results as an increase of efficiency of broiler chickens breeding after prebiotic feed supplementation were described by Bednarczyk et al. (2011).

The aim of this study was to estimate the effect of applying prebiotic preparations containing hemicellulose in the form of extracts from the cell wall of *Saccharomyces cerevisiae* yeast and butyric acid on the status of intestinal microflora of broiler chickens.

MATERIAL AND METHODS

The experiment was conducted at a poultry farm of the Experimental Station (RZD) Wilanów-Obory on 800 broi-

ler chickens of ROSS 308 breed. One-day chicks were at random allocated to four groups of 200 chickens: control and three experimental ones. The birds were kept on the litter, according to the standards for broilers management, as recommended by the Ross company. Over the rearing period, the chickens from the control group and all experimental groups were fed mixtures having the following nutritional value: Starter (till 18 day) – 21.0% crude protein and 12.2 MJ of ME; Grower (19–36 days) and Finisher (36–42 days) – 19.0% crude protein and 12.5 MJ of ME. Composition of feed is presented in Table 1. The content of various growth stimulating preparations in the Starter and Grower constituted the differentiating factor (Table 2). In the Finisher, no additives were employed. DSM premix included the preparations. The composition of the applied preparations was as follows: Biofit Booster – a complex of natural yeasts and minerals (MOS – mannanoligosaccharides and β -glucans, in the form of an extract from the cell walls of *Saccharomyces cerevi-*

siae); Alphamune – an extract from the cell walls of yeasts (β -glucans, mannans and polysaccharides); and Adimix – a sodium salt of n-butyric acid.

For microbiological analyses, a ten-centimeter-long sections of the small intestine (from duodenum) were collected from six 42-days old chickens from each group. In total, 24 samples of intestinal digesta were analyzed in the study. The aim of those analyses was to determine quantitative and qualitative factors of intestinal microflora in small intestine digesta of broiler chickens.

The quantitative determination of small intestine digesta, received from the four feeding groups, was done by standard Koch's plate method, with surface inoculation (Salyers and Whitt 2003), according to earlier papers (Gajewska et al. 2000, 2010). The following microbiological rates were determined: the Most Probable Number (MPN) of sulphite-reducing *Clostridium perfringens* bacteria, grown on Wilson – Blair's medium; the total number of heterotrophic mesophilic bacteria grown on nutrient

TABLE 1. Feed composition (%)

Component	Starter	Grower	Finisher
	%		
Wheat	37.3	36.4	47.4
Maize	22.0	25.0	18.0
Wheat bran	–	0.5	–
HP 300 – protein soybean concentrate	1.5	–	–
Soybean meal 46%	30.0	29.5	25.6
Soybean oil	3.6	4.6	–
Fodder chalk	0.6	–	–
Premix Br-S	5.0	–	–
Premix Br-G	–	4.0	–
Premix Br-F	–	–	4.0

TABLE 2. Scheme of experiment – content in the premix DSM

Group	1	2	3	4
Preparation	Biofit Booster	Adimix	Alphamune	Control
Dose (g·t ⁻¹)	500	1000	750	–

agar medium and nutrient agar medium with 5% of defibrinated sheep blood addition; the total number of bacteria from the *Enterobacteriaceae*, grown on McConkey's medium; the total number of lactic acid fermentation bacteria (LAB) from the *Lactobacillaceae*, grown on Sabouraud's medium; the total number of the microscopic fungi, grown on Sabouraud's medium; the *colilacto* index, as a ratio of the total number of bacteria from the *Enterobacteriaceae* to the *Lactobacillaceae*.

Further on, the macroscopic tests of the grown colonies were carried out. The microscopic tests were performed on the ground of observations of live fungi and bacteria or bacteria stained with the Gram's method, made using a Nikon E600 microscope with camera. On the basis of the morphological and physiological properties, the species of the isolated strains were identified according to Bergey's Manual of Systematic Bacteriology (Vos et al. 2009) and filamentous fungi and yeasts by Fassatióva (1983).

The results obtained were evaluated with a one-way variance analysis, calculated with the least square method in a statistical software SPSS 14.0 PL for Windows (SPSS 2006).

RESULTS

The results of the quantitative composition of intestinal microflora of broiler chickens, administered feed mixtures

supplemented with prebiotic preparations and acidifier (butyric acid) are presented in Figure 1 and Table 3.

The highest increase in the number of heterotrophic bacteria in small intestine digesta, which grew on nutrient agar medium, was demonstrated in a group of the Adimix complementation (2). A slightly lower increase in the number of bacteria was recorded in a group where the chickens were receiving the Biofit Booster preparation (1). The lowest number was found in group 3, where the chickens were administered Alphamune formulation. The differences were not statistically significant. Chickens were in good healthy condition.

The administration of all the formulas 1–3 to chickens was observed to reduce the total number of rods of the *Enterobacteriaceae*, grown on McConkey's medium, compared with the control group. Feeding broiler chickens with the Alphamune preparation affected a decrease in the total number of heterotrophic bacteria, grown on nutrient agar medium and bacteria from the *Enterobacteriaceae*, as well as a beneficial increase in the number of lactic acid rods of the *Lactobacillaceae*.

The obtained counts of the *Enterobacteriaceae* (grown on McConkey's medium) and the *Lactobacillaceae* (grown on Sabouraud's medium) bacteria enabled determining the *colilacto* index (Table 4).

The evaluation of the quantitative composition of bacteria in digesta of the small intestine of the broilers, based

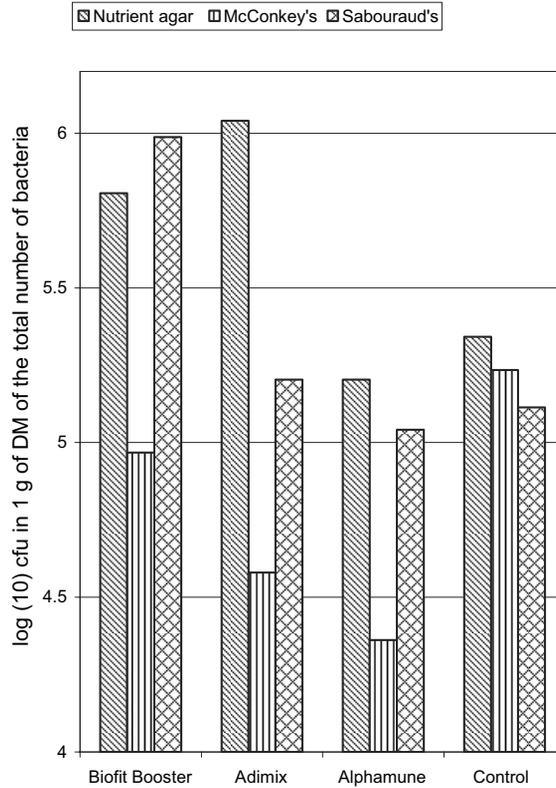


FIGURE 1. The average number of heterotrophic bacteria in intestinal digesta of broilers chicken grown on nutrient agar medium, McConkey's medium and Sabouraud's medium (log (10) cfu in 1 g of DM)

TABLE 3. Average number of of colonias of bacteria growth on nutrient agar medium, McConkey's medium and Sabouraud's medium (log (10) cfu in 1 g of DM)

Group	Specification	Nutrient agar medium	McConkey's medium	Sabouraud's medium
		log (10) cfu in 1 g of DM		
1	Biofit Booster	6.4×10^5	9.3×10^4	9.7×10^5
2	Adimix	1.1×10^6	3.8×10^4	1.6×10^5
3	Alphamune	1.6×10^5	2.3×10^4	1.1×10^5
4	Control	1.3×10^5	2.8×10^5	1.3×10^5
SE		1.22	9.93	5.00

on the obtained values of the *coli/lacto* index (the ratio of the number of bacteria from the *Enterobacteriaceae* to the *Lactobacillaceae*), showed that the best effects were achieved with the Biofit

Booster preparation (the lowest value – 0.10, compared to control – 1.39), and less favorable with the Alphamune preparation (*coli/lacto* index of 0.21). In the case of the Adimix preparation contain-

TABLE 4. Values of the *coli/lacto* index after prebiotics administration to broiler chickens

Group	<i>Coli/Lacto</i> Index
Biofit Booster	0.10
Adimix	0.24
Alphamune	0.21
Control	1.39

ing butyric acid, the *coli/lacto* index was observed to increase to a value of 0.24.

In all tested samples of the small intestine digesta there were no bacteria of the *Salmonella* sp. and *Shigella* genera, and β -hemolytic *Escherichia coli*, *Staphylococcus* sp., whereas analyses confirmed the presence of lac (-) *Proteus* sp., as well as of: *Pseudomonas* sp. and *Clostridium perfringens* bacteria, reducing sulphites, and actinomycetes of the *Streptomyces* genus. Hyphal fungi and yeasts occurred in less than 10^2 cfu in 1 g of DM of the small intestine digesta.

DISCUSSION

A positive effect of the applied prebiotics on the composition of intestinal microflora was indicated by the calculated *coli/lacto* index, which is the proportion of the number of intestinal bacteria of the *Enterobacteriaceae* to lactic acid rods from the *Lactobacillaceae*. This beneficial effect resulted in the improved production performance, including: body weight gain, feed consumption and survival rate. Niemiec et al. (2006) reported the higher body weight gains in a group of broilers treated with butyric acid, in the form of Adimix preparation; this group was also characterized by the lowest mortality. In each group of broilers, which were fed prebiotic preparations,

analyses showed lower feed conversion ratio and lower mortality, compared with the control group. Also Bednarczyk et al. (2011) found an increase of efficiency of broiler chickens after fed the diet supplemented with prebiotic. The addition of Adimix and Alphamune preparations exerting the most positive effect on the composition of intestinal microflora of broiler chickens, may consequently be of a positive – sanitary and microbiological significance for manure, used as a fertilizer, because neither sulphite-reducing pathogenic bacteria of the genus *Clostridium perfringens* nor *Salmonella* sp. were detected in any of the groups examined. Likewise Gajewska et al. (2000) did not reveal actinomycetes, filamentous fungi nor yeasts (below 10^2 cfu in 1 g of DM) and observed beneficial effects of prebiotic preparations on the composition of the indigenous intestinal microflora and parameters of rearing. In a study by Lipiński (2009), a preparation containing mannanoligosaccharides administered to chickens experimentally infected with *Salmonella typhimurium* has contributed to reducing the number of pathogens in the intestinal digesta. For better production performance, as well as more intense degree of colonization and multiplication of lactic acid bacteria (LAB) in the small intestine, use should be made of the coupled action of prebiotic preparations with probiotic preparations containing selected LAB, with strictly defined features referring to all criteria stimulated for probiotics. Ghareb and Bohm (2009) observed, that addition during 5 weeks of synbiotic Biomin IMBO preparation (a combination of *Enterococcus faecium* bacteria), a prebiotic (derived from chicory) and immune

modulating substances (derived from see algae), caused that broiler chickens stress responses (e.g. Heterophil/Lymphocyte ratio – H/L, after animals transportation) were evaluated. However, the action of butyric acid may be advantageously increased by utilizing the synergistic action of certain herbal extracts, essential oils, vitamin B₁₂ and carnitine (Różański 2010). These alternative feed additive, include products as enzymes, herbal products, microphlora enhancers, immunomodulators, organic acid, probiotics, prebiotics or combinations of these products (Midilli et al. 2008). Favourable effects of addition of prebiotics reflect in presence of antagonism towards pathogens, competition with pathogens, promotion of enzyme reaction, reduction ammonia and phenol products and increase oh pathogens resistance to colonization (Hajati 2010). Because probiotics can stimulate production of natural antibodies in chickens (Haghighi et al. 2006), additives of prebiotic preparations respectively chosen for probiotis, named together as synbiotics, probably can assure the best production performance.

CONCLUSIONS

All preparations tested had a positive impact on the composition of the bacterial of intestinal digesta of broiler chickens. The most beneficial effect on microbial intestinal state was achieved by using the prebiotic feed additive Alphamune containing glucans and mannans in the form of an extract from cell walls of *Saccharomyces cerevisiae* yeasts (decrease of total number of the heterotrophic bacteria and the low *coli/lacto* index – 0.21).

REFERENCES

- BEDNARCZYK M., URBANOWSKI M., GULEWICZ P., KASPERCZYK K., MAIORANO G.M., SZWACZKOWSKI T. 2011: Field and in vitro study on prebiotic effect of raffinose sp. oligosaccharides in chickens. *Bulletin of the Veterinary Institute in Pulawy* 55: 465–469.
- FASSATIOVA O. (1983) *Grzyby mikroskopowe w mikrobiologii technicznej*, Wyd. Nauk.-Tech., Warszawa.
- GAJEWSKA J., BUCKA J., ŻABIK A., RIEDEL J., MICHALCZUK J. 2009: Wpływ naturalnych preparatów roślinnych na stan mikroflory jelitowej brojlerów kurzych. *Ochrona Środowiska i Zasobów Naturalnych* 41, 302–309.
- GAJEWSKA J., GAZDA M., GORCZYŃSKA K. 2003: Oznaczenie wrażliwości wybranych szczepów bakterii i grzybów na wyciągi przypraw i ziół: Rozkład i korozja mikrobiologiczna materiałów technicznych. *Wyd. Politechnika Łódzka*, Łódź, 318–321.
- GAJEWSKA J., MICHALCZUK M., ŁUKASIEWICZ M., WILCZYŃSKA-CZYŻ K., NIEMIEC J. 2010: Influence of Aminokarnifarm preparate on composition of intestinal microflora of chicken broilers. *Ann. Warsaw Univ. of Life Sci. – SGGW* 47, 25–30.
- GAJEWSKA J., REKOSZ BURLAGA H., NIEMIEC J. 2000: Effect of addition of “Greenline” preparations to feed mixtures for broilers on composition of their intestinal microflora. *Acta Microbiol. Pol.* 51, 71–78.
- GHAREB K., BOHM J. 2009: Stress indicators to pre-slaughter transportation of broiler chickens fed diets supplemented with a synbiotic. *Int. J. Poultry Sci.* 8 (7), 621–625.
- GORNOWICZ E., KOŚLACZ J. 1998: Preparat enzymatyczny BioFeed Wheat i Alpha w żywieniu kurcząt brojlerów. *Post. Drob.* 36, 17–22.
- HAGHIGHI R.H., GONG J., GYLES L.C., HAYESA M.S., ZHOU H., SANEI B.,

- JAMES R., CHAMBERS R.J., SHARIF S. 2006: Probiotics stimulate production of natural antibodies in chickens. *Clin. Vacc. Immunol.* 13, 975–980.
- HAJATI H., REZAEI M. 2010: The application of prebiotics in poultry production. *Int. J. Poultry Sci.* 3, 298–304.
- JÓZEFIAK D., RUTKOWSKI A., FRĄCZAK M., FIDACH T. 2002: Zastosowanie wybranych zamienników antybiotykowych stymulatorów wzrostu w żywieniu kurcząt rzeźnych. *Rocz. Nauk. Zootech. (suplement)* 16, 211–215.
- LIPIŃSKI K. 2009: Rola dodatków paszowych w profilaktyce salmonelloz u drobiu. *Indyk Polski* 4, 7–12.
- MICHALCZUK M., GAJEWSKA J., GÓRSKA A., NIEMIEC J., REKOSZ-BURLAGA H. 2008: Efektywność preparatu roślinnego i kwasu 3-hydroksy-3-metylomasłowego w żywieniu kurcząt brojlerów. *Rocz. Nauk. PTZ* 4 (2), 65–72.
- MIDILLI M., ALP M., KOCABAGH N., MUGLALI O.H., TURAN N., YILMAZ H., CAKIR S. 2008: Effects of dietary probiotic and probiotic supplementation on growth performance and serum IgG concentration of broilers. *South African Journal of Animal Science* 38 (1), 21–27.
- NIEMIEC J., RIEDEL J., STĘPIŃSKA M., BORUTA A., STANISŁAWSKI P. 2006: Effect of the selected replacers of antibiotic growth stimulators on the results of rearing of broiler chickens. XVIII International Poultry Symposium PB WPS “Science for poultry – practice for science”, Rogów, 4–6 September, 153–157.
- RÓŻAŃSKI H. 2010: Kwasy organiczne i nieorganiczne jako alternatywa antybiotykowych stymulatorów wzrostu i kokcydiostatyków, <http://luskiewnik.strefa.pl/stymulatory/preview/pages/p16.htm>.
- SALYERS A.A., WHITT D.D. 2003: Mikrobiologia, różnorodność, chorobotwórczość i środowisko. Wyd. Nauk. PWN, Warszawa.
- SESKEVICIENE J., KOZŁOWSKI K., JANKOWSKI J., FARUGA A. 2005a: Use of a *Sanguinaria canadensis* L. preparation in turkey feeding. *Pol. J. Natur. Sci.* 18 (1), 83–91.
- SESKEVICIENE J., KOZŁOWSKI K., JANKOWSKI J., FARUGA A., JEROCZ H. 2005b: Erste Untersuchungen mit einem Präparat aus der Pflanze *Sanguinaria canadensis* L. bei Putenküken. *Vet. Zootech.* 30 (52), 88–91.
- SPSS, 2006: 14.0 for Windows user’s guide. SPSS Inc., USA.
- VOS P., GARRITY G., JONEM D., KRIEG N.R., LUDWIG W., RAINEY F.A., SCHLEIFER K.H., WHITMAN W.B. (eds) 2009: *Bergey’s Manual of Systematic Bacteriology. The Firmicutes. Vol. III.* Springer, New York.
- WĘŻYK S., URBAŃCZYK J. 2002: Roślinne dodatki w żywieniu drobiu. *Przegl. Hod.* 9, 18–21.

Streszczenie: *Wpływ prebiotyków i kwasu masłowego na stan mikroflory jelitowej brojlerów kurzych.* Celem pracy było zbadanie wpływu wybranych preparatów prebiotycznych firmy DSM Nutritional Products (Biofit Booster i Alphamune), zawierających hemicelulozy w postaci wyciągów ze ściany komórkowej drożdży *Saccharomyces cerevisiae* oraz preparatu zakwaszającego Adimix (kwas masłowy) na ilościowy i jakościowy skład mikroflory jelitowej brojlerów kurzych. Stwierdzono bardzo korzystne działanie prebiotycznego dodatku paszowego Alphamune zawierającego glukany i mannany w postaci wyciągu ze ścian komórkowych drożdży *Saccharomyces cerevisiae* (wskaźnik *coli/lacto* – 0,21).

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A comparison of honey production in bee colonies with instrumentally inseminated and naturally mated queens

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Abstract: *A comparison of honey production in bee colonies with instrumentally inseminated and naturally mated queens.* The study compares honey production in bee colonies with instrumentally inseminated and naturally mated queens. Poland has a special place in the world in terms of the numbers of instrumentally inseminated queens. Our country uses more of them than all other countries taken together. The dense distribution pattern of apiaries in Poland prevents any control over natural mating, while at the same time many bee-keepers who look for valuable breeding material decide to use instrumentally inseminated queens in their honey-making colonies. The cost of purchasing an instrumentally inseminated queen should be compensated by an increased productivity of the colony. The study was aimed at determining whether that was possible. The comparison was carried out at a large professional apiary in the season of 2008. Two comparison groups were created of 12 colonies each. Honey was harvested 3 times during the season. The average production of honey in the group with instrumentally inseminated queens significantly exceeded average production in that with naturally mated queens.

Key words: queen bee, honey production, instrumentally inseminated queen.

INTRODUCTION

Instrumental insemination of queen bees in the conditions prevalent in our country is the only method of individual selection (Jasiński 1972). It has resulted in a major progress in bee breeding (Prabucki

and Chuda-Mickiewicz 1998, 2000a, 2000b). The procedure has become a routine mating method (Jasiński et al. 1985). It has the disadvantage of a delayed onset of brood development compared to naturally mated queens (Ruttner 1975, Moritz and Kuhnert 1984, Kono-packa 1989). Instrumentally inseminated queens, which have not begun the brood cycle yet, are more reluctantly received by the colonies, which increases the risk of losing some of the queens (Marcinkowski 1982, Skubida and Pohorecka 2000). Instrumental insemination enables the creation of crossbreeds, however, which are characterised by heterosis (Strabel 2005). Same-breed lines can be crossed as well when the offspring is expected to display the desirable characteristics of both lines (Paleolog 2006). Jasiński ed. (1998) reports that instrumental insemination may result in obtaining hybrid bees which are much more productive than colonies with the same queens mating naturally. Similar results have been obtained by Cale and Gowen (1956) in the natural environment conditions of the U.S.A. The goal of the study was to determine whether colonies with instrumentally inseminated queens in average nectar flow conditions could produce more honey than colonies of similar strength with naturally mated

queens. That would enable an economic evaluation of using instrumentally inseminated queen bees in honey-making colonies.

MATERIALS AND METHODS

The study was carried out at a large apiary with 280 colonies, located in the south-eastern part of the country, in the Roztocze region. 2 groups of 12 colonies each were selected: one with an instrumentally inseminated queen, the other with a naturally mated queen. The mothers were sister Carniolan bees. Instrumental insemination was performed with Caucasian drones, and a cross-bred hybrid was obtained. Naturally inseminated queens mated with randomly selected drones.

The study period covered the season of 2008. Honey production was measured with respect to 3 honey flows: orchard, rape, and linden. Honey from each flow was harvested and weighted separately. The average for each group was subsequently determined, and the significance of differences was measured using Student's *t*-test. Statistical calculations were performed using the SPSS programme.

RESULTS AND DISCUSSION

Colonies with instrumentally inseminated queens produced: 8 kg from orchard, 13.5 kg from rape, 15.9 kg from linden flow (with 0.1 kg accuracy). Colonies with naturally mated queens produced: 5.6, 10, 12.6 kg, respectively. In each case, the average production of honey in the group with instrumentally inseminated queens was higher than in the group with naturally mated queens (Table 1). The differences between average production in both groups were statistically significant.

The average honey production for the entire season was 37.34 kg in the group with instrumentally inseminated queens, and was approximately 32% higher compared to the group with naturally mated queens. In the Cale and Gowen study (1956) conducted in American conditions, in colonies with instrumentally inseminated queens producing 99.4 kg the difference was lower, at about 24%. Jasiński ed. (1998) reports that Prabucki and Chuda-Mickiewicz harvested the average of 80 kg honey from hybrid bees cross-bred between the Central European bee and the Caucasian bee. The same au-

TABLE 1. Honey production from individual honey flows

Nectar flows	Average (kg)		From – to (kg)		Standard deviation	
	Instrumentally inseminated	Natural mating	Instrumentally inseminated	Natural mating	Instrumentally inseminated	Natural mating
Orchards	7.96	5.75	5.5–10	3–8	1.60	1.66
Rape	13.46	10.00	10–15	7–13.5	1.74	2.27
Linden	15.92	12.63	14–18	9.5–15.5	1.44	1.93
Total	37.34	28.38	–	–	–	–

thor reports that cross-breeds of selected Norwegian lines and selected lines of the Caucasian bee produced more than 150 kg of honey per colony. Wilde et al. (2002) harvested 29.5 kg honey from instrumentally inseminated queens of the Kortówka line, which was 4.4 kg more than in the control colonies.

The production of honey and differences between colonies with instrumentally inseminated and naturally mated queens vary depending on natural conditions and the bee-keeping management approach. Jasiński ed. (1998) believes that instrumentally inseminated queens realize their potential only with intensive bee-keeping. Thus, their introduction in extensive apiaries will not produce the desired results. An experimental apiary is a rationally managed professional apiary, where the cost of purchasing instrumentally inseminated queen bees was more than compensated by the increased yield.

REFERENCES

- CALE G.H., GOWEN J.W., 1956: Heterosis in the honeybee (*Apis mellifera* L.). *Genetics* 41, 292–303.
- JASIŃSKI Z., 1972: Badania nad naturalnym i sztucznym unasienianiem matek pszczelich. Praca doktorska SGGW, Warszawa, typework.
- JASIŃSKI Z. (ed.), 1998: Naturalny i kontrolowany dobór u pszczół. *Pszczelnictwo*. Wydawnictwo Albatros, Szczecin.
- JASIŃSKI Z., TROSKIEWICZ J., ZAWILSKI A., 1985: Naturalne czy sztuczne unasienianie matek pszczelich. *Pszczelarstwo* 36 (5/6), 5–7.
- KONOPACKA Z., 1989: Czynniki wpływające nawyniki sztucznego unasieniania matek pszczelich. Rozprawa habilitacyjna. IsiK, Puławy.
- MARCINKOWSKI J., 1982: Porównanie kilku sposobów poddawania matek do rodzin pszczelich. *Pszczel. Zesz. Nauk.* 26, 48–50.
- MORITZ R.F.A. KUHNERT M. 1984: Seasonal effects of artificial insemination of honeybee queens (*Apis mellifera* L.). *Apidologie* 15 (2), 223–231.
- PALEOLOG J., 2006: Wybrane zagadnienia z hodowli pszczół. Materiały konferencyjne. Uniwersytet Przyrodniczy Lublin.
- PRABUCKI J., CHUDA-MICKIEWICZ B. 1998: Results of the middle European bee improvement in Western Pomerania. *Fol. Univ. Agric. Stet.* 36, 27–37.
- PRABUCKI J., CHUDA-MICKIEWICZ B., 2000a: Doskonalenie towarowe użytkowanych w Polsce ras pszczół. *Fol. Univ. Agric. Stet. Zootechnika* 39, 131–142.
- PRABUCKI J., CHUDA-MICKIEWICZ B., 2000b: Podnoszenie wydajności miodowej użytkowanych w Polsce ras pszczół. Część I, II. *Pszczelarstwo* 51 (10), 5–6; 52 (11), 5–7.
- RUTTNER F., 1975: Die instrumentelle Besamung der Bienenköniginnen. II Aufl. Apimondia. Verlag. Buckarest.
- SKUBIDA P., POHORECKA K., 2000: Wykorzystanie różnych typów klateczek i mateczników do poddawania matek. *XXXVII Nauk. Konf. Pszczel. Puławy*, 85–87.
- STRABEL T., 2005: Genetyka cech ilościowych zwierząt w praktyce. Materiały do zajęć. Uniwersytet Przyrodniczy w Poznaniu, Poznań.
- WILDE J., WILDE M., KOBYLŃSKI A. 2002: *Biuletyn Naukowy* nr 18.

Streszczenie: *Porównanie produkcji miodu w rodzinach pszczelich z matkami sztucznie i naturalnie unasienionymi.* W pracy porównano produkcję miodu w rodzinach pszczelich z matkami sztucznie unasienionymi i z matkami naturalnie unasienionymi. Pod względem ilości sztucznie unasienionych matek pszczelich Polska zajmuje w świecie wyjątkowe miejsce. W naszym kraju wykorzystuje się ich więcej niż we wszystkich pozostałych krajach łącznie. Gęste rozmieszcze-

nie pasiek w Polsce uniemożliwia jakąkolwiek kontrolę naturalnego kojarzenia, a z kolei wielu pszczelarzy, poszukując wartościowego materiału hodowlanego, decyduje się na wykorzystanie sztucznie unasienionych matek w rodzinach przeznaczonych do produkcji miodu. Koszt zakupu sztucznie unasienionej matki pszczelej powinien być rekompensowany zwiększoną produktywnością rodziny pszczelej. W badaniu starano się uzyskać odpowiedź, czy jest to możliwe. Porównanie przeprowadzono w dużej pasiece towarowej, w sezonie 2008. Utworzono dwie grupy porównawcze po 12 rodzin. Miód odbierano 3 razy w ciągu sezonu. Średnia produkcja miodu grupy

z matkami sztucznie unasienionymi przewyższała istotnie średnią dla grupy z matkami naturalnie unasienionymi.

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The occurrence of entomopathogenic fungi in two catchment basins of the Zagożdżonka and Zwolenka Rivers in Masovian Province, Poland

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Abstract: *The occurrence of entomopathogenic fungi in two catchment basins of the Zagożdżonka and Zwolenka Rivers in Masovian Province, Poland.* The aim of the study was determination of number and species composition of entomopathogenic fungi for two river catchments: Zwolenka and Zagożdżonka. Both are left side tributaries of the Vistula River and have different geological structure. The area is dominated by mosaic of extensive agriculture and forests. Three species of entomopathogenic fungi were found: *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea*. The dominant species in both catchment basins was *I. fumosorosea*. Infections caused by other factors were also noted (48% by entomopathogenic nematodes of the family Steinernematidae, remaining 19% by mites, saprophytic fungi, non-fruiting mycelia and other unidentified factors).

Key words: entomopathogenic fungi, soil.

INTRODUCTION

Polish landscape is dominated by agriculturally used areas. These lands are cut by running waters and artificial barriers like retention reservoirs or traffic routes that affect agricultural ecosystems. Agriculture intensification has recently resulted in unfavourable changes in the natural environment. This is particularly true for soil micro- and mesofauna. Natural dis-

asters like floods and long droughts may also trigger environmental changes.

There are more than 200 species of Entomophthorales, Hypocreales and Deuteromycota fungi in Poland. Above half of them are the species rarely isolated and sporadically infecting insects being thus of little economic importance (Bałazy 2000). Hyphomycetes from among other Deuteromycota are relatively common in soils and play a key role in limiting the density of pest insects in various types of ecosystems. Recent studies confirm a negative effect of some measures applied by humans on the diversity of these entomopathogenic fungi. We still know little about the factors affecting their occurrence, persistence, population structure and virulence to potential hosts (Tkaczuk 2008). Fungi of the genera *Beauveria*, *Metarhizium* and *Isaria* belong to most common species of Hyphomycetes in our country (Boczek and Lipa 1978). Species composition of entomopathogenic fungi in Poland is recognized rather well though not equally in different parts of the country (Bałazy 2000, 2004).

Another group of entomophages consists of entomopathogenic nematodes represented by two families Steinernematidae and Heterorhabditidae which compete with fungi in infecting insects at various stages of their development.

Many of them are adapted to infect one or only few related host species (Ignatowicz 1998). The intensity of infection by entomopathogenic fungi and nematodes in soils depends on many biotic and abiotic factors. Soil structure is, apart from food availability, an important factor determining the occurrence of fungi and nematodes in a given area.

The aim of this study was to compare the density and species structure of entomopathogenic fungi in catchment basins of two rivers: the Zwoleńka and the Zagożdżonka. Both are left side tributaries of the Vistula River and have different geological structure (Jadczyń et al. 2011). Soil cover of both catchments is dominated by very light and light soils built of sands. A characteristic feature of the two catchments is the prevalence of very acidic (pH 4.5) and acidic (pH 4.6–5.5) soils (Jadczyń et al. 2011).

MATERIAL AND METHODS

Soil samples were studied with the method of trap insects proposed and elaborated by Zimmermann in 1986 (Górny and Grün 1993). The method allows for isolating the most virulent isolates of entomopathogenic fungi. The fungi cultured on selective media are much less virulent than the same species obtained from insects (Miętkiewski et al. 1991).

Soil was sampled from particular sites with a small spade to a depth of

30 cm. Soil samples were collected from arable lands in 28 sites of the Zwoleńka River catchment and from 16 sites of the Zagożdżonka River catchment basin. From 15 to 20 subsamples were taken from each site and mixed thereafter to obtain an averaged sample. Too dry soil was wetted with distilled water. When soil was too wet (bog soil), the sample was left for several days to let it dry.

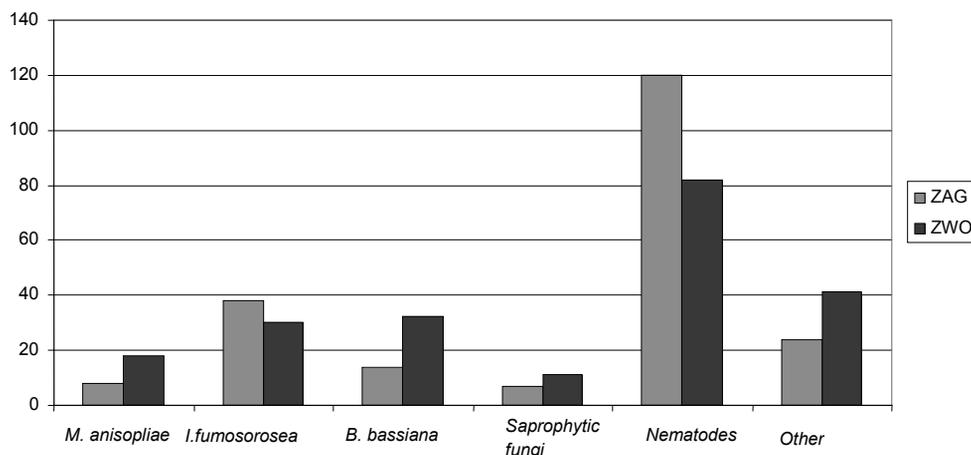
Soil samples were placed in plastic boxes of a volume of 250 cm³ and kept in an incubator for 1.5 months. Studies were carried out at a temperature of 20 and 25°C. Three repetitions were made for each site.

The greater wax moth (*Galleria mellonella* L., 1758) was used as a trap insect. First control of mortality was performed 5 days after placing boxes in the incubator and the next – every third day until the end of the experiment. Dead insects were sterilised, rinsed with distilled water and then laid out on sponges in glass Petri dishes filled with water. The dishes were kept in the incubators until the appearance of mycelium and production of spores.

Obtained results were analysed with the SPSS 14 software.

RESULTS AND DISCUSSION

Studies carried out in 2010 revealed 425 entomopathogenic infections in total. Thirty three percent of this number were caused by entomopathogenic fungi from the group Deuteromycota and 48% by entomopathogenic nematodes. The remaining 19% infections were caused by mites, saprophytic fungi, non-fruiting mycelia and other unidentified fac-



ZAG – the Zagożdżonka River catchment, ZWO – the Zwoleńska River catchment

FIGURE 1. Mortality of infected test insects *Galleria mellonella* in relation to the place of soil sampling

tors (Fig. 1). Part of the caterpillars of *G. mellonella* was infected by two or more infection factors. Three species of entomopathogenic fungi (*Beauveria bassiana* (Bals.-Criv.) Vuill., *Metarhizium anisopliae* (Metsh.) Sorokin, *Isaria fumosorosea* Wize) and nematodes of the family Steinernematidae were found and isolated in this study.

There was no site effect on the number of isolated fungi ($p > 0.05$). No statistically significant differences were noted between particular sites.

I. fumosorosea was the most often isolated species though in the Zwoleńska River catchment basin the infections caused by *B. bassiana* were more frequent. *M. anisopliae* was also isolated in this catchment. On the surface of larvae infected by *M. anisopliae* the presence of saprophagous mycelium was very often observed. Isolated fungi belonged to species that are numerous in various habitats in Poland and in other European countries (Vänninen 1995, Marjańska-

-Cichoń et al. 2005, Tkaczuk 2008). *B. bassiana* is typical for heavy soils rich in organic matter (Miętkiewski et al. 1991). Study area was dominated by light soils built of sands (Jadczyń et al. 2011), therefore, *B. bassiana* was isolated in smaller amounts. Thirty two mycelia were isolated from the Zwoleńska River catchment and only 14 from the Zagożdżonka River catchment. The third isolated fungus was *M. anisopliae* which prefers pastures, arable lands and meadows (Tkaczuk 2008). It was isolated from 8 sites in the Zwoleńska River catchment and from only 4 sites in the Zagożdżonka River catchment. Domination of this species in arable and meadow soils was noted by many authors e.g. Miętkiewski et al. (1988), Miętkiewski et al. (1991–1992), Tkaczuk (2008), Vänninen (1995). *M. anisopliae* is able to persist in soil longer than *B. bassiana*. The persistence of the latter species depends on the presence of its hosts, so the factors limiting host populations may affect the presence of this

fungus. In intensively used soils *B. bassiana* may have problems with finding appropriate host while conidia of *M. anisopliae* are able to persist on the primary host for a long period of time. Conidia of *M. anisopliae* are resistant to abiotic factors and biodegradation (Vänninen 1995, Tkaczuk 2008). Another factor limiting the presence of fungal colonies in soils is the availability of organic matter. The smaller amount of organic substances in soils the less units of entomopathogenic fungi are present there (Tkaczuk 2008). In most studied soils the content of organic matter was low ranging between 1.0 and 2.0% according to the assessment system adopted in Poland (Czyż and Reszkowska 2007).

Light soils prevailed in the study area. Such soils are more often dwelled by entomopathogenic nematodes. This is associated with a possibility of their movement in soil free spaces. Catchments of both rivers are also characterised by a high soil moisture facilitating the infection by nematodes which need less time to reach and infect the trap insect than do fungi. The fact that infected insect is less attractive for other pathogen is also of some importance. Temperature had no effect on isolation under laboratory conditions though in the natural environment it might be a factor determining the effective pathogenicity.

CONCLUSIONS

- Three species of entomopathogenic fungi were found: *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea*.

- The dominating species in both catchment basins was *I. fumosorosea*.
- Almost 50% of infections of test insects were caused by nematodes of the family Steinernematidae.

REFERENCES

- BAŁAZY S., 2000: Zróżnicowanie grup funkcjonalnych grzybów entomopatogenicznych. *Biotechnol.* 3 (50): 11–32.
- BAŁAZY S., 2004: Znaczenie obszarów chronionych dla zachowania zasobów grzybów entomopatogenicznych. *Kosmos* 53 (262): 5–16.
- BOCZEK J., LIPA J., 1978: Biologiczne metody walki ze szkodnikami roślin. PWN, Warszawa, 593 pp.
- CZYŻ E.A., RESZKOWSKA A., 2007: Próchnica w glebie. (in:) F. Woch (ed.). *Wademecum klasyfikatora gleb. IUNG-PIB w Puławach*, Puławy, 82–99.
- GÓRNY M., GRÜN L., 1993: *Methods in soil zoology*. PWN, Warszawa: 188–196.
- IGNATOWICZ S., 1998: Grzyby owadobójcze. *Nowoczesne Rolnictwo* 4: 44–45.
- JADCZYN T., IGRAS J., NOWOCIEŃ E., PODOLSKI B., WAWER R., 2011: Characteristic of soils in Zwolenka River watershed. *Sci. Rev. Eng. Env. Sci.* 52: 119–129.
- MARJAŃSKA-CICHOŃ B., MIĘTKIEWSKI R., SAPIEHA-WASZKIEWICZ A., 2005: Występowanie i skład gatunkowy grzybów owadobójczych w glebach z sadów jabłoniowych. *Acta Agrobot.* 58 (1): 113–124.
- MIĘTKIEWSKI R., DZIĘGIELEWSKA M., JANOWICZ K., 1988: Entomopathogenic fungi isolated in the vicinity of Szczecin. *Acta Mycol.* 33 (1): 123–130.
- MIĘTKIEWSKI R., TKACZUK C., ZASADA L., 1991–1992: Występowanie grzybów entomopatogenicznych w glebie ornej i łąkowej. *Acta Mycol.* 27 (2): 197–203.
- MIĘTKIEWSKI R., ŻUREK M., MIĘTKIEWSKA Z., TKACZUK C., 1991:

Przydatność wybranych gatunków owadów do wychwytywania grzybów owadobójczych z gleby. Zeszyty Naukowe WSR-P, Siedlce: 229–235.

TKACZUK C., 2008: Występowanie i potencjał infekcyjny grzybów owadobójczych w glebach agrocenoz i środowisk seminaturalnych w krajobrazie rolniczym. Rozprawa Naukowa, Siedlce in press: Akademia Podlaska: 1–160.

VÄNNINEN I., 1995: Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. Mycol. Research 100 (1): 93–101.

Streszczenie: Występowanie grzybów entomopatogenicznych na terenie dwóch zlewni rzek Zagożdżonki i Zwoleńka w województwie mazowieckim. Celem niniejszej pracy było poznanie liczebności i składu gatunkowego grzybów entomopatogenicznych występujących w dwóch zlewniach rzek Zwoleńka i Zagożdżonka. Zlewiska te charakteryzowała wysoka wilgotność, co sprzyjało bardziej infekcjom nicienieniowym niż grzybowym. Na badanym terenie zlewni obu

rzek stwierdzono występowanie w przewodzie *I. fumosorosea* nad innymi gatunkami grzybów entomopatogenicznych. W przypadku zlewni rzeki Zwoleńka przeważały infekcje wywołane przez *B. bassiana*. Izolowano także nicienie entomopatogeniczne z rodziny Steinernematidae (48%) a 19% stanowiły roztocza i grzyby saprofagiczne, nieowocujące grzybnie oraz inne czynniki.

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Changes in concentration of saturated fatty acid in milk of high performance Polish Holstein-Friesian cows during the lactation

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Abstract: *Changes in concentration of saturated fatty acid in milk of high performance Polish Holstein-Friesian cows during the lactation.* The purpose of this study was to examine the influence phase of lactation on the concentration of saturated fatty acid in milk of high performance dairy cows. The experiment was conducted on the twenty PHF cows at experimental dairy farm of the Warsaw University of Life Sciences. The cows were kept in a free-stall dairy shed and fed on TMR (*total mixed ration*) diet. The cows had continuous access to water, TMR diet was provided for ad libitum intake. Milk samples were collected 10-times during the first 10 month of lactation (once a month). Milk from the initial phase of lactation characterized by the lowest level of saturated fatty acid including: C_{12:0}, C_{14:0} and C_{16:0} and the highest level of BA compared with the final period. Stage of lactation significantly influenced the concentration of saturated fatty acids in milk of analyzed cows.

Key words: phase of lactation, saturated fatty acid, palmitic acid, miristic acid, cow's milk.

INTRODUCTION

Milk fat is synthesized either from fatty acid (FA) which are absorbed from the blood (about 60%) or by de novo synthesis in the mammary gland (about 40%). FA mainly are synthesized de novo from acetate and 3-hydroxybutyrate (3-HB). As the $\Delta 9$ desaturase has very low ac-

tivity with FA built of chains with less than 18 carbons, the most of the FA synthesized de novo are saturated fatty acids (SFA). According the Chilliard et al. (2000), only a small proportion of C_{14:0} and C_{16:0} is desaturated to C_{14:1} and C_{16:1}. The high concentration of SFA is typical for cow's milk, in which over 60–70% of the total fatty acids are saturated (Palmquist et al. 1993). Moreover, cow's milk contains a relatively low proportion of unsaturated fatty acids (UFA) and relatively high levels of LDL-cholesterol, what strongly affects the total content of FA, mainly C_{14:0} and C_{16:0}. Currently conducted studies indicated that SFA are etiological factor in many diseases. A diet high in SFA especially: lauric (C_{12:0}), myristic (C_{14:0}) or palmitic acids (C_{16:0}) significantly increases the risk of atherosclerosis and heart disease. More recent research showed that not all SFA have the same impact on serum cholesterol. For instance C_{12:0} and C_{14:0} acids have a greater total cholesterol raising effect than C_{16:0} acid. Additionally C_{18:0} has a neutral effect on the concentration of total serum cholesterol. It was found that these acids should not exceed more than 10% of total energy intake in daily ration for people.

The aim of the study was to examine influence of the stage of lactation on concentration of the SFA in cow's milk.

MATERIALS AND METHODS

The experiment was conducted on the twenty Polish Holstein-Friesian (PHF) cows (at 2nd lactation) at experimental dairy farm belonged to Warsaw University of Life Sciences. The cows were kept in a free-stall dairy shed and fed on TMR diet. The cows had continuous access to water, TMR (*total mixed ration*) diet was provided for ad libitum intake. The ingredient composition of the TMR

dose is presented in Table 1. The cows were milked daily at 05.30 and 17.30 and milk yield was recorded at each milking. Milk samples were collected 10-times during the first 10 months of lactation (once a month). The samples were collected at following stages of lactation: 8–30, 31–60, 61–90, 91–120, 121–150, 151–180, 181–210, 211–240, 241–270 and above 271 day of lactation.

Milk analyses

Basic parameters of the milk, i.e. fat and protein content, were determined by automated infrared analysis with a Milko-scan FT – 120 instrument (Foss Electric).

TABLE 1. Ingredient composition of the ration and concentrate (kg)

Ingredients	Feeding group		
	1	2	3
Feeding ration (kg)			
Maize silage	27.00	23.00	20.00
Alfaalfa silage	7.50	10.00	15.00
Maize corn silage	5.70	4.40	1.00
Concentrate	8.06	5.10	2.20
Straw	1.00	–	1.00
Total	49.26	42.50	39.20
Concentrate (kg)			
Hydropalm – by pass oil	0.60	–	–
Fodder chalk	0.20	0.15	0.10
NaHCO ₃	0.15	0.10	–
BetaLac – premix	0.20	0.10	0.10
NaCl	0.05	0.05	–
Rapeseed meal	2.40	2.30	2.00
Soya meal	3.10	1.50	–
Grain meal	1.30	1.30	–
Rumex	0.005	–	–
Ca ₃ (PO ₄) ₂	0.05	–	–
Total	8.06	5.50	2.20

For examination of the milk fat acid composition, extraction of fat was performed according to Röse-Gottlieb procedure (AOAC, 1990) at a room temperature. Fatty acid methylation was performed according to the transesterification method according to EN ISO 5509 method. The identification with fatty acid standards and quantitative determination of individual fatty acids in crude fat was conducted in Agilent Technologies 7890A GC with ChemStation software G2070BA, a flame-ionization detector (FID) and capillary column Mega-10; 60 m length, 0.25 mm in diameter and 0.32 µm thick. The separation was performed at pre-programmed temperature: 80°C for 1 min; 80–170°C at 6.5°C per min, 170°C for 11 min; 170–215°C at 2.75°C per min; 215°C for 3 min. Other parameters were: carrier gas (He) with constant flow rate 1.3 ml per min; split sample injector 50:1; injector temperature 250°C; and detector temperature 270°C.

Statistical analyses

The obtained data were analyzed statistically using the anova analysis of variance (least squares) by means of the SPSS 19.0 packet software by the model 1.

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

where:

Y_{ijk} – dependent variable,

A_i – stage of lactation effect ($i = 1-10$),

e_{ij} – standard error.

RESULTS AND DISCUSSION

Table 2 presents the influence of lactation stage on the content of protein and fat in cow's milk. The highest level of protein was observed in milk collected at the 9th and 10th collecting (in the final phase of the lactation) and the lowest level at the 2nd and 3rd one. The similar trend was noted in case of fat concentration. As the lactation progressed increased

TABLE 2. Changes in the protein and fat content in percent, during lactation

Collecting	Protein		Fat	
	LSM	SE	LSM	SE
1	3.52 ^{ABCD}	0.397	5.01 ^{ABCDEFghi}	0.755
2	3.03 ^{AEFGHIJK}	0.203	3.54 ^{AKLlmNOU}	1.081
3	3.12 ^{BILMNOUP}	0.241	2.83 ^{BKPRSTNXY}	1.333
4	3.31 ^{CEIRSTUWX}	0.278	2.92 ^{CLDZZZA'B'C'}	1.174
5	3.52 ^{FLRY}	0.196	3.97 ^{DRZD'}	0.698
6	3.55 ^{GMSz}	0.269	4.10 ^{ELSŻ}	0.720
7	3.55 ^{HNTz}	0.242	4.09 ^{FmTŻ}	0.591
8	3.53 ^{IOUa'}	0.252	4.36 ^{gNWA'}	0.640
9	3.68 ^{JUW}	0.269	4.48 ^{hOXB'D'}	0.669
10	3.76 ^{DKPXYzza'}	0.306	4.48 ^{iVYC'}	0.667

– SE – standard error of the mean;

– LSM – least square of mean;

– A,B,C,D $P \leq 0.01$; a,b,c,d $P \leq 0.05$.

concentration of fat and protein in milk were stated. DePeters and Cant (1992) in their studies observed that the reduction in protein concentration in milk is often caused by the dilution effect, rather than its reduced synthesis in mammary gland and it's always is associated with an increase in the milk yield.

Milk FA composition also seems to be influenced by the stage of lactation. At the onset of lactation, cows are most often mobilizing adipose FA, which are partially incorporated into milk fat. The greater uptake of long chain fatty acid during this period decreases the proportion of short and medium chain fatty acid (SMCFA) in milk fat, due to both a dilution effect and inhibition of de novo synthesis of FA (Chilliard et al. 2000). Therefore, proportions of SMCFA are relatively low at the beginning of the lactation and increase until at least 8th to 10th week of lactation.

The highest level of SFA (Table 3) were found in the 4th, 5th, 7th and 8th collection, so in the winter months. Also Talpur et al. (2008) showed higher by 5–10% level of SFA in the winter compared to the summer season. However, Kuczyńska (2001) came to different conclusion, she observed the highest level of saturated fatty acids up to the 100th day of the lactation.

In case of C_{14:0} the highest level was shown in the 9th collection. The peak of the concentration of C_{16:0} was observed at the 4th collecting (September and February). The lowest levels of miristic and palmitic were stated at the 1th and at 2nd stage of lactation respectively (June, July) (Table 3). Talpur et al. (2008) noticed that the highest concentration of C_{14:0} acid occurred in the winter, and

C_{16:0} in the summer season. However, Lock and Garnsworthy (2003) observed higher level of C_{12:0} and C_{14:0} (by 10%) and C_{16:0} (by 23%) in fat of milk collected in the spring period compared with the winter.

Significant changes in C_{18:0} level was stated during the experiment. Its the highest levels was noticed at the 1st collection (June), and the lowest at the 7th (December) (Table 3). Researches of Auld et al. (1998) also confirmed changes selected SFA in cow's milk during lactation. Milk collected at the initial stage of lactation (30 day) contained the lowest level of acids of C_{4:0} to C_{12:0} chain length, compared to milk from middle (120 day) and late phase of the lactation (210 day). According to above mentioned authors, the differences were caused by energy deficit, which often occurs at the peak of lactation rather than season of the feeding. Additionally, Palmquist et al. (1993) have stated that the synthesis of C_{4:0} to C_{12:0} fatty acids in mammary gland increases during the initial stage of lactation, with a simultaneous intensify mobilization of FA from the adipose tissue, and then is subsequently reduced. Chilliard et al. (2003) classified fatty acid into 3 groups: short-chain (C4 to C8), middle-chain (C10 to C16) and long-chain (above C18) group. Authors reported that the concentration of middle-chain FA was negatively correlated with the long-chain FA group. When long-chain FA are available either from the diet, or from body fat mobilization, there is a decrease in the percentage of medium-chain FA (C_{8:0} to C_{16:0}) in milk fat.

Reduction in SFA content in the milk is beneficial for the consumer's health.

TABLE 3. Changes in the content of saturated fatty acids (g in 100 g of fat) in depending of the stage of lactation

Col- lec- ting	SFA		C _{4:0}		C _{12:0}		C _{14:0}		C _{16:0}		C _{18:0}	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
1	58.050 ^{ABCDEF} GHI	4.404	1.901 ^{abcdef}	0.293	1.649 ^{ABCDEF} GHI	0.133	7.117 ^{ABCDEF} GH	0.285	27.625 ^{ABCDEF} GH	0.533	14.484 ^{ABCDEF} GHI	0.346
2	59.615 ^{IJKLLMNO}	3.610	2.321 ^{AGHIJKL}	0.323	2.147 ^{JKLMNO}	0.133	8.011 ^{IJKLLMNO}	0.285	27.607 ^{IJKLLMNO}	0.533	13.214 ^{JKLMNO}	0.346
3	63.888 ^{AJ}	2.254	1.858 ^{GMLNO}	0.252	2.611 ^{BJPQRSTUX}	0.133	9.598 ^{AJ}	0.285	31.600 ^{AJ}	0.533	11.578 ^{AJPRSTWXY}	0.346
4	65.428 ^{BUlp}	2.804	2.502 ^{BLUPRST}	0.339	2.943 ^{CKzA' b'c'D'}	0.133	10.286 ^{BJPr}	0.285	32.926 ^{BUlp}	0.533	8.475 ^{BKP}	0.346
5	65.788 ^{CKRst}	3.661	1.911 ^{HUWxyz}	0.477	3.394 ^{DLpZ}	0.133	10.523 ^{CK}	0.285	32.582 ^{CKr}	0.533	8.553 ^{CLR}	0.346
6	62.446 ^{DLURwx}	2.819	1.061 ^{CJMPWA'B'C'D'}	0.624	3.498 ^{ELRA'}	0.133	10.720 ^{PL}	0.285	31.728 ^{DL}	0.533	8.542 ^{DLS}	0.346
7	65.138 ^{ELw}	2.624	2.152 ^{dNRxA'E'F'}	0.421	3.392 ^{FMSB'}	0.133	10.889 ^{EL}	0.285	32.779 ^{ELSt}	0.533	8.254 ^{EMT}	0.346
8	65.026 ^{FMx}	3.889	2.167 ^{oYB'}	0.402	3.325 ^{GNtc'}	0.133	11.117 ^{PM}	0.285	32.146 ^{FM}	0.533	8.849 ^{FNW}	0.346
9	63.123 ^{GWgp}	4.126	1.667 ^{KSzC'E'}	0.217	3.539 ^{HOWD'}	0.133	11.284 ^{GNPr}	0.285	30.738 ^{GNLrs}	0.533	8.627 ^{GOX}	0.346
10	63.334 ^{HOl}	5.470	1.875 ^{LTD'F''}	0.152	3.308 ^{VX}	0.133	11.217 ^{HO}	0.285	31.180 ^{HOpt}	0.533	9.079 ^{HOY}	0.346

– SE – standard error of the mean

– LSM – least square of mean

– A,B,C,D $P \leq 0.01$; a,b,c,d $P \leq 0.05$

Moreover, it was stated that among the SFA only BA (C_{4:0}) had a health-promoted properties (Parodi 1999; Chilliard et al. 2001). The study showed significant influence of phase of the lactation on the concentration of these fatty acid. The highest level was found at beginning of the lactation (Table 3). Nałęcz-Tarwacka (2006) also noticed significant influence of the stage of the lactation on the level of butyric acid, however the author recorded different tendencies. The highest level of butyric acid was observed between 121–151 day of lactation – 3.438 g per 100 g of fat, and the lowest at the beginning of lactation (7–30 day) – 3.237 g per 100 g of fat. Talpur et al. (2008) stated its the highest level in milk collected in the spring (March, April, May).

CONCLUSIONS

There were significant influence of the stage of lactation on the concentration of SFA. Milk from the initial stage of lactation (early lactation) characterized by the lowest level of saturated fatty acid including: C_{12:0}, C_{14:0} and C_{16:0} and the highest level of BA compared with the end of lactation. The differences were caused by physiological inability of cows in early lactation to consume enough dry matter to meet energy requirements.

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REFERENCES

- AOAC, 1990: Official Methods of Analysis (15th edn), Vol. 1. Association of Official Analytical Chemists, Washington, DC.
- AULDIST M.J., WALSH B.J., THOMSON N.A., 1998: Seasonal and lactation influences on bovine milk composition in New Zeland. *J. Dairy Res.* 65: 401–411.
- CHILLIARD Y., FERLAY A., MANSBRIDGE R.M., DOREAU M., 2000: Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. *Ann. Zootech.* 49: 181–205.
- CHILLIARD Y., FERLAY A., DOREAU M., 2001: Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest. Prod. Sci.* 70: 31–48.
- CHILLIARD Y., FERLAY A., ROUEL J., LAMBERET G., 2003: A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *J. Dairy Sci.* 86: 1751–70.
- DePETERS E.J., CANT J.P., 1992: Nutritional factors influencing the nitrogen composition of bovine milk: A review. *J. Dairy Sci.* 75: 2043–2070.
- KUCZYŃSKA B. 2001: Badanie czynników warunkujących zmiany jakości tłuszczu mleka krowiego i koziego. Praca doktorska SGGW, maszynopis, Warszawa.
- LOCK A.L., GARNSWORTHY P.C., 2003: Seasonal variation In milk conjugated linoleic acid and delta-9 desaturase activity in dairy cows. *Liv. Prod. Sci.* 79: 47–59.
- NAŁĘCZ-TARWACKA T., 2006: Wpływ wybranych czynników na zawartość funkcjonalnych składników tłuszczu mleka krów. Rozprawa Habilitacyjna – Rozprawy Naukowe i Monografie. Wydawnictwo SGGW, Warszawa.
- PALMQUIST D.L., BEAULIEU A.D., BARBANO M., 1993: Feed and animal

- factors influencing milk fat composition. *J. Dairy Sci.* 76: 1753–1771.
- PARODI P.W., 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *J. Dairy Sci.* 82: 1339–1349.
- PUPPEL K., 2011: The influence of fish oil and linseed supplementation on the fat and the protein fraction content of cows milk. *Doctoral thesis*, typework, Warsaw.
- TALPUR F.N., BHANGER M.I., KHOOHARO A.A., ZUHRA MEMON G., 2008: Seasonal variation in fatty acid composition of milk from ruminants reared under the traditional feeding system of Sindh, Pakistan. *Sci. Direct Liv. Sci.* 118: 166–172.
- Badania przeprowadzono w rolniczym zakładzie doświadczalnym SGGW na dwudziestu krowach rasy PHF. Zwierzęta utrzymywane były w oborze wolnostanowiskowej i objęte żywieniem TMR. Krowy miały stały dostęp do wody, a TMR zadawany był ad libitum. Próbkę mleka pobierane były 10-krotnie w trakcie pierwszych 10 miesięcy laktacji (raz w miesiącu). Mleko w początkowym okresie laktacji charakteryzowało się najniższym poziomem nasyconych kwasów tłuszczowych, w tym: C_{12:0}, C_{14:0}, C_{16:0} jak również najwyższym BA w porównaniu z końcowym okresem. Podsumowując, można zatem stwierdzić, że faza laktacji wpływa znacząco na stężenie nasyconych kwasów tłuszczowych w mleku krow.

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Streszczenie: *Zmiany w stężeniu nasyconych kwasów tłuszczowych w mleku wysokowydajnych krow rasy Polskiej Holsztyńsko-Fryzyjskiej w trakcie trwania laktacji. Celem prezentowanej pracy było określenie wpływu fazy laktacji na kształtowanie się zawartości nasyconych kwasów tłuszczowych w mleku wysokowydajnych krow.*

Effect of beef from different fattening systems and fish meat on the selected indicators of diet utilization and health state of rats as model animals

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Abstract: *Effect of beef from different fattening systems and fish meat on the selected indicators of diet utilization and health state of rats as model animals.* The effect of beef from different fattening systems and of meat of marine fishes, as being employed and the sole protein and fat source in rats' diets on the selected parameters of their growth and health state was examined. The rats of the experimental groups received meat-free diet (group I – control); groups II–IV – diets with 20% of meat: in group II – meat of marine fishes, in group III – beef coming from the animals extensively fed the pasture forage and in group IV – beef from the animals intensively fed the maize silage and concentrates. The body gains of the rats and feed utilization conversion per body weight gain were studied. In blood, the selected morphological and biochemical parameters were determined. The weight of the collected (after killing) internal organs: liver, heart, kidneys and spleen and their percentage participation in body weight were determined. In case of the rats fed the beef-containing diet (groups II, IV), significantly higher body weight gains and lower consumption of diet for 1 g of the gain was recorded as compared to the groups fed the meat-free diet and diet with the participation of fish. In the blood of the rats, receiving beef in their diet, significantly higher level of erythrocytes was found, as well. The remaining examined parameters in all groups were found on a similar level.

Key words: beef, fish meat, rats – model animals, blood morphological and biochemical parameters.

INTRODUCTION

Nutritive and health-promoting value of foodstuffs becomes more and more important master for the contemporary consumer. From among the consumed kinds of meat, beef rises special controversies, being perceived as diet component which increases a risk of hypercholesterolemia and circulatory system diseases (Fraser 1999, Kontogianni et al. 2008). It introduces, however, other valuable bioactive components and minerals, and especially iron, to the diet (Gibson and Ashwell 2002, Johnston et al. 2007).

Properly selected cattle nutrition permits to modify chemical composition, so, in consequence, nutritive values of beef. The results of the studies indicate that pasture grazing of cattle as compared to feeding based upon preserved roughages and high quantities of concentrates leads to decrease of fat content in the meat and increase of the participation of unsaturated fatty acids, including conjugated linoleic acid (CLA) (Nuernberg et al. 2005, De la Fuente 2009, Łozicki et al. 2012). Besides it, pasture feeding affects the increase of vitamin E content as well

as of other antioxidants in the meat (Realini et al. 2004, De la Fuente et al. 2009, Łozicki et al. 2012).

Evaluation of chemical composition of beef coming from pasture fattening of the animals indicates, therefore, its more favourable dietetic values. The discussed product is, however, subject to digestion and further metabolic transformations in the body and a real effect of meat consumption may be difficult to be determined at *in vitro* studies. It seems, therefore, that the studies on model animals should be the successive stage of evaluation of nutritive and dietetic value of meat (Brandsch et al. 2006).

The aim of the studies was to perform the preliminary bioevaluation of beef, obtained from the cattle, extensively fed the pasture forage and from the animals intensively fed the maize silage and concentrates, and to consider its effect on the growth of model animals, utilization of diet and selected parameters of health state. The evaluation of the tested beef was carried out in comparison to the group of the animals fed the meat of marine fishes and to the group receiving semi-synthetic meat-free diet.

The further studies dietetic value of beef from different feeding systems will consider the effect of the employed meat on lipid management and antioxidative state of the body model animals.

MATERIAL AND METHODS

The study was carried out following the procedures approved by the Local Ethical Commission for experiments with animals.

The experiment was conducted with 40 growing rats of Wistar with the initial body weight ca. 108 g. The animals were randomly allocated to 4 experimental groups, 10 rats each. The rats were kept individually in plastic cages in a room with 12-hours light – dark cycle, at temperature of 22°C and humidity of 50–60%. The experiment lasted for 6 weeks. Over the experimental period, the animals had a free access to feed and water. The rats from group I were fed a diet without meat – the source of protein was casein, and instead of fat – rapeseed oil. Animals from group II received diet with meat (fillet) of saltwater fish – walleye pollack. Fish meat originated from a fish shop. The rats from groups III and IV received diets with beef that originated from the animals fed in the extensive fattening system with pasture forage (group III) and in the intensive fattening system with maize silage and feed concentrate (group IV). Beef was obtained from Hereford beef cattle fed in the intensive (conventional farm) and extensive (eco-farm) systems. Young bulls in the intensive system were fed maize silage, hay and feed concentrate throughout the fattening period. In the extensive system, in last 4 months before slaughter, the animals were fed only pasture forage (Łozicki et al. 2012).

In the groups where meat was administered (II–IV), it was the sole source of protein and fat (Table 1). Meat in the quantity of 20% was added to diets after lyophilization and comminution. The diets were isoprotein but had differed fat content (Table 2).

The chemical composition of diets were determined according to AOAC methods (AOAC 2005).

TABLE 1. Chemical composition, fatty acid profile and contents of selected minerals in the analyzed meat and rapeseed oil (Łozicki et al. 2012¹)

Specification	Beef – pasture feeding ¹	Beef – maize silage and concentrate feeding ¹	Fish meat	Rapeseed oil
Dry matter (g·kg ⁻¹)	228	231	197	–
Crude protein (g·kg ⁻¹)	218.20	220.00	230.45	–
Crude fat (g·kg ⁻¹)	20.61	27.83	7.79	–
SFA (% total fatty acid)	46.864	46.485	32.06	7.35
MUFA (% total fatty acid)	37.947	41.752	15.29	61.45
PUFA (% total fatty acid)	12.560	9.669	45.42	28.37
Fe (mg·kg ⁻¹)	37.1	29.7	2.79	–
Zn (mg·kg ⁻¹)	54.9	45.6	8.37	–
Cu (mg·kg ⁻¹)	1.1	1.2	0.655	–

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

TABLE 2. Composition and nutritive value of experimental diets

Specification	Group			
	I	II	III	IV
	g·kg ⁻¹			
Beef – pasture feeding	–	–	200	–
Beef – maize silage and concentrate feeding	–	–	–	200
Fish	–	200	–	–
Casein	197	–	–	–
Starch	674	711	711	711
Cellulose	40	40	40	40
Rapeseed oil	40	–	–	–
AIN-93G Mineral Mix (MX)	35	35	35	35
AIN-93-VX Vitamin Mix	10	10	10	10
Choline chloride	2	2	2	2
Methionine	2	2	2	2
In kg of feed mixture (g)				
Dry matter	904	910	911	906
Crude ash	28.3	29.6	31.4	32.9
Crude protein	163.6	167.4	166.5	162.2
Crude fat	39.89	10.2	17.4	23.4

During the duration of the experiment, the rats were weighed once a week and their daily gains were calculated. Daily intake of the experimental mixtures was controlled and conversion of the mixture per 1 g of body gain was calculated.

At the end of the experiment, the rats were anaesthetized with ketamine overdose and killing. Rats were fasted for 12 h before anaesthetized. Blood samples were collected from the left cardiac ventricle to plastic test tubes. Immediately after rats killing the selected internal organs (liver, heart, kidneys and spleen) were collected and next their percentage participation in body weight was calculated. Standard laboratory methods were used to measure morphological blood parameters – red blood cells count, hematocrit, mean volume of red blood cells, hemoglobin concentration, white blood cells count. The concentration of biochemical blood markers (glucose, total protein, albumins, urea, ALT, AST) in blood serum were analyzed with the spectrometric method, using a Vitros – DT II analyzer (Johnson & Johnson), with a slide kit (Johnson & Johnson Clinical Diagnostics).

The obtained results were statistically developed, using a one-way analysis of variance with the least square method.

The attention was paid to the effect of feeding on the analyzed parameters. Computations were performed with Statgraphics 6.0 Plus statistical package.

RESULTS AND DISCUSSION

In the rats fed the beef-containing diets, significantly higher ($P \leq 0.01$) body weight gains were recorded as compared to the groups of the animals fed the meat-free and fish-containing diets (Table 3). In the groups which received beef in their diet, lower consumption of the diet per 1 g of body gain was also observed. In comparison to the group fed the fish-containing diet, the discussed differences were statistically significant ($P \leq 0.05$).

Any differences in concentration of glucose, total protein, urea and albumins in blood serum between the groups were not recorded what indicates a similar utilization of energy and protein from the particular diets (Table 4). The analysed parameters were found within the limits of reference intervals for the rats, as given by Wolfensohn and Lloyd (2003). The concentration of glucose is also effected by lipid management in the organism what has an influence on secretion and effect of insulin. It is connected with the

TABLE 3. Effect of diets on growth performance of rats

Specification	Group				SE	P-value
	I	II	III	IV		
Initial body weight	108.6	108.9	108.2	108.6	6.079	0.999
Final body weight	273.8b	273.3b	304.6a	292.9	9.361	0.061
Average daily gain	3.79B	3.95Dd	4.80AC	4.51Ac	0.173	0.0005
The use of feed on 1 g daily gain	4.23a	4.67b	4.21a	4.39	0.152	0.131

AB – differences between the selected rows ($P \leq 0.01$); ab – differences between the selected rows ($P \leq 0.05$).

TABLE 4. Morphological and biochemical blood parameters of the rats

Specification	Group				SE	P-value
	I	II	III	IV		
	Biochemical blood markers					
Glucose (mmol·l ⁻¹)	7.78	8.03	8.68	8.34	0.386	0.355
Albumins (g·l ⁻¹)	34.56	34.32	33.46	32.61	0.870	0.418
Urea (g·l ⁻¹)	5.11	4.80	4.78	4.69	0.231	0.604
Total protein (g·l ⁻¹)	66.30	68.67	65.74	64.02	1.975	0.441
ALT (U·l ⁻¹)	43.90	44.0	44.13	48.32	3.104	0.349
AST (U·l ⁻¹)	262.90	284.43	292.32	326.12	28.441	0.272
Morphological blood markers						
Erythrocytes (10 ⁶ μl ⁻¹)	7.96A	7.85a	7.80a	7.52Bb	0.134	0.017
Leukocytes (10 ³ μl ⁻¹)	8.73	8.44	7.83	7.67	0.591	0.546
Hematocrit (%)	44.20	46.16	45.26	44.75	0.702	0.256
Blood cells (10 ³ μl ⁻¹)	825	806	884	836	43.883	0.586
Hemoglobin (g·dl ⁻¹)	16.14	16.08	15.89	15.42	0.260	0.213

AB – differences between the selected rows ($P \leq 0.01$); ab – differences between the selected rows ($P \leq 0.05$).

insulin-resistance of hepatocytes which may result from excessive accumulation of lipids in the liver. It is especially supported by the diet, rich in saturated fatty acids (Konstantynowicz et al. 2011). In own studies, the higher intake of saturated fatty acids occurred in the groups of the animals which received beef in the diets (Table 1).

Any effect of the diet on liver enzymes – alanine transaminase (ALT) and aspartate transaminase (AST), as being determined in the blood serum, was not found (Table 4). The mentioned enzymes are indicators of damage of liver cells which may occur e.g. as a result of fatness of the liver. Lack of differences between the groups indicates that the examined diets did not differ in their effect on functioning of the liver.

When comparing the analyzed morphological blood markers of the experi-

mental animals (Table 4), we may say that significant differences occurred only in the case of erythrocytes, the highest concentration of which was recorded in group III and the lowest one – in group I ($P \leq 0.05$).

The highest number of erythrocytes in group III may result from the highest Fe level in the diets of the discussed group. It originated from the higher content of the mentioned element in the meat of the animals fed the pasture forage as compared to the meat of the intensively fed animals and meat of fish (Table 1). Morphological parameters of blood were found within the limits of reference values (Wolfensohn and Lloyd 2003).

Any differences between the groups in respect of the percentage participation of the analysed organs in relation to body weight of the rats were not found (Table 5). Therefore, the type of the diet did not

TABLE 5. The percentage participation of the selected organs in relation to body weight

Specification	Group				SE	P-value
	I	I	III	IV		
	%					
Liver	2.77	2.75	2.47	2.77	0.125	0.278
Heart	0.351	0.344	0.324	0.318	0.016	0.077
Kidneys	0.728	0.691	0.633	0.701	0.031	0.173
Spleen	0.173	0.191	0.161	0.172	0.011	0.287

AB – differences between the selected rows ($P \leq 0.01$); ab – differences between the selected rows ($P \leq 0.05$).

have any influence on the growth of the analysed organs. The comparison of the participation of liver in the body weight of the animals seems to be important. Its functioning is affected by the quantity and type of the fat, consumed from the diet. The lowest fat content occurred in the fish-containing diet and the highest one was recorded in the diets with beef and meat-free mixtures. The participation of the particular groups of fatty acids in the diet was also different. However, lack of differences between the groups in respect of the liver weight as well as activity of the liver enzymes does not indicate the distinct effect of the examined components of diets on the discussed parameters.

CONCLUSIONS

The examined diets had an influence on the growth of the rats and utilization of the administered diets by the animals. Significantly higher body weight gains and lower consumption of diets for 1 g of the gain was recorded in groups fed mixture with beef as compared to the groups fed meat-free control mixture and mix-

ture with the participation of fish. In the blood of the rats, receiving beef in their diet, significantly higher level of erythrocytes was found, as well. The remaining examined parameters in all groups were found on a similar level.

REFERENCES

- AOAC, 2005: Official Methods of Analysis of AOAC International 16th Ed. Association of Analytical Chemists, Arlington, VA, USA.
- BRANDSCH C., SHUKLA A., HIRCH F., STANGL G.I., EDER K., 2006: Effect of proteins from beef, pork, and turkey meat on plasma and liver lipids of rats compared with casein and soy protein. *Nutrition* 22: 1162–1170.
- FRASER F., 1999: Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-Day Adventists. *Am. J. Clin. Nutr.* 70: 532–538.
- De La FUENTE J., DIAZ M.T., ALVAREZ I., OLIVER M.A., FONT K., FURBOLS M., SANUDO C., CAMPO M.M., MONTOSSI F., NUTE G.R., CANEQUE V., 2009: Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. *Meat Sci.* 82: 331–337.

- GIBSON S., ASHWELL M., 2002: The association between red and processed meat consumption and iron intakes and status among British Adults. *Public Health Nutr.* 6 (4): 341–350.
- JOHNSTON J., PRYNNE C.J., STEPHEN A.M., WADSWORTH M.E.J., 2007: Haem and non-haem iron intake through 17 years of adults life of a British Birth Cohort. *Br. J. Nutr.* 98: 1021–1028.
- KONSTANTYNOWICZ K., MIKŁOSZ A., STEPEK T., CHABOWSKI A., 2011: Akumulacja lipidów (triacylo-, diacylogliceroli i ceramidów) wewnątrz hepatocytów, a rozwój insulinooporności wątrobowej. *Postępy Hig. Med. Dośw.* 65: 236–243 [in Polish].
- KONTOGIANNI M.D., PANAGIOTAKOS D.B., PITSAVOS C., CHRYSOHOOU C., STEFANADIS C., 2008: Relationship between meat intake and the development of acute coronary syndromes: The CARDIO2000 case-control study. *Eur. J. Clin. Nutr.* 62: 171–177.
- ŁOZICKI A., DYMNICKA M., ARKUSZEWSKA E., PUSTKOWIAK H., 2012: Effect of pasture or maize silage feeding on the nutritional value of beef. *Ann. Anim. Sci.* 12, 1: 81–93.
- NUERNBERG K., DANNENBERGER D., NUERNBERG G., ENDER K., VOIGT J., SCOLLAN N.D., WOOD J.D., NUTE G.R., RICHARDSON R.I., 2005: Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. *Live Prod. Sci.* 94: 137–147.
- REALINI C.E., DUCKETT S.K., BRITO G.W., DALLA RIZZA M., De MATOS D., 2004: Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Sci.* 66: 567–577.
- WOLFENSOHN S., LLOYD M., 2003: *Handbook of Laboratory Animal, Management and Welfare*, 3rd Ed., Blackwell Publishing Ltd.
- Streszczenie:** *Wpływ mięsa wołowego z różnych systemów opasu oraz mięsa ryby na wybrane wskaźniki wykorzystania diety i stanu zdrowia szczurów jako zwierząt modelowych.* Badano wpływ mięsa wołowego z różnych systemów opasu i mięsa ryb morskich zastosowanych jako jedyne źródło białka i tłuszczu w dietach szczurów na wybrane wskaźniki ich rozwoju oraz stanu zdrowia. Szczury grup doświadczalnych otrzymywały: dietę bezmięsną (grupa I – kontrolna), grupy II–IV diety w których mięso stanowiło 20% diety, w grupie II – mięso ryb morskich, w grupie III – mięso wołowe od zwierząt żywionych ekstensywnie zielonką pastwiskową i w grupie IV – mięso wołowe od zwierząt żywionych intensywnie kiszoną z kukurydzy i paszą treściwą. Badano przyrosty szczurów oraz wykorzystanie diety na przyrost masy ciała. We krwi oznaczono wybrane wskaźniki morfologiczne i biochemiczne. Określano masę pobranych po dekapitacji narządów wewnętrznych: wątroby, serca, nerek i śledziony i ich procentowy udział w masie ciała. U szczurów żywionych dietami z wołowiną (grupy III i IV) stwierdzono istotnie wyższe przyrosty masy ciała oraz niższe zużycie mieszanek na 1 g przyrostu w stosunku do grup żywionych mieszką bezmięsną kontrolną oraz mieszką z udziałem mięsa ryby. We krwi szczurów otrzymujących w dietach wołowinę stwierdzono również istotnie wyższy poziom erytrocytów. Pozostałe badane parametry we wszystkich grupach były na podobnym poziomie.

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Effect of genetic stock on production results and growth rate of chickens

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Abstract: *Effect of genetic stock on production results and growth rate of chickens.* The experiment was carried out with 320 chickens (fast-growing Hubbard Flex and slow-growing Hubbard JA 957) divided into two experimental groups, each having 160 birds in four replicates, with 40 birds per replicate. During the experiment, body weight was measured initially and at weekly intervals, and feed intake and flock health were determined to calculate feed conversion (kg per 1 kg gain) and percent mortality in the flock. The aim of the study was to compare body weight, growth rate and production results in fast-growing Hubbard Flex (42 days) and slow-growing Hubbard JA 957 (42 and 63 days) chickens designed for free-range husbandry. It was found that fast-growing Hubbard Flex chickens reared to 42 days of age were characterized by higher body weight and better feed conversion compared to Hubbard JA 957 chickens, at both 42 and 63 days of age. However, the flock of slow-growing Hubbard JA 957 chickens was in better health. Until 42 days of growth, mortality in this flock was 1.87% compared to 3.38% in the Hubbard Flex flock. Over the whole rearing period (63 days), Hubbard JA 957 chickens had a mortality of 3.16%. Because of their better resistance and lower mortality, especially during the final period of growth, slow-growing chickens should be a more desirable choice for alternative husbandry systems.

Key words: Hubbard Flex, Hubbard JA 957, production results, growth rate.

INTRODUCTION

Recent years have seen an increasing number of studies investigating alterna-

tive, less intensive housing systems for broiler chickens, in which breeders use slower growing chickens adapted for free-range production (Fanatico et al. 2005, Fanatico et al. 2007, Fortomarjts et al. 2007, Castellini et al. 2008). The birds available in Poland are hybrids derived from multipurpose hens in conservation flocks of Sussex or Greenleg Partridge breeds and Cobb 500 or Ross 308 meat-type cocks. These chickens are resistant to unfavourable weather conditions, are willing to use outdoor runs, and their flocks are not affected by diseases found in intensive broiler production systems, such as sudden death syndrome and leg injuries.

In most European countries, however, breeders use fast-growing chickens in free-range production systems to ensure higher body weights (Network for Animal Health and Welfare in Organic Agriculture 2002). At 42 days of age, fast-growing chickens have a mean body weight of 2.40 kg compared to 1.94 kg in slow-growing chickens (Castellini et al. 2002a, Pietrzak et al. 2006). The use of fast-growing chickens is associated with lower physical activity on free-range, increased mortality due to sudden death syndrome, and culling due to leg injuries (Castellini et al. 2002a, b).

Organic production requirements impose high production costs that translate into the price level, which restricts demand (Gornowicz 2009). Therefore, selection of the right stock for raising chickens to 63 or 81 days of age will help to make these products more available.

The aim of the study was to compare body weight, growth rate and production results in fast-growing Hubbard Flex and slow-growing Hubbard JA 957 chickens designed for free-range husbandry.

MATERIAL AND METHODS

The experiment was carried out with 320 chickens (fast-growing Hubbard Flex and slow-growing Hubbard JA 957) divided into two genetic groups, each having 160 birds in four replicates, with 40 birds per replicate. Chickens were obtained from the same poultry hatchery in Kisielany. They were banded with tags and housed under standard hygienic conditions at a stocking rate of 11.4 birds per m². Hubbard Flex chickens were raised to 42 days, and Hubbard JA 957 to 63 days like free-range chickens. Rearing of Hubbard Flex broilers was terminated at 6 weeks of age due to the risk of leg abnormalities (tibial dyschondroplasia, femoral head necrosis) and other disease entities (sudden death syndrome, ascites), which increases with age.

Hubbard Flex chickens were kept until 42 days of age using three-phase feeding (starter, grower I, finisher), and Hubbard JA 957 chickens were kept until 63 days using four-phase feeding (starter, grower I, grower II, finisher). The diets had the following nutritive value: starter (days 1–14): 21–22% crude protein, 1.17%

lysine, 0.98% Met. + Cys. and 11.90 MJ ME; grower I (days 15–35): 20% crude protein, 1.02% lysine, 0.88% Met. + Cys. and 12.13 MJ ME; grower II (days 36–56, only for slow-growing chickens): 19% crude protein, 0.98% lysine, 0.80% Met. + Cys. and 12.34 MJ ME; finisher (days 36–42 for fast-growing chickens and days 57–63 for slow-growing birds): 18% crude protein, 0.90% lysine, 0.78% Met. + Cys. and 12.40 MJ ME. The starter, grower I and grower II diets contained the coccidiostat monensin at 2500 mg per 1 t of feed.

During the experiment, body weight was measured at one day of age and over subsequent weeks of rearing, and feed intake and flock health were determined to calculate feed conversion (kg per 1 kg gain) and percent mortality in the flock.

The results were analysed statistically with one-way analysis of variance calculated by the least squares method using SPSS 19.0 PL for Windows (SPSS 2010).

RESULTS AND DISCUSSION

On placement day, Hubbard JA 957 chicks were 2.0 g heavier than Hubbard Flex chicks and the difference was statistically significant (Table 1). The lower body weight of Hubbard Flex chickens resulted from the fact that eggs for hatching were obtained from young broiler parent stock. Sokołowicz and Herbut (2000), who investigated the effect of hens' age on performance of their offspring, showed that chicks obtained from younger parent stock (31 weeks old) were 7 g lighter than chicks obtained from older parent stock (55 weeks old).

TABLE 1. Body weight of broiler chickens – Hubbard Flex and Hubbard JA 957 (g)

Day	Hubbard Flex		Hubbard JA 957	
	LSM	SE	LSM	SE
1	39 ^B	0.30	41 ^A	0.30
7	143 ^A	1.25	129 ^B	1.26
14	367 ^A	4.40	311 ^B	4.35
21	759 ^A	8.00	637 ^B	7.95
28	1215 ^A	13.79	999 ^B	13.49
35	1818 ^A	24.14	1438 ^B	23.47
42	2544 ^A	28.13	1876 ^B	27.19
63	–	–	3487	–

A, B – means with different superscripts differ significantly at $P \leq 0.01$.

After one week of rearing, Hubbard Flex chickens were significantly heavier than Hubbard JA 957 birds. Such a rapid increase in body weight results from long-term selection of these chickens for fast growth rate. Hubbard JA 957 chickens do not attain such a high body weight within such a short time (42 days), which makes them ideal material for raising under organic or free-range conditions. Hubbard Flex chickens were less efficient in feed conversion (kg per 1 kg gain) compared to slow-growing Hubbard JA 957 chickens (Table 2). The difference of 0.5 kg results from the fact that the latter were raised three weeks longer than Hubbard Flex chickens. Mortality during the whole rearing period was higher in Hubbard Flex birds (Table 2). The number of deaths in Hubbard JA 957 chickens was lower, which may indicate that these birds had better immunity (all of them were obtained from the same Poultry Hatchery). Mikulski et al. (2011) observed a similar tendency when comparing fast-growing Hubbard F-15 with slow-growing Hubbard JA 957 chickens. The mean body weight of Hubbard F-15

broilers was about 0.8 kg higher than that of Hubbard JA 957 chickens (4.4 vs. 3.6 kg). A higher body weight of the chickens at slaughter compared to the results obtained in the experiment is due to the chickens being reared for 23 days longer. The results show that the strongest effect on growth rate in chickens is exerted by the genotype, on which the body weight obtained depends, regardless of the duration of rearing. This is supported by Sosnowka-Czajka et al. (2004), who reported that chicken genotype determines the growth rate and final body weight, but has no effect on feed conversion and chicken mortality. Mikulski et al. (2011) found mortality to be higher in fast-growing Hubbard F-15 compared to slow-growing Hubbard JA 957 chickens (6.03 vs. 2.50%). Sudden death syndrome accounted for 90% of mortality in Hubbard F-15 broilers. The death losses were due to the Hubbard F-15 broilers being kept longer (to 65 days of age), despite the fact that in terms of protein and energy, the feed was balanced to account for a longer period of rearing.

TABLE 2. Feed conversion and mortality for Hubbard Flex and Hubbard JA 957

Group	Feed consumption (kg per 1 kg gain)	Mortality (%)
Hubbard Flex – FG (42 day of age)	1.5 ^b	3.38 ^a
Hubbard JA 957 – SG (42 day of age)	1.8 ^a	1.87 ^b
SE	0.7	1.1
Hubbard JA 957 – SG (63 day of age)	2.0	3.16

a,b – means with different superscripts differ significantly at $P \leq 0.05$.

On the first day of growth, Hubbard Flex chickens weighed less than 40 g. It is not profitable to rear fast-growing chickens for a longer period of time because of the higher feed intake during the entire growth period and the high mortality of chickens in the final stage of the production cycle. When comparing the body weight of Hubbard Flex and Hubbard JA 957 chickens, Wilanowska et al. (2002) obtained similar results to ours. At the end of rearing (day 45), Hubbard Flex chickens had a higher body weight compared to Hubbard JA 957 chickens (2.2 vs. 1.8 kg). Feed conversion was less efficient in Hubbard JA 957 (2.17 kg per 1 kg) compared to Hubbard Flex chickens (2.04 kg per 1 kg). Mortality was higher in fast-growing birds (8.91%) compared to Hubbard JA 957 chickens (2.97%). The three-fold higher mortality in fast-growing Hubbard Flex chickens was caused by their high body weight in the final period of growth, which causes birds health problems and makes them less resistant to pathogenic agents.

Similar findings were reported by Polak (2004) who compared ISA 215 and Anak Titan broiler chickens characterized by a slower rate of growth. At 42 days of growth, ISA 215 chickens had a body weight of 2.40 kg and Anak Titan chickens weighed 2.30 kg. Feed conver-

sion during the entire rearing period was 1.56 kg in Anak Titan and 1.53 kg in ISA 215 broilers.

In our study, we obtained higher body weights than those stated in the management guide for Hubbard JA 957 chickens (available at <http://www.hubbardbreeders.com>). At 42 days of age, Hubbard JA 957 broilers should weigh 1.69 kg. The higher body weights could be due in part to very good housing conditions. All the hygienic measurements made during the course of the experiment did not exceed the standards stated in the Regulation of the Minister of Agriculture and Rural Development of 15 February 2010 (Polish Journal of Laws No. 56 Item 344). The Hubbard management guide specifies that feed conversion should be 1.71 kg per 1 kg in Hubbard Flex at 42 days of age, and 2.28 kg per 1 kg in Hubbard JA 957 chickens at 63 days. When rearing chickens with outdoor access for 3 weeks longer, it is important to ensure better production results than those stated in the management guide for a flock of Hubbard JA 957 chickens. Such information will encourage poultry producers to choose these chickens for free-range production.

Different results were obtained by Berri et al. (2005), who compared fast-, medium- and slow-growing chickens.

Depending on their growth potential, the birds were grown until 6, 8 and 12 weeks and fed a properly formulated diet adjusted to the growth rate of a given genetic line. The hybrids grown until 8 weeks of age were most similar in terms of production results to Hubbard JA 957 chickens. At 12 weeks of age, chickens with a slow growth potential reached a body weight of 2.88 kg, those with a medium growth potential weighed 2.65 kg, and broilers 2.50 kg. Statistically significant differences were found in body weight between the chickens. The duration of rearing was the factor that caused slow-growing chickens to reach higher body weights compared to fast- and medium-growing chickens.

The weekly measurements of body weight enabled the growth and growth rate of the chickens to be presented graphically (Fig. 1). The growth rate of fast-growing chickens is higher until 2 weeks of age compared to that of slow-growing birds. Chickens had similar growth rate until 6 weeks of age, after

which it continued to decline until the end of rearing (Hubbard JA 957). Because the growth rate of birds decreases with age, the longer rearing of slower growing chickens compared to broiler chickens increases maintenance costs associated with greater amounts of feed purchased for the entire production period and poorer feed conversion at the end of rearing.

CONCLUSIONS

It was found that fast-growing Hubbard Flex chickens reared to 42 days of age were characterized by higher body weight and better feed conversion compared to Hubbard JA 957 chickens grown to 42 days. However, the flock of slow-growing Hubbard JA 957 chickens was in better health, with only 1.87% mortality to 42 days of growth and 3.16% mortality (compared to 3.38% in Hubbard Flex) for the entire growth period. Because of their better resistance and lower mortal-

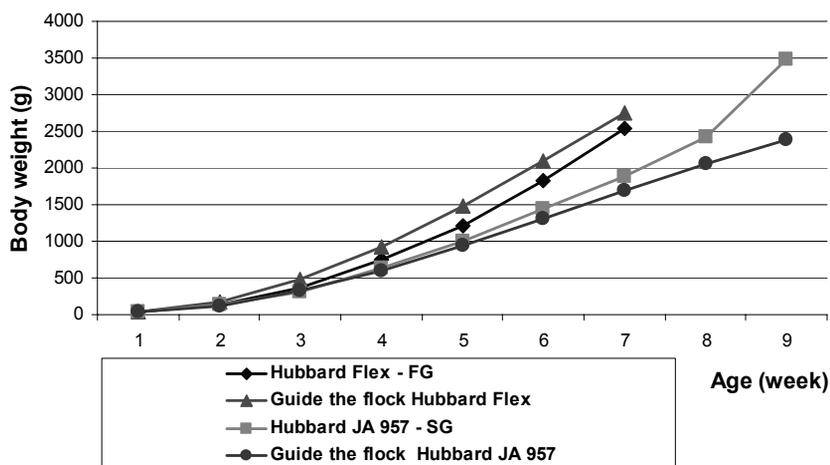


FIGURE 1. Body weight of fast- and slow-growing chickens in comparison to guide the flock – Hubbard Flex and Hubbard JA 957 (g)

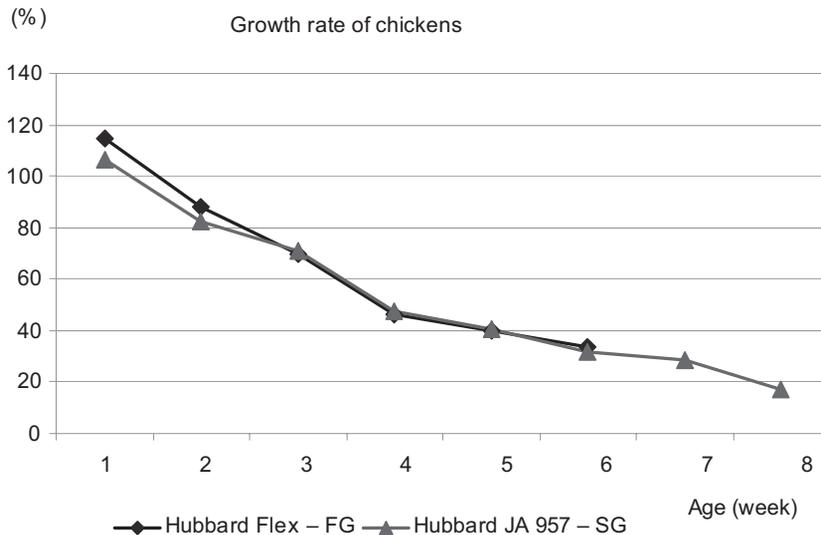


FIGURE 2. Growth rate of fast- and slow-growing chickens – Hubbard Flex and Hubbard JA 957 (%)

ity, especially during the final period of growth, slow-growing chickens should be a more desirable choice for alternative husbandry systems.

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REFERENCES

- BERRI C., Le BIHAN-DUVAL E., BAEZA E., CHARTRIN P., PICGIRARD L., JEHL N., QUENTIN M., PICCARD M., DUCLOS M., 2005: Further processing characteristics of breast and leg meat from fast-, medium- and slow-growing commercial chickens. *Animal Research*, 5: 123–134.
- CASTELLINI C., BERRI C., LE BIHAN-DUVAL E., MARTINO G., 2008: Qualitative attributes and consumer perception of organic and free range poultry meat. *World. Poultry Sci. J.*, 64 (4): 500–512.
- CASTELLINI C., DAL BOSCO A., MUGNAI C., BERNARDINI M., 2002a: Performance and behaviour of chickens with different growing rate reared according to the organic system. *Ital. J. Anim. Sci.* 1: 291–300.
- CASTELLINI C., MUGNAI C., DAL BOSCO A., 2002b: Meat quality of three chicken genotypes reared according to the organic system. *Ital. J. Food Sci.* 14: 401–412.
- FANATICOA.C., CAVITTL.C., PILLAI.P.B., EMMERT J.L., OWENS C.M., 2005: Evaluation of slower-growing broiler genotypes grown with and without outdoor access: meat quality. *Poultry Sci.* 84 (11): 1785–1790.
- FANATICOA.C., PILLAI.P.B., EMMERT J.L., OWENS C.M., 2007: Meat quality of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. *Poultry Sci.* 86 (10): 2245–2255.
- FORTOMARJS P., ARSENOS G., TSERVENI-GOUSHI A., YANNAKOPOULOS A., 2007: Performance and behaviour of broiler chickens as affected by the housing system. *Arch. Geflügelkd.* 71 (3): 97–104.

- GORNOWICZ E., 2009: Ocena jakości mięsa kurcząt brojlerów i jaj oraz analiza efektywności ich pozyskiwania w aspekcie rolnictwa ekologicznego. Sprawozdanie z prowadzenia w 2009 r. badań podstawowych na rzecz rolnictwa ekologicznego. Instytut Zootechniki PIB, Kraków.
- GORNOWICZ E., LEWKO L., 2010: Effectiveness of broiler meat production in the light of ecological farming system. *Poultry Rabbits, Nutria, Fish Modern Trends in Meat Production*: 502–507.
- MIKULSKI D., CELEJ J., JANKOWSKI J., MAJEWSKA T., MIKULSKA M., 2011: Growth performance, carcass traits and meat quality of slower-growing and fast growing chickens raised with and without outdoor access. *Asian-Aust. J. Anim. Sci.* 24 (10): 1407–1416.
- Network for Animal Health and Welfare in Organic Agriculture, 2002: Final recommendation and comments. <http://www.veeru.reading.ac.uk/organic> (Accessed 27.07.2012).
- PIETRZAK D., MROCZEK J., LEŚNIK E., ŚWIERCZEWSKA E., 2006: Quality of meat and fat from three breeding lines of chickens served feed with or without antibiotic growth stimulator. *Med. Wet.* 62: 917–921.
- POLAK M., 2004: Wartość rzeźna kurcząt brojlerów różniących się pochodzeniem, wiekiem uboju oraz płcią. *Zeszyty naukowe Przeglądu Hodowlanego* 4.
- Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 15 lutego 2010 roku w sprawie wymagań i sposobu postępowania przy utrzymywaniu gatunków zwierząt gospodarskich, dla których normy ochrony zostały określone w przepisach Unii Europejskiej [Dz.U. z 2010 r. nr 56, poz. 344].
- SOKOŁOWICZ Z., HERBUT E., 2000: Wpływ wieku kur na wyniki produkcyjne ich potomstwa. *Zeszyty Naukowe Przeglądu Hodowlanego* 49: 515.
- SOSNÓWKA-CZAJKA E., HERBUT E., SKOMOROCHA I., POŁTOWICZ K., 2004: Wpływ genotypu na wyniki produkcyjne i ekonomiczne odchowu kurcząt brojlerów. *Materiały 69 Zjazdu PTZ* 21–23 września: 79–80.
- SPSS 2010: SPSS, 10.0 for Windows user's guide, 2010. SPSS Inc., USA.
- WILANOWSKA M., ŚWIERCZEWSKA E., NIEMIEC J., SKOMIAŁ J., 2002: Porównanie wyników chowu kurcząt rzeźnych certyfikowanych I 957 oraz ISA i Hubbard. *Roczniki Naukowe Zootechniki Suplement* 16: 167–172.

Streszczenie: *Wpływ materiału genetycznego na wyniki produkcyjne i tempo wzrostu kurcząt.* Doświadczenie przeprowadzono na 320 kurczętach, w dwóch grupach genetycznych (szybkorosnące (FG) Hubbard Flex i wolnorosnące (SG) Hubbard JA 957) po 160 szt. w 4 powtórzeniach po 40 szt. W czasie trwania doświadczenia kontrolowano masę ciała: w 1. dniu oraz w cotygodniowych odstępach czasu, sprawdzano również spożycie paszy i zdrowotność stada, celem wyliczenia zużycia paszy kg na 1 kg przyrostu masy ciała i procentu śmiertelności w stadzie. Celem pracy było porównanie masy ciała i tempa wzrostu oraz wyników produkcyjnych szybkorosnących kurcząt Hubbard Flex (42 dzień życia) i wolnorosnących Hubbard JA 957 (42 i 63 dzień życia), przeznaczonych do chowu wolno wybiegowego. Stwierdzono, że szybkorosnące kurczęta Hubbard Flex odchowywane do 42. dnia życia uzyskiwały większą masę ciała i lepszy wskaźnik zużycia paszy w porównaniu z kurczętami Hubbard JA 957 zarówno w 42. jak i 63. dniu życia. Jednak stado wolnorosnących kurcząt Hubbard JA 957 charakteryzowało się lepszą zdrowotnością. Do 42. dnia odchowu w stadzie tym śmiertelność wyniosła 1,87 vs. 3,38% w stadzie Hubbard Flex. W całym okresie odchowu (63 dni) kurcząt Hubbard JA 957 śmiertelność wyniosła 3,16%. Wolnorosnące kurczęta ze względu na lepszą odporność i mniejszą liczbę padnięć, zwłaszcza w końcowym okresie odchowu, powinny być chętniej wybierane przez producentów alternatywnych systemów chowu.

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Effect of L-carnitine on performance and dressing percentage of broiler chickens

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Abstract: *Effect of L-carnitine on performance and dressing percentage of broiler chickens.* The study was conducted on 640 chickens randomly divided into two groups, each having four replications (4 control and 4 experimental – L-carnitine) with 80 chickens per pen. Chickens in the control (C) and experimental groups (E) received a feed of the same nutritional value, and chickens from the experimental group were supplemented with Aminocarnifarm (43.68% of L-carnitine) in drinking water (62.5 g per 100 l) during three periods: from 1 to 7, 21 to 28, and 36 to 42 days of age. The study evaluated the effect of L-carnitine supplemented to chickens on production results and results of slaughter analysis. It was found that Aminocarnifarm supplemented to drinking water improved feed conversion during the whole rearing period, reduced mortality, and contributed to increases in average body weight, dressing percentage and proportion of leg muscles (males), reduced the proportion of breast muscles (males and females), and decreased carcass fatness (males).

Key words: broiler chickens, L-carnitine, production results, slaughter analysis.

INTRODUCTION

In recent years, broiler chickens have been intensively selected for increased weight gain. This strategy improved the rate of growth and feed conversion but had undesirable effects in the form of increased deposition of abdominal fat and greater incidence of metabolic diseases, such as ascites. Excess carcass fat is un-

attractive to healthy-eating consumers who reach for poultry meat because of its nutritional properties. At the same time, increased carcass fatness reduces the profits of poultry producers. The problem can be addressed through proper selection, but this is a long-term process and breeders look for quick solutions. One solution is to provide broilers with dietary supplements such as L-carnitine (Buyse et al. 2001).

L-carnitine is synthesized *in vivo* from lysine and methionine, and it is formed with contributions from vitamins B3 (niacin), B6 (pyridoxine), B12 (cyanocobalamin), C (ascorbic acid) and folic acid, as well as iron (Fe²⁺) (Golzar Adabi et al. 2011). This substance is needed to transport long-chain fatty acids into mitochondria, these acids taking part in β -oxidation that leads to production of energy (Carter et al. 1995, Brooks 1998). L-carnitine was discovered in the early 20th century by Gulewitsch and Krimberg, who isolated it from muscle tissue (Arslan 2006). L-carnitine prevents fatty tissue buildup, thus reducing obesity and atherosclerosis. It decreases the calorie requirement and increases the tolerance to effort (Pietrzak and Opala 1998). Many experiments and clinical observations showed that L-carnitine takes part in regulating the body's lipid levels. It

also has the ability to reduce the level of triacylglycerols and cholesterol (Calvani et al. 2000).

L-carnitine is known to increase antioxidant status during aging. It is accepted that L-carnitine represents the second line of cell defence against reactive oxygen species and their derivatives as it breaks free-radical chain reactions (termination of peroxidation) and prevents undesirable oxidation reactions (Arenas et al. 1998). By reducing the amount of oxidative damage that occurs as a result of peroxidation of polyunsaturated fatty acids found in membrane phospholipids, L-carnitine plays a major role in stabilizing cell membranes and in regulating the function of ion channels (role in calcium transport) (Kalaiselvi and Panneerselvam 1998).

Over the last twenty years, many experiments were performed to test the use of L-carnitine in broiler nutrition. Researchers studied its effect on production parameters such as body weight, rate of growth, feed consumption and conversion, content of abdominal fat, proportion of breast and leg muscles, and giblets percentage. It was also investigated if L-carnitine has an effect on chicken health. The results obtained were inconsistent. Some authors provided conclusive evidence that L-carnitine has a beneficial effect on these parameters, while others held that L-carnitine has no effect on, or even adversely affects production results and mortality (Golzar Adabi et al. 2011).

One of the many commercially available L-carnitine supplements is Aminocarnifarm, which also contains taurine, vitamins and amino acids. It is a complex preparation that stimulates growth,

resistance and body condition and can be used in all farm animals. According to the manufacturer, Aminocarnifarm performs multiple functions in broiler nutrition: it shortens the growth period and makes chickens more resistant while reducing flock mortality.

The objective of the study was to investigate the effect of Aminocarnifarm preparation on performance of broiler chickens and results of slaughter analysis.

MATERIAL AND METHODS

The experiment was conducted at the farm of the Warsaw University of Life Sciences in Obory (Poland) using Cobb 500 broiler chickens, which were reared to 42 days of age in accordance with flock management guidelines. A total of 640 chickens were randomly divided into two groups, each having four replications (4 control and 4 experimental – L-carnitine) with 80 chickens per pen. Chickens in the control (C) and experimental groups (E) received a feed of the same nutritional value, and chickens from the experimental group were supplemented with Aminocarnifarm in water (62.5 g per 100 l) during three periods: from 1 to 7, 21 to 28, and 36 to 42 days of age. Aminocarnifarm contains vitamins (B6, B7, B12), calcium, amino acids and L-carnitine (43.68% of the preparation).

Chickens were fed starter (days 1 to 21), grower (days 22 to 35) and finisher diets (days 36 to 42). The nutritive value of the diets, provided by the manufacturer, and the results of analysis are presented in Table 1.

TABLE 1. Nutritive value of basal diet in broiler feeding

Specification	Type of a diet/Age of chicken (days)		
	Starter (1–21)	Grower (22–35)	Finisher (36–42)
EM _N (MJ·kg ⁻¹)	12.00	12.10	12.20
Total protein (%)**	19.96	18.98	18.60
Lysine (%)	1.39	1.30	1.18
Met. + Cys. (%)	0.97	0.90	0.87
Threonine (%)	0.82	0.75	0.66
Tryptophan (%)	0.23	0.22	0.20
Crude fibre (%)**	2.20	2.25	2.24
Crude fat (%)**	4.10	4.60	4.70
Crude ash (%)**	5.50	5.50	5.50
Nitrogen – free extract (%) **	55.84	56.47	57.12

**analysis of nutritive value of fodders applied in the nutrition of the broiler chickens.

During the experiment, birds were monitored for individual body weight at 1, 21, 35 and 42 days of age, feed consumption each time the feed changed, and health status (mortality and culling).

At the end of the rearing period on day 42, 6 males and 6 females with body weight similar to the mean body weight of a given sex in the group were chosen from each group for slaughter, weighed and subjected to a 12-hours feed withdrawal. After slaughter, carcasses were chilled, weighed and subjected to simplified dissection. Abdominal fat, edible giblets, and breast and leg muscles were collected and weighed. The results obtained were used to calculate dressing percentage and the percentage of carcass components.

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

RESULTS

Tables 2 and 3 present the average body weight of broiler chickens at 1, 21, 35 and 42 days of rearing. The experimental group, in which chickens were supplemented with L-carnitine in Aminocarni-farm achieved higher body weights compared to the control group. Significant differences were noted in males at 35 and 42 days of rearing. The preparation also had an effect on the body weight of females, but the differences were not significant. It was found that L-carnitine preparation slightly reduced mortality and improved feed conversion (kg·kg⁻¹) in chickens (Table 4). Mortality to 14 days of age was due to omphalitis, yolk sac inflammation and gout. Between 15 and 42 days of age, pulmonary congestion, exudative diathesis and crop impaction were found in necropsied birds.

TABLE 2. Mean body weight of broiler chicken (g)

Group	Age of chicken							
	1 day		21 days		35 days		42 days	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Control	39.4	0.1	1015	9.0	2147 ^b	30.8	2774 ^b	48.0
Experimental	39.3	0.1	1025	9.0	2173 ^a	33.5	2796 ^a	48.5

a, b – means with different superscripts differ significantly at $P \leq 0.05$ (in column).

TABLE 3. Mean body weight of broiler chicken at 35 and 42 days (g)

Group	35 days male		35 days female		42 days male		42 days female	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Control	2355 ^b	29.8	1940	33.9	3050 ^b	45.9	2498	36.8
Experimental	2385 ^a	31.5	1962	33.3	3086 ^a	48.0	2506	36.8

a, b – means with different superscripts differ significantly at $P \leq 0.05$ (in column).

TABLE 4. Production results of broiler chicken

Item	Control	Experimental
Feed conversion ratio ($\text{kg} \cdot \text{kg}^{-1}$)	2.1	2.0
Mortality rate (%)	3.5	3.0

Dressing percentage, the proportion of breast muscles, leg muscles and giblets, and abdominal fat content are being constantly improved by broiler breeders. The breast and leg muscles of today's broilers are much better developed than in old-type broilers, with an approximately 90% increase in dressing percentage compared to the 1950s. However, the increased rate of growth had a negative impact on broilers. The weight of digestive tract decreased, which impaired digestion, and chickens became fatter. Fat is predominantly deposited in the abdominal cavity, in the vicinity of the cloaca, and under the skin, and because it is a waste product efforts are made to reduce its content through the use of dietary supplements.

Dressing percentage of males was higher in the experimental group that received L-carnitine compared to the control group (Table 5), but the difference was not significant. A similar relationship was observed in females, in which the difference was significant at $P \leq 0.01$ (Table 6).

Feeding L-carnitine to the experimental group of males caused a non-significant decrease in the proportion of breast muscles and a significant increase in the proportion of leg muscles compared to the control group (Table 5). The proportion of breast muscles also decreased in the experimental group of females (Table 6). The proportion of leg muscles was similar in both groups.

L-carnitine supplementation decreased carcass abdominal fat in males (Table 5),

TABLE 5. Results of slaughter analysis – male (%)

Item	Dressing percentage	Breast muscle	Leg muscle	Abdominal fat
	%			
Control group	74.95	30.96	19.82b	1.94
Experimental group	75.97	29.40	22.52a	1.89
SE	0.49	0.56	0.78	0.16

a, b – means with different superscripts differ significantly at $P \leq 0.05$ (in column).

TABLE 6. Results of slaughter analysis – female (%)

Item	Dressing percentage	Breast muscle	Leg muscle	Abdominal fat
	%			
Control group	73.17 ^B	31.59 ^a	20.20	2.09
Experimental group	75.88 ^A	29.97 ^b	20.15	2.56
SE	0.58	0.46	0.65	0.16

a, b – means with different superscripts differ significantly at $P \leq 0.05$ (in column); A, B – means with different superscripts differ significantly at $P \leq 0.01$ (in column).

but increased it in females (Table 6). The inclusion of L-carnitine to the diets of chickens from the experimental group had little effect on the proportion of gizzard, liver and heart. In males, the proportion of gizzard remained almost unchanged and the proportion of liver and heart slightly decreased in the L-carnitine-supplemented group compared to the control group. In females from the experimental group, the proportion of gizzard decreased, the proportion of liver increased significantly, and the proportion of heart remained almost unchanged (Table 7).

DISCUSSION

The experimental group in which chickens were supplemented with L-carnitine had higher body weights compared to the control group. It was shown that the increased body weight of chickens may result from the contribution of L-carnitine to the metabolism of long-chain fatty acids in cell mitochondria. L-carnitine transports fatty acid molecules, present in the form of acetyl-CoA, from cytosol to the mitochondrial matrix where they are oxidized, resulting in the production

TABLE 7. Results of slaughter analysis of male and female – giblets (%)

Item	Males			Females		
	Gizzard	Liver	Heart	Gizzard	Liver	Heart
Control group	0.84	2.22	0.52	1.16	2.15b	0.53
Experimental group	0.83	2.02	0.48	1.00	2.42a	0.54
SE	0.05	0.13	0.02	0.06	0.08	0.02

a, b – means with different superscripts differ significantly at $P \leq 0.05$ (in column).

of energy in the form of ATP in body cells (Hoppel 2003).

Rodehutsord et al. (2002) reported a significant effect of L-carnitine supplementation on energy and protein utilization by Ross broilers given different levels of dietary energy. They fed L-carnitine from 1 to 21 days of age and noted an increase in body weight in the experimental group compared to the control group of chickens, when 4 and 8% of fat was added to the diet. In their study on the effect of L-carnitine, Buyse et al. (2001) showed a non-significant increase in average body weight of chickens receiving L-carnitine at 14, 21, 28 and 42 days of rearing. In males, the average body weight to 28 days was non-significantly lower in the experimental compared to the control group, but at the end of rearing (day 42) the results were the same in both groups.

Nouboukpo et al. (2009), who investigated the effect of L-carnitine supplemented in drinking water on the growth of broiler chickens, observed at 7 days of rearing that chickens from the control group had significantly lower body weight compared to the experimental groups receiving 30 and 60 mg of L-carnitine in 1 l of drinking water.

In a study investigating the effect of L-carnitine supplementation of diets differing in energy levels on performance, Rabie and Szilagyi (1998) found that L-carnitine had a positive effect on the body weight of chickens at 53 days, but the differences were not significant.

Other authors who studied the effect of L-carnitine on broiler performance found that it had no effect on body weight. None of the levels of L-carnitine: 0 and 200 mg per 1 kg of feed (Leibet-

seider 1995); 0 and 160 mg per 1 kg of feed (Lien and Horng 2001); 0, 25, 50, 75 and 100 mg per 1 kg of feed (Xu et al. 2003); 0, 50, 100 and 150 mg per 1 kg of feed (Cevik and Ceylan 2005) increased the body weight of chickens.

L-carnitine was found to slightly reduce mortality and improve feed conversion (1 kg of feed per 1 kg gain). The decreased mortality could be due to the fact that L-carnitine increases the production of antibodies, thus enhancing body immunity (De Simone et al. 1982). Furthermore, L-carnitine has antioxidant effects as it prevents oxidative stress and regulates nitric oxide (NO) (Brown 1999) and influences the activity of enzymes that play a role in defence against oxidative agents (Kremser et al. 1995). The improvement in feed conversion could be due to the fact that L-carnitine enhances fatty acid burning, thus decreasing calorie requirements (Czeczot and Ścibor 2005).

Geng et al. (2004, 2007), who studied the effects of L-carnitine (added daily to feed from 1 to 42 days of age) and coenzyme Q10 on productivity of males, found that the supplements improved feed conversion ratio (FCR). In the experiment from 2004, the authors showed FCR to decrease non-significantly, and in the experiment from 2007, FCR decreased significantly in the group of males supplemented with 100 mg of L-carnitine per 1 kg of feed compared to the other groups.

Similar results were obtained by Rabie and Szilagyi (1998) who fed L-carnitine from 18 to 53 days of age and feed conversion improved regardless of the amount of dietary energy. The effect of L-carnitine was statistically significant.

The results obtained are not supported by the studies of Rezaei et al. (2007) and Buyse et al. (2001), in which L-carnitine supplemented to chickens had no effect on feed conversion.

In our experiment, mortality decreased in the L-carnitine-supplemented group compared to the control group. Similar findings were reported by Daskirian and Teeter (2001), who observed a significant decrease in mortality in broilers receiving dietary L-carnitine.

In two studies by Geng et al. (2004, 2007), L-carnitine had an effect on mortality due to ascites (pulmonary hypertension). This disease is assessed using the ascites heart index (AHI), which is the ratio of right ventricular weight to total ventricular weight (Burton et al. 1968). Broilers with $AHI \geq 0.30$ are considered to suffer from pulmonary hypertension (Cawthon et al. 2001). The results of these two experiments were similar and showed that the L-carnitine supplement caused a significant decrease in ascites mortality.

Dressing percentage of males was higher in the experimental group supplemented with L-carnitine compared to the control group. Similar results were obtained by Daskirian and Teeter (2001) for Cobb broilers, the dressing percentage of which increased as a result of L-carnitine supplementation, but the differences were not significant.

Improved dressing percentage was also reported by Zhang et al. (2010), who studied the effect of acetyl-L-carnitine on meat quality and lipid metabolism in broilers. Dressing percentage increased with the increasing acetyl-L-carnitine supplementation, but the differences were not significant.

Different results were obtained by Celik and Ozturkcan (2003), who investigated the effect of supplemental L-carnitine and ascorbic acid on carcass composition, carcass yield, and plasma L-carnitine concentration of broiler chickens reared under different temperature; by Celik et al. (2003) in an experiment studying the effects of L-carnitine and niacin supplied by drinking water on rearing performance, carcass composition and plasma L-carnitine concentration of broiler chickens; and by Kidd et al. (2009), who determined the effect of L-carnitine on thigh yield in broilers. The three studies showed that L-carnitine supplementation had no effect on dressing percentage.

Xu et al. (2003) revealed that supplemental L-carnitine increases the proportion of breast and thigh muscles in the carcass. Best results for breast muscles were obtained when L-carnitine was added at 50 and 75 mg per 1 kg of feed. These results were significantly different compared to the other groups of broilers. The increase in leg muscles was not statistically significant.

The addition of acetyl-L-carnitine caused a non-significant increase in the proportion of breast and leg muscles (Zhang et al. 2010).

Different results were reported by Daskirian and Teeter (2001) for broilers, in which dietary L-carnitine exerted no effect on the proportion of breast muscles.

L-carnitine should decrease body fatness. Bremer (1983) proves that increased oxidation of fatty acids by L-carnitine makes them less available during esterification to triacylglycerols, which are deposited in adipose tissue. Xu et

al. (2003) also found a decrease in the abdominal fat of carcasses from males. In the group supplemented with L-carnitine, the abdominal fat content decreased significantly in relation to the control group.

Similar results were obtained by Wang et al. (2003), who found fat content to decrease in the experimental groups supplemented with L-carnitine, with statistically significant differences. Opposite results to those in the L-carnitine study were obtained by Buyse et al. (2001), who observed the proportion of abdominal fat to increase in the experimental group of males and to decrease in females. Different results were reported by Corduk et al. (2007) in an experiment investigating the effects of dietary energy density and L-carnitine supplementation on growth performance. Control broilers and those supplemented with L-carnitine in the experimental group (100 mg per 1 kg of feed) had the same abdominal fat content of 15 g per 1 kg of body weight. Kamińska (2003), who studied the effect of sex on fat content and other production parameters obtained similar results, which provided conclusive evidence that fat content was higher in pullets (2.9%) than in males (2.05% of their weight).

The dietary supplementation of chickens from the experimental group with Aminocarnifarm had little effect on the proportion of gizzard, liver and heart in the carcass. A study by Arslan et al. (2004), who added L-carnitine to goose diets, showed a significant ($P \leq 0.05$) increase in liver percentage in this group. Buyse et al. (2001) observed average liver and heart weight to increase in both males and females, but the differences were not significant. Decreased gizzard

weight was found by Rabie and Szilagy (1998), who reported that gizzard weight averaged 28.28 g in the control group of broilers and 27.39 g in the experimental group supplemented with 50 mg of L-carnitine per 1 kg of feed. These differences were not significant.

An increase in average liver weight was also reported by Celik et al. (2003), but it was not significant. Different results were obtained by Rezaei et al. (2007) in an experiment with broiler males, in which liver weight decreased and heart percentage remained unchanged in the experimental group receiving L-carnitine.

CONCLUSIONS

In summary, Aminocarnifarm (43.68% of L-carnitine) had a beneficial effect on weight gains, mortality and feed conversion, and increased dressing percentage and the proportion of leg muscles.

REFERENCES

- ARENAS J., RUBIO J.C., MARTIN M.A., CAMPOS Y., 1998: Biological roles of L-carnitine in perinatal metabolism. *Early Human Development*, 53, 43–50.
- ARSLAN C., 2006: L-carnitine and its use as a feed additive in poultry feeding a review. *Revue de Médecine Vétérinaire* 157, 3, 134–142.
- ARSLAN C., CITIL M., SAATCI M., 2004: Effects of L-carnitine administration on growth performance, carcass traits, serum lipids and abdominal fatty acid compositions of geese. *Revue de Médecine Vétérinaire* 155, 6, 315–320.

- BREMER J., 1983: Carnitine: metabolism and function. *Physiological Reviews* 63, 1420–1480.
- BROOKS G.A., 1998: Mammalian fuel utilization during sustained exercise. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 120, 89–107.
- BROWN G.C., 1999: Nitric oxide and mitochondrial respiration. *Biochemica and Biophysica Acta* 1411, 351–369.
- BURTON R.R., BESCH E.L., SMITH A.H., 1968: Effect of chronic hypoxia on the pulmonary arterial blood pressure of the chicken. *American Journal of Physiology* 214, 1438–1442.
- BUYSEJ., JANSSENSG.P., DECUYPEREJ., 2001: The effects of dietary L-carnitine supplementation on the performance, organ weights and circulating hormone and metabolite concentrations of broiler chickens reared under a normal or low temperature schedule. *British Poultry Science* 42, 230–241.
- CALVANI M., REDAE., ARRIGONI-MARTELLI E., 2000: Regulation by carnitine of myocardial fatty acid and carbohydrate metabolism under normal and pathological conditions. *Basic Research of Cardiology* 95, 75–83.
- CARTER A.L., ABNEY T.O., LAPP D.F., 1995: Biosynthesis and metabolism of carnitine. *Journal of Child Neurology* 10, 3–7.
- CAWTHON D., BEERS K., BOTTJE W.G., 2001: Electron transport chain defect and inefficient respiration may underlie pulmonary hypertension syndrome (ascites)-associated mitochondrial dysfunction in broilers. *Poultry Science* 80, 474–484.
- CELIK E., OZTURKCAN O., 2003: Effects of dietary supplemental L-carnitine and ascorbic acid on performance, carcass composition and plasma L-carnitine concentration of broiler chicks reared under different temperature. *Archives of Animal Nutrition* 57, 27–38.
- CELIK L., OZTURKCAN O., INAL T.C., CANACANKATAN N., KAYRIN L., 2003: Effects of L-carnitine and niacin supplied by drinking water on fattening performance, carcass quality and plasma L-carnitine concentration of broiler chicks. *Archives of Animal Nutrition* 57 (2), 127–136.
- CEVIK A.F., CEYLAN N., 2005: Effects of dietary L-carnitine supplementation on performance and carcass traits of broiler chickens. 3rd Ulusal Hayvan Besleme Kongresi, Adana, 391–396.
- CORDUK M., CEYLAN N., ILDIZ F., 2007: Effects of dietary energy density and L-carnitine supplementation on growth performance, carcass traits and blood parameters of broiler chickens. *South African Journal of Animal Science* 37 (2), 65–73.
- CZECZOT H., ŚCIBOR D., 2005: Rola L-karnityny w przemianach, żywieniu i terapii. *Postepy Higieny i Medycyny Doświadczalnej* 59, 9–19.
- DASKIRIAN M., TEETER R.G., 2001: Effects of dietary L-carnitine (Carniking) supplementation on overall performance and carcass characteristics of seven-week-old broiler chickens. Oklahoma Agricultural Experiment Station, 986.
- GENG A., BAOMING L., YUMING G., 2007: Effects of dietary L-carnitine and coenzyme Q10 at different supplemental ages on growth performance and some immune response in ascites-susceptible broilers. *Archives of Animal Nutrition*, 61 (1), 50–60.
- GENG A., YUMING G., JIANMIN Y., 2004: Effects of dietary L-carnitine and coenzyme Q10 supplementation on performance and ascites mortality of broilers. *Archives of Animal Nutrition* 58 (6), 473–482.
- GOLZAR ADABI S.H., COOPER R.G., CEYLAN N., CORDUK M., 2011: L-carnitine and its functional effects in poultry nutrition. *Worlds Poultry Science Journal* 67, 277–288.
- HOPPEL C., 2003: The role of carnitine in normal and altered fatty acid metabolism.

- American Journal of Kidney Diseases 41, 4–12.
- KALAISELVI C.J., PANNEERSELVAM C., 1998: Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. *Journal of Nutritional Biochemistry* 9, 575–581.
- KAMIŃSKA B.Z., 2003: Wpływ obniżenia poziomu białka w mieszanke typu grower na wyniki produkcyjne brojlerów i bilans azotu. *Roczniki Naukowe Zootechniki* 30 (2), 343–351.
- KIDD M. T., GILBERT J., CORZO A., PAGE C., VIRDEN W.S., WOODWORTH J.C., 2009: Dietary L-carnitine influences broiler thigh yield. *Asian-Australasian Journal of Animal Science* 22, 681–685.
- KREMSER K., STANGL H., PAHAN K., SINGH I., 1995: Nitric oxide regulates peroxisomal enzyme activities. *European Journal of Clinic Chemistry and Clinic Biochemistry* 33, 763–774.
- LEIBETSEDER J., 1995: Studies on effects of L-carnitine in poultry. *Archives of Animal Nutrition* 48, 97–108.
- LIEN T.F., HORNG Y.M., 2001: The effect of supplementary dietary L-carnitine on the growth performance, serum components, carcass traits and enzyme activities in relation to fatty acid β -oxidation of broiler chickens. *British Poultry Science* 42, 92–95.
- NOUBOUKPO K.E., TONA K., AGBONON A., GBEASSOR M., BUYSE J., DECUYPERE E., 2009: Effects of L-carnitine supplementation in drinking water on layer-type chick juvenile performance. *Archive für Geflügelkunde* 74 (2), 116–120.
- PIETRZAK I., OPALA G., 1998: Role of carnitine in human lipid metabolism. *Wiadomości Lekarskie* 51, 71–75 (in Polish).
- RABIE M. H., SZILAGYI M., 1998: Effects of L-carnitine supplementation of diets differing in energy levels on performance, abdominal fat content, and yield and composition of edible meat of broilers. *British Journal of Nutrition* 80, 391–400.
- REZAEI M., ATTAR A., GHODRATNAMA A., KERMANSHAHI H., 2007: Study the effects of different levels of fat and L-carnitine on performance, carcass characteristics and serum composition of broiler chicks. *Pakistan Journal of Biological Sciences* 10 (12), 1970–1976.
- RODEHUTSCORD M., TIMMLER R., DIECKMANN A., 2002: Effect of L-carnitine supplementation on utilization of energy and protein in broiler chickens fed different dietary fat levels. *Archives of Animal Nutrition*, 56, 431–441.
- De SIMONE C., FERRARI M., LOZZI A., MELI D., RICA D., SORICE F., 1982: Vitamins and immunity: II. Influence of L-carnitine on the immune system. *Acta Vitaminology and Enzymology* 4, 135–140.
- SPSS, 2006: SPSS, 14.0 for Windows user's guide, 2006. SPSS Inc., USA.
- WANG J., DU R., QIN J., WANG S., WANG W., LI H., PANG Q., 2003: Effect of yeast chromium and L-carnitine on lipid metabolism of broiler chickens. *Asian-Australasian Journal of Animal Sciences* 12, 1809–1815.
- XU Z.R., WANG M.Q., MAO H.X., ZHAN X.A., HU C.H., 2003: Effects of L-carnitine on growth performance, carcass composition, and metabolism of lipids in male broilers. *Poultry Science* 82, 408–413.
- ZHANG Y., MA Q., BAI X., ZHAO L., WANG Q., JI C., LIU L., YIN H., 2010: Effects of dietary acetyl-L-carnitine on meat quality and lipid metabolism in Arbor Acres broilers. *Asian-Australasian Journal of Animal Sciences* 12, 1639–1644.

Streszczenie: Wpływ L-karnityny na wyniki produkcyjne i wydajność rzeźną kurcząt brojlerów. Badania przeprowadzono na 640 kurczątach podzielonych losowo na dwie grupy, każda w czterech powtórzeniach (4 kontrolne i 4 doświadczalne – L-karnityna), liczące po 80 ptaków. Kurczęta z grupy kontrolnej (K) i z grupy doświadczalnej (E) otrzymywały mieszanke o tej samej wartości odżywczej, natomiast kurczętom z grupy

doświadczalnej podano dodatek Aminocarnifarmu (43,68% L-karnityny) do wody – 62,5 g na 100 l w 3 okresach: od 1 do 7, od 21 do 28 oraz od 36 do 42 dnia życia. Oceniano wpływ preparatu z L-karnityną dla kurcząt brojlerów na wyniki produkcyjne i wyniki analizy rzeźnej. Stwierdzono, że dodatek do wody Aminocarnifarmu spowodował w całym okresie odchowu poprawę wykorzystania paszy, obniżenie śmiertelności oraz wpłynął na wzrost średniej masy ciała (35, 42 dzień ♂, $P \leq 0,05$), wydajności rzeźnej ($P \leq 0,01$, ♀), udziału mięśni nóg ($P \leq 0,05$, ♂), udziału wątroby ($P \leq 0,05$, ♀).

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Preliminary characterization of the structure of race, sex, and color of the Polish population of alpacas

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Abstract: *Preliminary characterization of the structure of race, sex, and color of the Polish population of alpacas.* Alpacas are known worldily for their very fine, luxurious fiber which is three times more hardy and six times more thermal than sheep fiber. Aside from fiber, alpacas are also bred for meat and used in the tourism and recreational industry and animal assisted therapy. Possibility of comprehensive use of these animals caused alpacas' popularity to grow immensely over the past few decades all over the world, also in Poland, where they adapted very well and have been bred since 2004. The objective of the analysis was to study the stocks of the Polish alpaca population, sex structure, also interesting was the inquiry of the distribution of various fleece colour. In Poland the majority of alpaca breedings are concentrated in voivodeships: Silesia, Podlasie and Wielkopolska. According to questionnaire surveys carried out in 2012 on 9 farms, alpaca population in Poland numbers 651 and consists of: Suri (12%) and Huacaya (88%) which differ in coat type. In the surveyed population 86% consisted females and 14% males, in Suri population, 65% consisted females and 35% males. Alpaca fleece occurs in 22 natural colours, all shades of beige, brown and grey. Distribution of coat colour in the surveyed population, according to the methodologically amended criterium was established as follows: white 58%, fair brown 12%, dark brown 9%, cream-coloured 6% and grey 6%, black 5%, multicoloured 4%. In the surveyed population of Huacaya alpacas the majority consisted white-coloured animals (63%), fair brown-coloured – 11%, dark brown-coloured – 8%, grey and black – 5% each, multicoloured – 3%. The Suri population was dominated by cream-coloured animals, whereas fair brown-coloured and dark brown-co-

loured consisted 20% each, white-coloured – 12%, grey, black and multicoloured – 9% each. Results obtained can be considered satisfying compared with results obtained in Peru, where the biggest alpaca population is.

Key words: alpaca, population, race, gender, color, fiber.

INTRODUCTION

Alpacas are part of South American Camelidae family and are known all over the world for their fine, luxurious fiber. Alpaca fiber is a highly ecological product, three times as hardy and six times as thermal as sheep fiber, also, it does not contain lanoline (Quispe 2010). Alpacas are also bred for their meat and used in the tourism and recreational industry and animal assisted therapy (Morales Villavicencio 2010). In Poland alpacas adapted very well and have been bred since 2004. Two species were distinguished: Suri and Huacaya which differ significantly in coat type. Huacaya alpacas' fleece is short, spongy and crimped whereas Suris' fleece is long, falling loosely on the sides making it seem like a kind of coat (Wuliji et al. 2000). Research (Renieri et al. 2004, Oria et al. 2009) indicates 22 natural alpaca fleece colours have been established, among them: white, black

many shades of beige, brown and grey and different colour schemes. The objective of the analysis was to study the stocks of the Polish alpaca population, sex structure, also interesting was the inquiry of the distribution of various fleece colour. The results of this study will make it possible for further scientific study relating herd selection to be carried out.

MATERIAL AND METHODS

Questionnaire surveys carried out in 2012 among alpaca breeders who belong to Polski Związek Hodowców Alpak (Polish Association for Alpaca Breeders). 651 specimen from 9 farms have been surveyed. The information obtained in the surveys concerned: number of the animals, race, sex and fleece color (white, cream-color, fair brown, dark brown, grey, black and multicolour-spotted)

Data obtained can be found described and presented on diagrams. It has also been compared with the data obtained in Peru.

RESULTS AND DISCUSSION

The majority of the alpaca breedings in Poland are located in voivodeships: Silesia, Wielkopolska and Podlasie. 651 specimen are bred in the aforementioned breedings which constitutes 90% of the entire Polish alpaca population. In Poland there can be found both alpaca races: Huacaya (88%) and Suri (12%) (Figure 1). In the surveyed population 86% was male and 14% female (Figure 2) Among Huacaya population 89% constitute female specimen and 14% – male, whereas among Suri 65% is female and 35% – male.

The basic fleece color distribution in surveyed population according to the methodologically amended criterium was established as follows: white 58%, fair brown 12%, dark brown 9%, cream-coloured 6% and grey 6%, black 5%, multicoloured 4% (Figure 3). In the surveyed population of Huacaya alpacas the majority consisted white-coloured animals (63%), fair brown-coloured – 11%, dark brown-coloured – 8%, grey and black-5% each, multicoloured – 3% (Figure 4). The Suri population was dominated

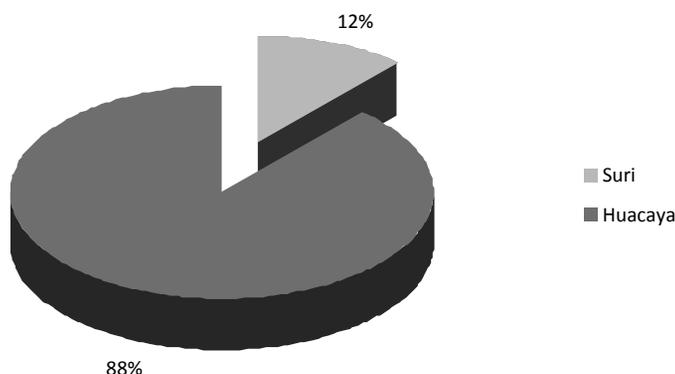


FIGURE 1. Alpaca breed structure in the Polish population in 2012, in percent

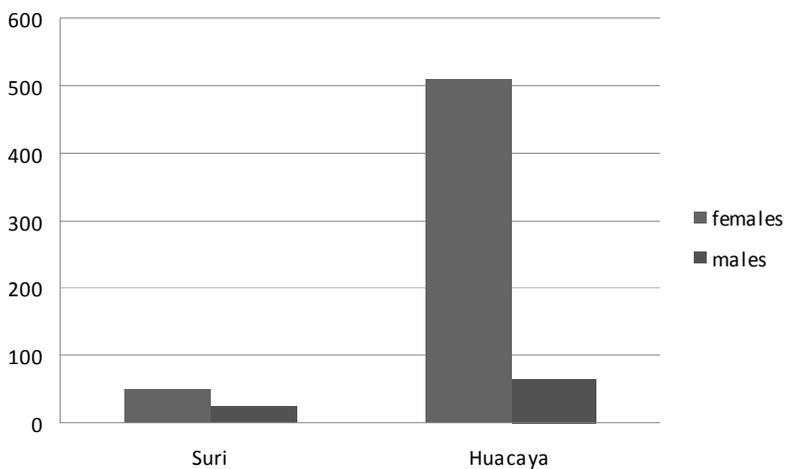


FIGURE 2. The gender structure of alpacas in the Polish population in 2012

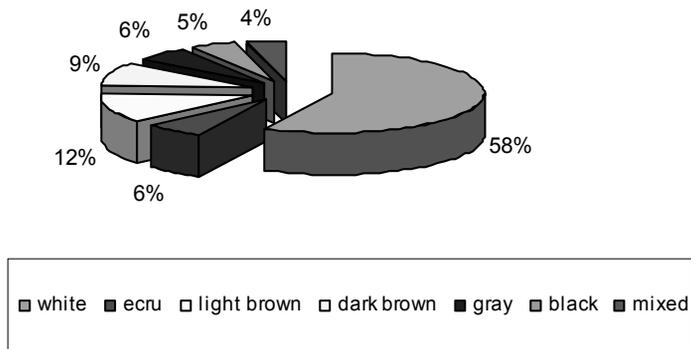


FIGURE 3. Percentage distribution of coat color in alpacas in the Polish population in 2012

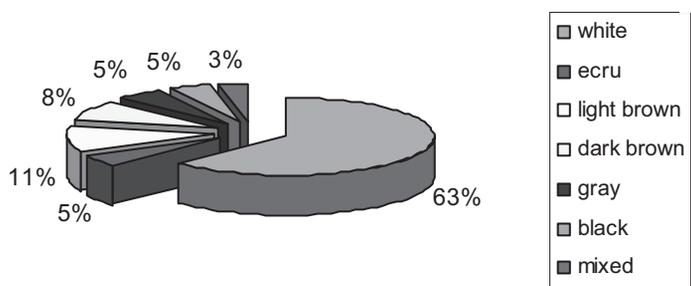


FIGURE 4. Percentage of coat color in alpacas Huacaya breed in the Polish population in 2012

by cream-coloured animals, whereas fair brown-coloured and dark brown-coloured constituted 20% each, white-coloured – 12%, grey, black and multicoloured – 9% each (Figure 5).

established that multicoloured (spotted) fleece constitutes 13% whereas Cáceres and Díaz (2007) research established this number to be 30%. Both researches were carried out in Puno – a region hold-

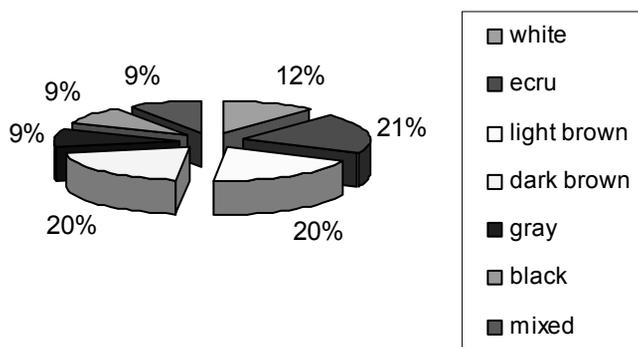


FIGURE 5. Percentage of coat color in alpacas Suri breed in the Polish population in 2012

Statistics gathered around the world showing the state of the alpaca population, do not take into account the division due to sex and color. From information provided by the Peruvian Ministry of Agriculture, Peru being the country which holds 80% of the world alpaca population, it follows that the Suri race constitutes 15% of the world alpaca population and its population decreases. The alpaca population is dominated by Huacaya race which constitutes 85% of it (Enriquez 2003, Minag 2010). The augmented demand for white fiber led to a decrease of the population of alpacas of different color in Peru (Fernandez-Baca 1994).

From data gathered by Caballero and Flores (2004) it follows that 80% of alpacas in Peru is white coloured. Multicoloured (spotted) fleece is considered undesirable, therefore it is important for population of such specimen to be kept at its lowest or even completely absent. In Huanca et al. (2007) research it was

ing the most numerous Peruvian alpaca population.

In the past years in the international market, there has been recorded a great interest in alpaca fiber (Bustinza 2001). The world market takes interest in natural (not dyed) fiber, therefore the rising demand for colour-coated alpacas, which are in minority in the world alpaca population.

CONCLUSION

Alpacas imported from South America adapted very well in Poland. The number of specimen bred (651) and the number of breeders increase every year. Polish alpaca population consists of Huacaya alpacas (88%) and Suri alpacas (12%), which is comparable with the Peruvian data: 85% Huacaya and 15% Suri, Peru being the country holding the biggest number of alpacas.

Fleece color distribution in the Polish alpaca population is as follows: white – 58%, fair brown – 12%, dark brown – 9%, cream-colour and grey – 6% each, black – 5%, multicoloured – 4%. The Peruvian research indicates that white colored alpacas constitute the majority of the population (80%), therefore the Polish population is more varied in the fleece colour distribution, which makes this result much better than the Peruvian one. With regard to multicoloured fleece, the result in Poland (4%) is better than the one obtained in Peru (13 and 30%).

There has been a great interest in alpacas in Poland. In 2012 the Association of Alpaca Breeders was founded and it shall specify further direction of research and development.

REFERENCES

- BUSTINZA V., 2001: La alpaca, conocimiento del gran potencial andino. Universidad Nacional del Altiplano, Puno, Perú: 493 pp.
- CABALLERO W., FLORES A., 2004: La sierra: primera prioridad para salir de subdesarrollo agrario. CONCYTEC, Lima, Perú: 272 pp.
- CÁCERES M., DÍAZ G., 2007: Estructura poblacional y variabilidad fenotípica de alpacas (*Vicugna pacos*) en el distrito de Paratia, Provincia de Lampa-Puno. Arch. Latinoam. Prod. Anim. 15 (Suppl. 1): 480–481.
- ENRIQUEZ P., 2003: La alpaca suri de colores naturales: ¿una raza en proceso de extinción? LEISA Revista de Agroecología 19 (3): 22–25.
- FERNANDEZ-BACA S., 1994: Genetic erosion on Camelidae. Animal Genetic Resources Information 14: 97–105.
- HUANCA T., APAZA N., GONZALES M., 2007: Defectos congénitos y hereditarios visibles en alpacas de dos zonas representativas de la región Puno. Arch. Latinoam. Prod. Animal. 15 (Supl. 1): 186–194.
- MINAG – Ministry of Agriculture of Perú, 2010: Estadística Agraria. <http://www.minag.gab.pe/portal/herreamientas/estadisticas/estadistica-agraria-mensual83?start=2>
- MORALES VILLAVICENCIO A., 2010: Chów alpak. MULTICO, Warszawa: 84 pp.
- ORIA I., QUICAÑO I., ALFONSO E.Q.L., 2009: Variabilidad del color de la fibra de alpaca en la zona altoandina de Huancavelica-Perú. Animal Genetic Resources Information 45: 79–84.
- QUISPE E., 2010: Evaluación de características productivas y textiles de la fibra de alpacas Huacaya de la región de Huancavelica, Perú. Libro de Conferencias Magistrales del International Symposium on Fiber South American Camelids. Huancavelica-Perú: 119–169.
- RENIERI C., ANTONININI M., FRANK E., 2004: Fibre recording systems in camelids. In: Current status of genetic resources, recording and production systems in African, Asian and American Camelids. ICAR Technical Series 11: 131–141.
- WULIJI T., DAVIS G.H., DODDS K.G., TURNER P.R., ANDREWS R.N., BRUCE G.D., 2000: Production, performance, repeatability and heritability estimated for live, weight, fleece weight and fiber characteristics of alpacas in New Zealand. Small Rumin. Rs. 37: 189–201.

Streszczenie: *Wstępna charakterystyka struktury pogłowia alpak w Polsce pod względem ras, płci, występujących typów koloru. Alpaki znane są na świecie z produkcji bardzo cienkiego luksusowego włókna, które jest trzy razy trwalsze od wełny owczej i sześć razy cieplejsze. Oprócz włókna wykorzystywane są też do produkcji*

mięsa, w turystyce i rekreacji oraz alpakoterapii. Wszechstronna możliwość wykorzystania tych zwierząt spowodowała, że w ostatnich dekadach znacznie wzrosło zainteresowanie ich hodowlą na całym świecie w tym w Polsce. Celem pracy było wstępne scharakteryzowanie polskiego pogłowia alpak pod względem ras, płci, występujących typów koloru. W naszym kraju alpaki zaaklimatyzowały się bardzo dobrze i hodowane są od 2004 roku. W Polsce hodowle alpak skoncentrowane są w przeważającej części w województwach: śląskim, wielkopolskim i podlaskim. Z badań ankietowych przeprowadzonych w 2012 roku na 9 farmach wynika, że krajowa populacja liczy 651 szt. i składa się z alpak: Suri (12%) i Huacaya (88%) różniących się rodzajem runa. W badanej populacji samice stanowiły 86%, a samce 14%. Wśród alpak Huacaya 89% stanowiły samice i 11% samce, u Suri udział samic wynosił 65% a samców 35%. Runo alpak występuje w 22 naturalnych kolorach od białego do czarnego ze wszystkimi możliwymi odcieniami beżu, brązu i szarego. Udział podstawowych kolorów runa w badanej populacji wg przyjętego kryterium w metodyce był następujący: biały 58%, jasny

brąz 12%, ciemny brąz 9%, kremowy i szary po 6%, czarny 5%, wielokolorowy 4%. W badanej populacji alpak Huacaya największy udział stanowiły zwierzęta w kolorze białym 63%, jasny brąz 11%, ciemny brąz 8%, a kremowy, szary i czarny po 5%, wielokolorowy 3%. Dominującym kolorem runa Suri był kremowy 21%, jasny brąz i ciemny brąz po 20%, biały 12%, natomiast szary, czarny i wielokolorowy po 9% każdy. Uzyskane wyniki są satysfakcjonujące w porównaniu z badaniami prowadzonymi w Peru, które posiada największą na świecie populację tych zwierząt.

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Analysis of body measurements and pelvis area index of Limousine cows

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Abstract: *Analysis of body measurements and pelvis area index of Limousine cows.* The aim of the research was to analyse the basic body measurements and to define the relationship between pelvis area index (IPM) and body weight (M), height at sacrum (WK) as well as chest circumference (OKP) of Limousine beef cows. Data concerning 634 Limousine cows were taken as a material for investigation. The measurements of chest circumference, height at sacrum and cows body weight were carried out. To calculate pelvis area index the following body measurements were collected: width at hips *Tuber coxarum* (Tc), width at pins *Tuber ischium* (Ti), length of pelvis (TcTi). Correlation coefficients between pelvis area index (IPM) and body weight (M), height at sacrum (WK), chest circumference (OKP) were calculated using Spearman method by SPSS 12.0. The high variability of cow body weight for Limousine breed was observed. Variability coefficients (CV) of cow body weight ranged from 18.3 to 20% and were much higher than variability coefficients of body measurements which were similar to those obtained by the other authors. All of correlation coefficients between pelvis area index and the basic body measurements and cow body weight were positive and statistically significant ($p \leq 0.01$). Statistically significant ($p \leq 0.01$) correlation between IPM and cow body weight (M) and chest circumference (OKP) as well as height at sacrum (WK) justify the Polish breeders aspirations to enlarge the body weight and caliber of beef cows.

Key words: cow body weight, body measurements, pelvis area index, correlations.

INTRODUCTION

The body measurements are the tool to compare and evaluate the body conformation of animals of different breeds. In order to assess the proper proportion of the particular body parts the body indices are used (Nogalski 2003, Litwińczuk and Szulc 2005). They are often used to draw some conclusions concerning proportionality and maturity and stay in relation with the other traits (such as life weight) (Bene et al. 2007). Body measurements may also be used as an important selection factor.

Body measurements can be also applied for animal body weight estimation, which is often useful when preparing individual feeding ration or for determining medicine dose (Andersen 1978, Dickerson 1978, Fitzhugh 1978). Based on the conformation, beef production can be better estimated than other production traits. The heritability of the body measurements is relatively high (Arango et al. 2002). According to Bene et al. (2007), heritability of the live weight of the mature animals and of the height at sacrum ranged 0.47–0.51 and 0.62–0.88 respectively, and there is also close genetic cor-

relation ($r = 0.80$) between those traits. It should be noticed, that in the scientific literature there are very few comparative examinations of the body measurements of the various beef cattle breeds. The published data are mostly based on data from technical books, and refer to the animals kept in the various husbandry systems and at various times.

MATERIAL AND METHODS

The experiment was carried out at the beef farms associated to the Polish Beef Cattle Breeders and Producers Association keeping purebred herds of the most popular in Poland beef breed – Limousine. The data for 634 Limousine (at different age) cows were considered. All of the examined animals were weighed on the commercial scale.

Moreover, the following body measurements were done: chest circumference (OKP) and body length (DT). The above mentioned measurements were used to calculate the body weight according to Truchanowski method (MT) using a following formula:

$$MT = \frac{DT \cdot OKP \cdot K}{100}$$

where:

DT – body length (measured from the top of the withers to external part of pin),

OKP – chest circumference,

K – coefficient for beef breeds = 2.5.

That way of body weight calculation was used to evaluate the correlation between the methods. To calculate pelvis area index (IPM) the following body measurements were carried out: width at

hips *Tuber coxarum* (Tc), width at pins *Tuber ischium* (Ti), rump length (TcTi). IPM was calculated according to a formula:

$$IPM = \frac{TcTc + TiTi \cdot TcTi}{2}$$

Cow caliber of Limousines was evaluated in 11 point scale by McKirnan (2000) based on height at sacrum (WK).

RESULTS AND DISCUSSION

Cow body weight, body measurements and IPM were presented in Table 1. Limousine cows weight ranged from 326 to 990 kg with high average means 626 kg. The comparison of body weights and the basic body measurements of mature cows shows that the traditional classification on small, average and big caliber cows should be verified for the Polish beef cattle population.

The average of the particular body measurements, i.e. OKP, DT, WK, WW confirm, that the modern type of Limousine cow is the cow of big caliber, tall and long-sized, which means that the selection gives desired results. The measurements connected to calving ease like Tc, Ti, TcTi and IPM confirmed predispositions of examined breed in terms of calving difficulty, which is one of the most important traits in the beef cattle selection. Many authors agreed, that one of the most important reasons of calving difficulty is a disproportion between foetus size and pelvis area, especially in case of heifers. Pelvis area heritability for beef cows is high ($h^2 = 0.56$) (Morrison et al. 1986), which means that this trait

TABLE 1. Cow body weight, body measurements and indices

Specification	Limousine cows					
	<i>n</i>	min.	max.	\bar{x}	SD	CV
Body weight on balance – M (kg)	420	326	990	626	116.4	18.6
Body weight calculated on chest circumference – OKP (kg)	632	280	986	651	119.8	18.4
Body weight calculated according to Truchanowski method – MT (kg)	634	388	965	666	83.8	12.6
Chest circumference – OKP (cm)	634	152	237	199	12.8	6.4
Body length – DT (cm)	634	100	166	133	9.9	7.4
Height at withers – WW (cm)	634	122	156	139	4.7	3.4
Height at sacrum – WK (cm)	634	118	153	135	4.6	3.4
Width at hips – Tc (cm)	634	32	68	54	4.3	8.0
Width at pins – Ti (cm)	634	18	36	25	3.0	12.1
Rump length – TcTi (cm)	634	39	66	55	3.4	6.2
Pelvis area index – IPM	634	1150	3154	2207	279.6	12.7

could be improved just by the selection itself. It was stated by Philipsson (1976) and Weiher et al. (1992), that the pelvis area is highly connected to the measurements describing its width and length. It was also proved (Nogalski 2004), that internal pelvis measurements are highly correlated with pelvis area measured as in the presented study. According to the above mentioned author, correlation coefficient between internal and external measurements was high ($r = 0.73$). The similar results were presented by Weiher et al. (1992), who found strong relation between width at hips and the internal pelvis width.

Caliber traits, defined as the skeletal development of examined cows, was evaluated by 11 point scale based on height at sacrum measurements (McKirnan 2000) are shown in Table 2. According to Litwińczuk and Szulc (2005) and Wójcik (2006) a high correlation between that measurement and chest

circumference and body weight makes height at sacrum one of the best indices of beef cattle maturity grade.

Correlations between the real cow body weight (M) and the different measurements and IPM are presented in Table 3.

Very high and highly significant correlation coefficients between the real body weight (measured on balance) and body weight estimated on the base of chest circumference (OKP) (0.926) and body weight estimated according to Truchanowski method (MT) (0.825) confirm usefulness of some body measurements for estimation of approximate body weight of the animal.

Body weight calculated in that way is sufficient to prepare feeding ration or medicine dose, but is not for beef cattle recording scheme, where the real body weight measured on scale is needed.

Very high and highly significant correlation coefficients between the real body weight (M) and pelvis area index

TABLE 2. Percentage of caliber note according to height at sacrum – WK measurement according to McKirnan's scale

Percentage of caliber note according to height at sacrum WK measurement											
Hight at sacrum – WK (cm)	Up to 113	114–118	119–122	123–127	128–132	133–137	138–142	143–146	147–151	152–155	156–160
McKirnan's category	1	2	3	4	5	6	7	8	9	10	11
Limousine cows	–	–	0.5	3.5	23.8	41.2	21.8	8.2	1.0	0.2	–

TABLE 3. Correlation between body weight measured on balance (kg) and estimated by other methods

Specification	Body weight on balance – M (kg)	
Body weight calculated on chest circumference – OKP (kg)	<i>n</i>	419
	<i>r</i>	0.926**
Body weight calculated according to Truchanowski method – MT (kg)	<i>n</i>	420
	<i>r</i>	0.825**
Pelvis area index – IPM	<i>n</i>	420
	<i>r</i>	0.782**
Height at sacrum – WK (cm)	<i>n</i>	420
	<i>r</i>	0.553**
Height at withers – WW (cm)	<i>n</i>	420
	<i>r</i>	0.610**

**Correlation significant at 0.01.

(IPM) (0.782) as well as between real body weight (M) and height at sacrum (WK) (0.553) and withers (WW) (0.610) fully justifies breeders' tendency to increase body weight and caliber of cows in the beef herds. The results obtained Morris and Wilton (1976).

Correlations between pelvis area index and the basic body measurements are presented in Table 4. All calculated correlations were high and statistically significant ($p \leq 0.01$). Obtained results are similar to the figures presented by other authors (Andersen 1978, Berger et al. 1992, Dickerson 1978).

CONCLUSIONS

- Very high and highly significant correlation coefficients between the real body weight (measured on balance) and pelvis area index as well as between real body weight and height at sacrum and withers fully justifies the tendency of breeders to increase the body weight and caliber of cows in the beef herds.
- The simple body measurements like OKP, WK, WW and/or body weight examination before breeding time may help to avoid the problems and consequences linked to calving difficulties.

TABLE 4. Correlations between pelvis area index and the basic body measurements

Specification	Pelvis area index – IPM	
	Chest circumference – OKP (cm)	<i>n</i>
<i>r</i>		0.708**
Height at sacrum – WK (cm)	<i>n</i>	634
	<i>r</i>	0.556**
Height at withers – WW (cm)	<i>n</i>	634
	<i>r</i>	0.545**

**Correlation significant at 0.01.

REFERENCES

- ANDERSEN B.B., 1978: Animal size and efficiency, with special reference to growth and feed conversion in cattle. *Anim. Prod.* 27, 381–391.
- ARANGO J.A., CUNDIFF L.V., Van OLECK L.D., 2002: Genetic parameters for weight, weight adjusted for body condition score, height and body condition score in beef cattle. *J. Anim. Sci.* 80, 3112–3122.
- BENE S., NAGY B., NAGY L., KISS B., POLAR J. P., SZABO F., 2007: Comparison of body measurements of beef cows of different Breed. *Arch. Tierz.* 50 4, 363–373.
- BERGER P.J., CUBAS, A.C., KOEHLER K.J., HEALEY M.H., 1992: Factors affecting distocia and early calf mortality in Angus cows and heifers. *J. Anim. Sci.* 70, 1775–1786.
- DICKERSON G.E., 1978: Animal size and efficiency: basic concepts. *Anim. Prod.* 27, 367–379.
- FITZHUGH H.A., 1978: Animal size and efficiency, with special reference to the breeding female. *Anim. Prod.* 27, 393–401.
- LITWIŃCZUK Z., SZULC T., 2005: Hodowla i użytkowanie bydła (praca zbiorowa). PWRiL, Warszawa.
- McKIRNAN B., 2000: Frame scoring of beef cattle. Agfact A2.3.4. Department of Primary Industries, State of New South Wales, Australia.
- MORRIS C.A., WILTON J.W. 1976: The influence of body size on the biological efficiency of cows: A review. *Can. Anim. Sci.* 56, 613–647.
- MORRISON D.G., WILLIAMSON W.D., HUMMES P.E., 1986: Estimates of heritabilities and correlations of traits associated with pelvic area in beef cattle. *J. Anim. Sci.* 63, 432–440.
- NOGALSKI Z., 2003. Relations between the course of parturition, body weights and measurements of Holstein-Friesian calves. *Czech J. Anim. Sci.* 48, 2, 51–59.
- NOGALSKI Z. 2004: Zootechniczne uwarunkowania jakości porodu jałówek i krów czarno-białych. *Rozpr. i Monogr.* 101, UWM, Olsztyn.
- PHILIPSSON J., 1976: Studies on calving difficulty, stillbirth and associated factors in Swedish cattle breeds. IV. Relationships between calving performance, pre-calving body measurements and size of pelvic opening in Friesian heifers. *Acta Agric. Scand.* 26, 221–229.
- WEIHER O., HOFFMANN G., SASS D., 1992: Untersuchungen ueber Beziehungen zwischen Beckeninnen – und Beckenaussenmassen bei Schwarzbuntkuehen. *Dt. Tierärztl. Wschr.* 99, 433–472.
- WÓJCIK P., 2006: Przydatność wyników punktowej oceny budowy ciała i pomiarów zoometrycznych miednicy w selekcji krów na łatwe porody. *Rocz. Nauk. Zoot., Monografie i Rozprawy, Instytut Zootechniki, Kraków.*

Streszczenie: Analiza pomiarów zoometrycznych i indeksu powierzchni miednicy krów rasy *limousine*. Celem badań była analiza podstawowych pomiarów zoometrycznych oraz określenie zależności między indeksem powierzchni miednicy (IPM) a rzeczywistą masą ciała (M), wysokością w krzyżu (WK) i obwodem klatki piersiowej (OKP) krów jednej z najbardziej popularnych w Polsce ras mięsnych tj. *limousine*. Badania objęto populację 634 krów rasy *limousine*. Określono masy ciała krów (M) oraz wykonano pomiary obwodu klatki piersiowej (OKP) i wysokości w krzyżu (WK). Dla oszacowania indeksu powierzchni miednicy u wszystkich krów oznaczono odległość pomiędzy guzami biodrowymi *Tuber coxarum* (Tc), odległość pomiędzy guzami kulszowymi *Tuber ischium* (Ti) oraz długość zadu (TcTi). Współczynnik korelacji między indeksem powierzchni miednicy a obwodem klatki piersiowej (OKP), wysokością w krzyżu (WK) i masą ciała krowy (M) oszacowano metodą Spearmana za pomocą pakietu SPSS 12.0. Stwierdzono dużą

zmienność masy ciała krów w obrębie rasy. Uzyskane współczynniki zmienności (CV) masy ciała krów były wyższe (od 18,3 do 20%), a współczynniki zmienności wymiarów zoometrycznych zbliżone do uzyskanych przez innych autorów. Wszystkie korelacje (r) między indeksem powierzchni miednicy a podstawowymi pomiarami zoometrycznymi i rzeczywistą masą ciała były dodatnie i istotne ($p \leq 0,01$). Wysokie i istotne statystycznie ($p \leq 0,01$) współczynniki korelacji między IPM a masą rzeczywistą (M) oraz obwodem klatki piersiowej (OKP) i wysokością w krzyżu (WK) w pełni uzasadniają dążenia hodowców bydła mięsnego do zwiększania kalibru i masy ciała krów matek.

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The fatty acid composition of *longissimus dorsi* muscle of Polish Lowland ram lambs fattening under overhead shelter versus those in a barn

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Abstract: *The fatty acid composition of longissimus dorsi muscle of Polish Lowland ram lambs fattening under overhead shelter versus those in a barn.* The aim of the study was the comparison of fatty acid composition in longissimus dorsi muscle of ram lambs fattening under overhead shelter versus those in a barn. The maintenance did not influence on main fatty acid composition of muscle tissue. The muscle tissue of lambs reared under overhead shelter compared to these kept in the barn was characterized by significantly higher C10:0, C12:0 ($P \leq 0.01$) but higher C18:2, c9, t11 ($P \leq 0.05$) content, which is desirable in human diet with regard to its peculiarities. The lambs rearing under overhead shelter does not decrease the nutritional value of meat and can reduce the cost of fattening.

Key words: fatty acid, lambs, maintenance.

INTRODUCTION

The fatty acid composition in the diet is very important in relation to consumer health. The saturated fatty acid and n-6 unsaturated fatty acid in dietary lipids increase the risk to contract coronary heart disease (Hu et al. 1999). The polyunsaturated fatty acids (PUFAs), particularly n-3 PUFAs exert beneficial effects against cardiovascular and cancer diseases, inflammatory and immune disorders, and neurological dysfunctions (Burns and Spector 1994, Calder 1998). The conjugated linoleic acids such as c9,

t11 and C18:2 have the same profitable effect on human health (Khanal and Dhi-man 2004). The lamb meat can be characterized by comparatively high amounts of beneficial conjugated linoleic acids from other ruminant meat (Schmid et al. 2006). Different factors influence lambs fatty acid profile. It has been shown that fat composition of lamb's tissues may be modified by diet and animal production system (Diaz et al. 2002, Wachira et al. 2002, Rhee et al. 2003).

The aim of the present study was examination if the maintenance affects the fatty acid composition in *longissimus dorsi* muscle of Polish Lowland ram lambs fattening under overhead shelter versus those in a barn.

MATERIAL AND METHODS

The experiment has been conducted on 20 lambs of the Polish Lowland sheep of dual wool and meat purpose. This strain of sheep with good body conformation and meat performance is also characterise by high prolificacy (175%) and is mainly used to slaughter lambs production (Kuźnicka et al. 2005).

After lambing single, ram lambs together with their nursing mothers were divided into two groups, experimental

(I) and control (II), comprised 10 lambs in each. During all period of the experiment, treated group (I) was kept under overhead shelter, while control group (II) was kept in the barn.

The barn was made of bricks with tin ridge roof, usable loft and gravitate ventilation, where temperature did not decrease below 12°C at 75% of relative humidity.

The overhead shelter was constructed of three wooden walls, wire-netting open-front from southern side and tin uninsulated roof. Both groups were kept on deep litter.

The mating season was held at typical term for that breed (September/October), so parturition on the turn of February and March.

The animals were fed standard diets recommended by INRA (2001). The ewes were fed with farm produced fodder (grass hay, corn grain, rapeseed grind, wheat bran, red carrot). The lambs during the rearing period to the age of 100 days were with mothers and milk was their basic food. From second week of age, they received additional concentrate (barley, oat, soya grind) and grass hay. The protein and energy content of the given feed are given in Table 1.

After the weaning (100th day of life) lambs from both groups has been kept at the same conditions as during the nursing period.

At the age of 120 days the lambs were slaughtered according to standard commercial procedures. Carcasses were chilled at 4°C for 24 h. The samples of meat were taken from *musculus longissimus dorsi* for fatty acid analysis.

Total lipids in lamb's muscle were extracted according to Folch et al. (1957). Fatty acid methyl esters were prepared according to method given by Kramer et al. (1997). Fatty acid composition of lipids was performed by gas-chromatograph analysis using Hewlett Packard GC 5890 instrument equipped with a fused-silica capillary column DB-23 (length 60 m, internal diameter 0.25 mm, film thickness 0.25 µm). Operating conditions were: helium flow 1.2 ml per 1 min; a FID detector at 240°C. The temperature programme of column was: 1 min at 130°C, an increase to 170°C by +10°C per 1 min; to 215°C by +2.5° per 1 min; to 230°C by +20° per 1 min. The fatty acids and fatty acid isomers were identified and quantified via following external FAME standards: 189-17, 189-19 – Supelco; octadecadienoic acid, conjugated methyl ester 0-5632 – Sigma-Aldrich) (Poznan, Poland). Results are expressed in g per 100 g of fat.

Data were estimated using the SPSS statistical package. The one-way analysis of variance procedures were used to examine the effect of maintenance on the

TABLE 1. The protein (g) and energy (MJ) content of the given feed

Specification	Protein (g)	Energy (MJ)
Ewe during the nursing	350	11.8
Lambs 31–50 day of age	39–56	1.4–1.9
Lambs 51–70 day of age	78–96	2.7–3.4
Lambs 71–100 day of age	115–159	4.2–5.9
Lambs 101–120 day of age	165	7.1

fatty acid composition of *musculus longissimus dorsi*.

The figures have been compared in tables in the form of the least square means (LSM) and the standard error (S_E).

RESULTS AND DISCUSSION

The fatty acid composition in intramuscular fat of lambs rearing under overhead shelter and in the barn are reported in Table 2.

The higher portion of saturated fatty acid (SFA) in the group of lambs reared under overhead shelter may be due to intensive synthesis de novo of those acids in this less favorable to human health medium-chain fatty acids (FA), such as C10:0 and C12:0.

Harsh rearing conditions (lower temperature) may cause higher feed intake and higher production of volatile fatty acids in the rumen, from which are the ruminants synthesized SFA and accumulate in tissues (Chilliard 1993).

In lambs reared under overhead shelter the increase of palmitic and stearic acids of the SFA has been noted, although the differences were not significant. The palmitic acid has a neutral effect on human health, while stearic effectively reduce blood cholesterol levels (Castelli 1993, Mensik 1993). Because of that, an increase of that acid content does not decrease health benefits of lamb meat.

In the muscle tissue of lambs kept under overhead shelter decreased monounsaturated fatty acids (MUFA) but increased polyunsaturated fatty acids (PUFA) content. MUFA significantly reducing the level of fraction LDL lipoproteins affecting atherosclerotic changes

(Renner 1995). The difference between the maintenance was not confirmed statistically. These two groups of fatty acids can attend similar functions in the body of animals and thus compete with each other, especially when it comes to take positions in the phospholipid molecule (Hames and Hooper 2004).

Therefore, the increase in PUFA content in muscle tissue of lamb kept under overhead shelter justifies the decrease of MUFA and vice versa in the group of lambs kept in the barn.

The PUFA fatty acids, especially long-chain of n-3 C18:3, C20:5 (EPA), C22:6 (DHA) are essential to the human health. They are not only influence beneficial on the cardiovascular system but also exert anti-cancer activity, reduce inflammation, and the toxicity of certain drugs (Drevon 1992, Fernandez and Venkatraman 1993, Burns and Spector 1994). Even a small increase in the content of these acids in the meat of lambs can be considered for improving health benefits.

In intramuscular fat of lambs reared under overhead shelter also the higher content ($P \leq 0.05$) of rumenic acid (C18:2 *c*9, *t*11 – conjugated linoleic acids, CLA) has been noted. The rumenic acid can be produced by bacteria in the rumen or be derived from endogenous synthesis of vaccenic acid, C18:1 *t*11 by $\Delta 9$ desaturase (Bessa et al. 2000, Schmid et al. 2006). This isomer has positive effect on preventing cancer, which results from the antioxidant properties of those isomer metabolites. In addition, conjugated linoleic acid *c*9, *t*11 has been characterizing by immunoregulatory, cytotoxic and anti-atherosclerosis properties (Bessa et al. 2000, Radzik-Rant 2005,

TABLE 2. Fatty acid composition (g per 100 g of fat) of *longissimus dorsi* muscle depending of maintenance

Fatty acids	Lambs fattening in the barn		Lambs fattening under overhead shelter		Significance
	LSM	S _E	LSM	S _E	
C10:0	0.25	0.02	0.36	0.02	**
C12:0	0.65	0.07	0.92	0.07	**
C14:0	5.61	0.35	6.38	0.35	
C14:1	0.24	0.02	0.25	0.02	
C15:0	0.71	0.03	0.82	0.03	*
C15:1	0.21	0.01	0.22	0.01	
C16:0	24.91	0.43	25.17	0.43	
C16:1	1.96	0.10	1.83	0.10	
C17:0	1.27	0.07	1.18	0.07	
C17:1	1.10	0.06	1.02	0.06	
C18:0	13.85	0.48	14.15	0.48	
C18:1 <i>t</i> 11	2.44	0.16	2.66	0.16	
C18:1 <i>c</i> 9	33.64	0.65	31.86	0.65	
C18:2 <i>n</i> 6	3.50	0.20	3.58	0.20	
C18:2 <i>c</i> 9, <i>t</i> 11	0.47	0.04	0.61	0.04	*
C18:3 <i>n</i> 3	1.08	0.09	1.30	0.09	
C18:3 <i>n</i> 6	0.45	0.05	0.49	0.05	
C20:4 <i>n</i> 6	0.63	0.16	1.01	0.16	
C20:3 <i>n</i> 3	0.58	0.10	0.57	0.10	
C20:5 <i>n</i> 3	0.12	0.02	0.10	0.02	
C22:5 <i>n</i> 3	0.18	0.03	0.15	0.03	
C22:6 <i>n</i> 3	0.10	0.02	0.13	0.02	
Σ SFA	47.24	0.71	48.97	0.71	
Σ MUFA	39.61	0.78	37.85	0.78	
Σ PUFA	7.11	0.45	7.94	0.45	
Σ <i>n</i> -3	2.06	0.18	2.26	0.18	
Σ <i>n</i> -6	4.58	0.33	5.08	0.33	
<i>n</i> -6/ <i>n</i> -3	2.65	0.18	2.63	0.18	

Statistical significance: * $P \leq 0.05$, ** $P \leq 0.01$.

Radzik-Rant et al. 2012). Slightly higher ratio of CLA *c*9, *t*11/C18:1, *t*11 indicates greater $\Delta 9$ desaturase activity in the body of lambs kept under overhead shelter.

CONCLUSIONS

The maintenance did not influence on main fatty acid composition of muscle tissue.

The muscle tissue of lambs reared under overhead shelter compared to keep in the barn was characterized by significantly higher C10:0, C12:0 ($P \leq 0.01$) but higher C18:2, *c*9, *t*11 ($P \leq 0.05$) content, which is desirable in human diet with regard to its peculiarities.

The lambs rearing under overhead shelter does not decrease the nutritional value of meat and can reduce the cost of fattening.

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REFERENCES

- BESSA R.J.B., SANTOS-SILVA J., RIBEIRO J.M.R., PORTUGAL A.V., 2000: Reticulo-rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. *Liv. Prod. Sci.* 63, 201–211.
- BURNS C.P., SPECTOR A.A., 1994: Biochemical effect of lipids on cancer therapy. *J. Nutr. Biochem.* 5, 114–123.
- CALDER P.C., 1998: Fat chance of immunomodulation. *Immunol Today* 19, 244–247.
- CASTELLI W.P., 1993: The fact and fiction of lowering cholesterol concentration in the primary prevention of coronary heart disease. *Brit. Heart J.* 69 (Suppl.), 70–73.
- CHILLIARD Y., 1993: Dietary fat and adipose tissue metabolism in ruminants, pigs and rodents. A review. *J. Dairy Sci.* 76, 3897–3931.
- DIAZ M.T., VELASCO S., CANEGUE V., LAUZURICA S., De HUIDORO F.R., PEREZ C., GONZALES J., MANZANARES C., 2002: Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. *Small Rum. Res.* 43, 257–268.
- DREVON A.Ch., 1992: Marine oil and their effects. *Scand. J. Nutr.* 36 (suppl.) 26, 38–45.
- FERNANDEZ G., VENKATRAMAN J.T., 1993: Role of omega-3 fatty acids in health and disease. *Nutr. Res.* 13, 19–45.
- HAMES B.D., HOOPER N.M., 2004: Biochemistry – krótkie wykłady. Wyd. Nauk. PWN, Warszawa.
- FOLCH J., LEES M., SLOANA-STANLEY G.H., 1957: A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 266, 497–510.
- HU F.B., STAMPFER M.J., MARSON J.E., ASCHIERO A., COLDITZ G.A., SPEIZER F.E., HENNEKENS CH., WILLET W.C., 1999: Dietary saturated fats and their food sources in ratio to the risk of coronary heart disease in women. *Am. J. Clin. Nutr.* 70, 1001–1008.
- INRA 2001: Normy żywienia bydła, owiec i kóz. Wartość pokarmowa pasz dla przeżuwaczy. Opracowanie według INRA (1988). Wyd. II. IZ Kraków.
- KHANAL R.C., DHIMAN T.R., 2004: Biosynthesis of conjugated linoleic acid (CLA). *Pak. J. Nutr.* 3, 2, 72–81.
- KRAMER J.K.G., FELLNER V., DUGAN M.E.R., SAUER F.D., MOSSOBA M.M. YURAWECZ P., 1997: Evaluating acid and base catalysts in the methylation of

- milk and rumen fatty acids with special emphasis on conjugated diens and total trans fatty acids. *Lipids* 32, 1219–1228.
- KUŹNICKA E., RANT W., NIŹNIKOWSKI R., 2005: The estimation of lifetime reproductive performance of Żelazneńska sheep. *Roczniki Naukowe Zootechniki (Supl.)* 21, 41–44.
- MENSIK R.P., 1993: Effectsof the individual saturated fatty acids on serum lipids and lipoprotein concentrations. *Am. J. Clin. Nutr.*, 57 (Suppl.), 711–714.
- RADZIK-RANT A., 2005: Modyfikowanie zawartości kwasów tłuszczowych w tkance mięśniowej jagniąt poprzez wzbogacenie diety olejami różnego pochodzenia. *Rozprawy Naukowe i Monografie*. Wydawnictwo SGGW, Warszawa.
- RADZIK-RANTA A., RANT W., KUŹNICKA E., 2012: The fatty acid composition of *longissimus lumborum* muscle of suckling and early-weaned dual-purpose wool/meat lambs. *Archiv. Tierzucht* 55, 3, 285–293.
- RENNER E., 1995: Importance of milk and its different components for humen nutrition and health. *Turku. Finland. Proceedings of NJF/NMR – Seminar*, 252, 7–22.
- RHEE K.S., LUPTON C.J., ZIPRIN Y.A., RHEE K.C., 2003: Carcass traits of Rambouillet and Merino × Rambouillet lambs and fatty acid profiles of muscle and subcutaneous adipose tissues as affected by new sheep production system. *Meat Sci.* 65, 693–699.
- SCHMID A., COLLOMB M., SIEBER R., BEE G., 2006: Conjugated linoleic acid in meat and meat products: *Meat Sci.* 73, 29–41.
- WACHIRA A.M., SINCLAIR L.A., WILKINSON R.G., ENSER M., WOOD J.D., FISHER A.V., 2002: Effect of dietary fat source and breed on the carcass composition n-3 polyunsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. *Br. J. Nutr.* 88, 697–709.

Streszczenie: Skład kwasów tłuszczowych w mięśniu *longissimus dorsi* u trzyczkówek polskiej owcy nizinnej utrzymywanych w owczarni i pod wiatą. Celem badań było porównanie składu kwasów tłuszczowych w mięśniu najdłuższym grzbietu jagniąt trzyczkówek tuczonych w ciepłej owczarni i pod półotwartą wiatą. Nie stwierdzono wpływu warunków odchowu na zawartość podstawowych grup kwasów tłuszczowych w tkance mięśniowej. W mięsie trzyczkówek tuczonych pod wiatą zanotowano istotnie większą ($P \leq 0,01$) zawartość kwasów C10:0, C12:0 przy jednocześnie wyższym ($P \leq 0,05$) poziomie CLA, który jest pożądany w diecie człowieka. Odchowywanie jagniąt w ekonomicznych pomieszczeniach półotwartych nie obniża walorów zdrowotnych pozyskiwanego od nich mięsa.

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The content of *cis-9, trans-11* CLA isomer determined by two methods in ewe's milk fat

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Abstract: The content of *cis-9, trans-11* CLA isomer determined by two methods in ewe's milk fat. The aim of this study was the quantitative determination of *cis-9, trans-11* isomer by two methods: gas chromatography (GC) and silver-ion high performance liquid chromatography (Ag⁺HPLC) in ewes milk fat. The study was carried out on 20 ewes of Wrzosówka and Żelazneńska sheep, which were maintained at the same environmental and nutritional conditions. The milk samples have been collected from ewes at the age of 3–4 years and in the 4th week of lactation. The chemical composition, as well as fat content, was similar in both studied breeds. The amount of C18 fatty acids connected with the major CLA isomer did not show statistical differences between breeds, besides of C18:3, which was higher ($P \leq 0.03$) for Wrzosówka. The activity of $\Delta 9$ desaturase, the enzyme responsible for endogenous synthesis, was also higher ($P \leq 0.01$) for Wrzosówka ewes. The content of *cis-9, trans-11* C18:2 isomer in milk fat of studied ewes determined by GC method did not differ with this obtained by Ag⁺HPLC. The difference has not been noted also within studied breeds. Obtained results suggest, that major CLA isomer in milk could be sensitively identified what allow to precisely determining its amount both by classic GC method and Ag⁺HPLC method.

Key words: sheep, milk, *cis-9, trans-11* isomer, gas chromatography, Ag⁺HPLC.

INTRODUCTION

Conjugated linoleic acids (CLA) represent a mixture of positional and geometric isomers of linoleic acid that contain

conjugated double bonds. The most recognizable as a biologically active isomers are *cis-9, trans-11* C18:2 called rumenic acid (RA) and *trans-10, cis-12* C18:2. The *cis-9, trans-11* is the major isomer and represents about 75–80% of total CLA in milk fat (Park et al. 2007). Milk is the rich source of this isomer and it is recognized that the fat of ewe's milk has the highest content in comparison to cow and goat. The *cis-9, trans-11* CLA exert positive effects on cancer, cardiovascular disease, diabetes, immune system and bone health in humans and animals (Belury 2002, Benjamin and Spener 2009).

This isomer is formed as intermediates of biohydrogenation of linoleic acid by rumen bacteria. Endogenous synthesis of *cis-9, trans-11* CLA is the predominant production pathway in the mammary gland and also occurs in other adipose tissue by the action of $\Delta 9$ desaturase enzyme on *trans*-vaccenic acid with is another intermediate in ruminal biohydrogenation (Collomb et al. 2006).

Analysis of CLA isomers in animal products is resolved combining different techniques: gas chromatography – mass spectrometry (GC-MS), gas chromatography with a flame – ionization detector (GC-FID) and silver-ion high performance liquid chromatography (Ag⁺HPLC)

are usually used (Adlof 2003, Crus-Hernandez et al. 2004, Aldai et al. 2006). The classic procedure by GC-FID allows determination of the total CLA content and permits the assignment of contents to the some isomers. However, Ag⁺HPLC method achieves a good separation of them.

The aim of this work was the quantitative determination of *cis*-9, *trans*-11 isomer by two methods: gas chromatography (GC) and silver-ion high performance liquid chromatography (Ag⁺HPLC) in ewe's milk fat and demonstration if application of one or the other method allows receive comparable results.

MATERIAL AND METHODS

The milk samples have been collected from 20 suckling ewes of two breeds (10 ewes for each breed): Żelazneńska and Wrzosówka sheep, which were maintained at the same environmental and nutritional conditions.

The feeding of the ewes of both breeds was based on local feeds: cereal meal, hay, straw and mineral mixture. The chemical composition and nutritional value of fodder are presented in Table 1.

All sampled sheep were at the age of 3–4 years and in the 4th week of lactation.

Two hours before milk collection, the lambs were separated from their dams, than ewes were hand-milked and 200 ml milk samples were collected for chemical analysis.

Each milk sample was analysed for fat, protein, lactose, total solids (TS) with IR spectrometry using Milkoscan FT-120. Chemical composition of fod-

der was analysed according to procedure of AOAC (1990). Milk fat was extracted according to the Rose-Gottlieb method. The fatty acids and *cis*-9, *trans*-11 CLA isomer were indicated using a gas chromatograph Hewlett Packard 5890, equipped with capillary column DD-23 (length 60 m, internal diameter 0.25 mm, film thickness 0.25 µm). Operation conditions were: helium flow 20 cm per 1 s; a FID detector at 240°C; a split-splitless injector at 220°C. The temperature program of column was: 1 min at 130°C, an increase to 210° C at 10°C per 1 min; held at this temperature for 25 min, than increased to 230°C at 2.5°C per 1 min and held for 18 min at 230°C. The identification of fatty acids was conducted based on retention time relatively to palmitic acid (16:0). For acids and their isomers quantitative analysis by external calibration was performed using standards of chosen acids (Sigma and Supleco). Results are expressed as g in 100 g of estrified FA.

The *cis*-9, *trans*-11 CLA isomer was also determined using a liquid chromatograph (Waters 625LC); the photodiode detector (DAD: model 996, Waters) and professional software Millennium 32 (ver. 4.00). The isomer was separated by two silver-ion columns (Chrom. Sphere, 5 µm Lipids: 250×4.6 mm; Chrompack), secured by the protective column (10 × 3 mm). The mobile phase consisted hexane (98.39% v/v), acetic acid (1.60% v/v) and acetonitrile (0.013% v/v), at flow rate of 1 ml per 1 min; the diode array detector was adjusted to 234 nm. The calibration of HPLC system conducted using individual standards of CLA isomers (Sigma, USA; Larodon Fine

TABLE 1. The chemical composition and nutritional value of fodder given for ewes

Specification	Cereal meal	Hay	Straw
Dry matter (%)	89.24	90.82	91.1
Crude protein (%)	11.8	8.8	3.06
Ether extract (%)	1.72	1.36	1.24
Crude fiber (%)	4.89	34.55	41.24
Ash (%)	2.00	6.81	6.23
EN MJ per 1 kg d.m.	7.54	4.27	–
UFL per 1 kg d.m.	1.06	0.6	–
UFV per 1 kg d.m.	1.02	0.5	–
PDI per 1 kg d.m.	69	68	–

EN – net energy; UFL – unit energy for milk production; UFV – unit energy for meat production; PDI – protein dig.

Chemicals AB, Sweden) (Czauderna et al. 2003).

Statistical treatment of the data of milk chemical composition, CLA isomer and C18 fatty acids was performed using the SPSS 14.0 software (2003) based on a linear model that included the effect of breed and the method of CLA isomer determination. All effects were tested against residual mi-squares to determine level of significance.

RESULTS AND DISCUSSION

The difference of chemical composition of Wrzosówka and Żelazneńska sheep milk has not been observed (Table 2). The fat content in milk of both breeds was at the similar level, although this component is very variable. The lower fat portion (5.84%) in milk of Wrzosówka breed has been recorded in earlier studies by Nowak and Niżnikowski (1996). Radzik-Rant (2005) in milk of Żelazneńska sheep in top of lactation reported similar content of this component compared to present study.

The amount of C18 fatty acids was determined by gas chromatography because of their common pathway with the major *cis*-9, *trans*-11 C18:2 isomer. The differences in vaccenic acid (TVA) and linoleic acids content between studied breeds were statistically insignificant, while linolenic acid was higher ($P \leq 0.03$) for Wrzosówka sheep (Table 2). In earlier studies of the fatty acids profile in milk of Wrzosówka sheep the similar content of linoleic acid have been recorded (Radzik-Rant 1996). The other study on the effect of fish oil supplementation on milk fat quality of Żelazneńska sheep showed comparable content of C18:2 (2.99 g per 100 g of fat) and higher amount of C18:3 (0.71 g in 100 g of fat) in control group (Radzik-Rant 2005). Compared to present results, also the higher content of C18:3 for Polish Mountain and Friesian sheep was obtained by Patkowska-Sokoła et al. (2005). The similar portion of C18:2 and C18:3 was reported in milk fat of crossbred (Friesian \times Polish Merino) sheep (Borys 2004). Radzik-Rant et al. (2011) in analogical period of lactation

TABLE 2. The chemical composition of studied ewe's milk (%)

Item	Wrzosówka sheep	Żelazneńska sheep	S _E	p-value
	LSM	LSM		
Fat	6.69	6.86	0.52	0.832
Protein	4.89	4.70	0.14	0.364
Lactose	5.54	5.70	0.07	0.126
TS	16.46	14.29	0.88	0.109
SNF	6.88	6.87	0.04	0.853

TS – total solids; SNF – solids not-fat.

for Polish Merino and Polish Mountain sheep indicated lower content of C18:2 (2.30 and 2.11 g in 100 g of fat, respectively) and similar value of C18:3 for Polish Merino (0.49 g in 100 g of fat) but much higher portion of that acid for Polish Mountain sheep (2.06 g in 100 g of fat). Compared to obtained results the similar value of vaccenic acid C18:1 *trans*-11 in milk of other sheep breeds was registered by Addis et al. (2005), De La Fuente et al. (2009) and Radzik-Rant et al. (2011).

The $\Delta 9$ desaturase activity index expressed as a CLA/TVA ratio was higher ($P \leq 0.01$) for Wrzosówka than for Żelazneńska sheep (Table 2). The content of *cis*-9, *trans*-11 CLA determined by gas chromatography was also higher in Wrzosówka milk, although the difference was statistically insignificant (Fig. 1). In spite of, that rumenic acid can be produced during rumen biohydrogenation of linoleic acid but the major portion of this isomer in milk (64–98%) originate though endogenous synthesis by $\Delta 9$ desaturase from C18:1 *trans*-11, which could be obtained by biohydrogenation from both linoleic and linolenic acids (Corl et al. 2001, Piperova et al. 2002).

The content of the *cis*-9, *trans*-11 CLA isomer determined by GC method in studied ewe's milk fat not differ ($P \leq 0.823$) from the content of that isomer determined by Ag⁺HPLC method (Fig. 2). The amount of RA in milk fat obtained by two analytical techniques not differs within studied Wrzosówka ($P \leq 0.623$) and Żelazneńska ($P \leq 0.445$) breeds (Fig. 1).

The preliminary results indicated that GC method less precise than Ag⁺HPLC can be sufficient to determine content of the major CLA isomer. The gas chromatography is not sufficient for identification and determination of other CLA isomers, which are masked by the RA peak (Luna et al. 2005). More precise silver-ion high performance liquid chromatography allows to determine other than minor CLA isomers, which content in milk is little or vestigial. This method can provide separation of CLA not attainable by other means (Delmonte et al. 2005).

CONCLUSIONS

The chemical composition of milk including fat content was similar for both studied breeds. The content of C18

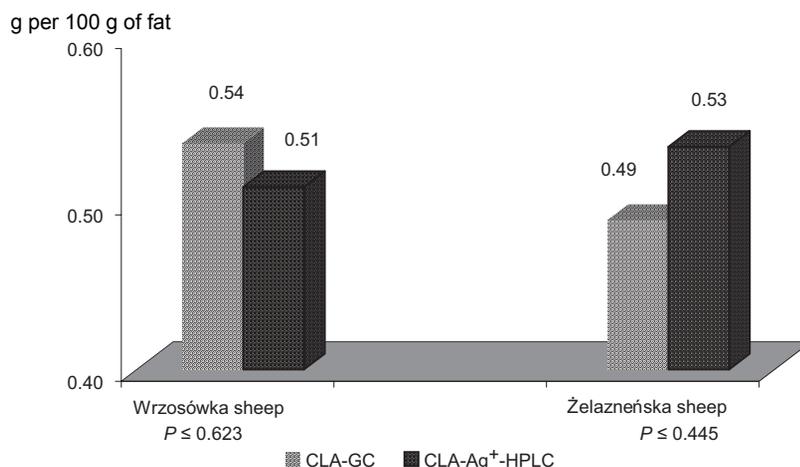


FIGURE 1. The content of *c*9, *t*11 C18:2 – CLA in milk fat of Wrzosówka and Żelazneńska sheep determined by gas chromatography – GC and silver-ion high performance liquid chromatography – Ag⁺HPLC (g per 100 g of fat)

fatty acids, which are connected with RA synthesis, did not differ in fat milk of Wrzosówka and Żelazneńska sheep but Δ 9 desaturase activity responsible for endogenous synthesis of *cis*-9, *trans*-11 CLA isomer has been higher in Wrzosówka breed.

The content of RA determined by GC was similar to this determined by

Ag⁺HPLC method, which suggest, that major CLA isomer in milk could be sensitively identified what allow to precisely determining its amount both by classic GC method and by silver-ion high performance liquid chromatography (Ag⁺HPLC). However, farther studies on larger number of samples are required to confirm obtained results.

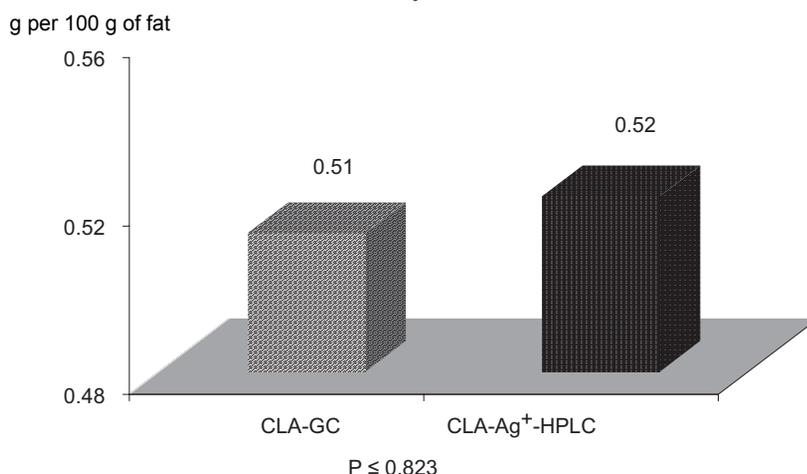


FIGURE 2. The content of *c*9, *t*11 C18:2 – CLA in milk fat determined by gas chromatography – GC and silver-ion high performance liquid chromatography – Ag⁺HPLC (g per 100 g of fat)

TABLE 3. The C18 fatty acids content in milk fat of studied ewes obtained by gas chromatography – GC (g in 100 g of fat)

Item	Wrzosówka sheep	Żelazneńska sheep	S _E	P-value
	LSM	LSM		
C18:1 <i>trans</i> -11 (TVA)	2.20	2.41	0.15	0.350
C18:2	2.68	2.89	0.16	0.378
C18:3 n-3	0.61	0.50	0.03	0.030
CLA/C18:1 <i>trans</i> -11	0.24	0.20	0.00	0.000

REFERENCES

- ADDIS M., CABIDDU H., PINNA G., DECONDA M., PIREDDA G., PIRISI A., MOLLE G., 2005: Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid *cis*-9, *trans*-11. *Journal of Dairy Science* 88, 3443–3454.
- ADLOF R.O. 2003: Application of silver-ion chromatography to the separation of conjugated linoleic acid isomers. (in) J.-L. Sébédio, W.W. Christie, R. Adlof (eds). *Advances in conjugated linoleic acid research*) Vol. 2. AOCS Press, Champaign, IL, USA, 37–55.
- ALDAI N., OSORO K., BARRON L.J.R., NAJERAA.I., 2006: Gas-liquid chromatographic method for analysis complex mixtures of fatty acids including conjugated linoleic acids (*cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) and long-chain (n-3 or n-6) polyunsaturated fatty acids. Application to the intramuscular fat of beef meat. *J. Chromatogr. A* 1110, 133–139.
- AOAC (Association of Official Analytical Chemists) 1990: *Food Composition Additives Natural Contaminants*. 2.4. Oils and Fats, 965.
- BELURY M.A., 2002: Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action. *Annual Rev. of Nutrition* 22, 505–531.
- BENJAMIN S., SPENER F., 2009: Conjugated linoleic acid as functional food: an insight into their health benefits. *Nutr. Metab.* 6, 1–3.
- BORYS B. 2004: Wpływ stosowania pełnotłustych nasion rzepaku i lnu w żywieniu dojonych owiec na ilość i jakość produkowanego mleka i sera twarogowego. *Zeszyty Nauk. Przeglądu Hodowlanego* 72, 3, 95–106.
- COLLOMB M., SCHMID A., SIEBER R., WECHSLER D., RYHANEN E., 2006: Conjugated linoleic acids in milk fat: Variation and physiological effects. *International Dairy Journal* 16, 1347–1361.
- CORL B.A., BAUMGARD L.H., DWER D.A., GRINARI J.M., PHILIPS B.S., BAUMAN D.E., 2001: The role of delta(9)-desaturase in the production of *cis*-9, *trans*-11 CLA. *Journal of Nutrition Biochemistry* 12, 622–630.
- CRUS-HERNANDEZ C., DENG Z., ZHOU J., HILL A.R., YURAWECZ M.P., DELMONTE P., MOSSOBA M.M., DUGAN M.E.R., KRAMERJ.K.G., 2004: Methods for analysis of conjugated linoleic acids and *trans*-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. *J. AOAC Int.* 87, 545–562.
- CZAUDERNA M., KOWALCZYK J., WĄSOWSKA I., NIEDŹWIEDZKA K.M., 2003. Determination of conjugated linoleic acid isomers by liquid chromatography and photodiode array detection. *J. of Anim. and Feed Sci.* 12, 369–382.

- De La FUENTE L.F., BARBOSA E., CARRIEDO J.A., GONZALO C., ARENAS R., FRESNO J.M., SAN PRIMITIVO F., 2009: Factor influencing variation of fatty acid content in ovine milk. *Journal Dairy Science* 92, 3791–3799.
- DELMONTE P., KATAOKA A., CORL B.A., BAUMAN D.E., YURAWECZ M.P. 2005: Relative retention order of all isomers of *cis/trans* conjugated linoleic acid FAME from the 6,8- to 13,15-positions using silver ion HPLC with two elution systems. *Lipids* 40 (5), 509–514.
- LUNA P., FONTECHA J., JUÁREZ M., De La FUENTE M.A. 2005: Identification of conjugated isomers of linoleic acid in ewes milk fat. *J. of Dairy Res.* 72, 415–424.
- NOWAK W., NIŻNIKOWSKI R. 1996. The influence of chosen factors on milk traits of Wrzosówka ewes nursing lambs. *Zeszyty Naukowe PTZ* 23, 145–160.
- PARK Y.W., JUÁREZ M., RAMOS M., HAENLEIN G.F.W. 2007: Physico-chemical characteristics of goat and sheep milk. *Small Rum. Res.* 68, 88–113.
- PATKOWSKA-SOKOŁA B., RAMADANI S., BODKOWSKI R., 2005. Skład chemiczny mleka polskiej owcy górskiej i owcy fryzyjskiej z okresu żywienia pastwiskowego. *Roczniki Naukowe Zootechniki (supl.)* 21, 73–75.
- PIPEROVA L.S., SAMPUGNA J., TETER B.B., KALSCHEUR M.P., YURAWECZ M.P., KU Y., MOREHOUSE K.M., ERMAN R.A. 2002: Duodenal and milk trans octadecanoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of *cis-9*-containing CLA in lactating dairy cow. *J. of Nutr.* 132, 1235–1241.
- RADZIK-RANT A., 1996: Analiza składu kwasów tłuszczowych w mięsie, tłuszczu i mleku owiec rasy wrzosówka. *Zesz. Nauk. PTZ* 23, 133–144.
- RADZIK-RANT A., 2005: Modyfikowanie zawartości kwasów tłuszczowych w tkance mięśniowej jagniąt poprzez wzbogacanie diety olejami różnego pochodzenia. *Monografie i Rozprawy. Warszawa.*
- RADZIK-RANT A., ROZBICKA-WIECZOREK A., CZAUDERNA M., RANT W., KUCZYŃSKA B., 2011: The chemical composition and fatty acid profile in milk of Polish Mountain Sheep and Polish Marino. *Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci.* 49, 163–172.
- SPSS, 2003: SPSS, 14.0 for Windows user's guide. SPSS, Inc., USA.
- Streszczenie:** Zawartość *cis-9, trans-11 CLA* w tłuszczu mleka maciorek oznaczanego przy użyciu dwóch metod. Celem pracy było określenie zawartości izomeru *cis-9, trans-11 CLA* dwiema metodami: chromatografii gazowej (GC) i argentometrycznej chromatografii cieczowej (Ag^+HPLC), w tłuszczu mleka maciorek. Badania przeprowadzono na 20 maciorkach rasy wrzosówka i żelazna, które były utrzymywane w jednym stadzie w tych samych warunkach środowiskowych i żywieniowych. Próby mleka pobierano od 3–4- letnich matek w 4 tygodniu laktacji. Skład chemiczny mleka jak i zawartość tłuszczu były podobne u obu badanych ras. Zawartość C18 kwasów tłuszczowych pochodnych głównego izomeru CLA nie wykazała różnic pomiędzy badanymi rasami, oprócz kwasu linolowego, którego zawartość była wyższa ($P \leq 0,03$) u wrzosówki. Aktywność $\Delta 9$ desaturazy, enzymu odpowiadającego za endogenną jego syntezę była także wyższa ($P \leq 0,01$) u tej rasy. Zawartość izomeru *cis-9, trans-11 C18:2* określona przy użyciu GC nie różniła się od zawartości tego izomeru oznaczonego metodą Ag^+HPLC w mleku badanych maciorek. Różnic tych nie odnotowano także w obrębie badanych ras. Uzyskane wyniki świadczą, iż główny izomer CLA kwasu żwaczowego może być precyzyjnie identyfikowany, co pozwala na dokładne określenie jego zawartości w mleku zarówno przy użyciu klasycznej chromatografii gazowej jak i wysoko-sprawnej argentometrycznej chromatografii cieczowej Ag^+HPLC .

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Influence of Ag nanoparticles, ATP and biocomplex of Ag nanoparticles with ATP on morphology of chicken embryo pectoral muscles

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Abstract: *Influence of Ag nanoparticles, ATP and biocomplex of Ag nanoparticles with ATP on morphology of chicken embryo pectoral muscles.* Increase of genetic potential of fast growing chicken broilers to enlarge size of muscles may lead to decrease quality of the meat because of non-adequate amount of nutrient and energy, stored within the eggs, for providing optimal development. Deliver to the chicken embryo additional amount of energy as ATP or ATP attached to the nanoparticles of silver, as a transporting molecule, may promote growth and development of breast muscle. The objective of the investigation was to evaluate the effect of Ag nanoparticles and ATP, administrated in ovo to the embryo, on the morphology of pectoral muscle. The fertilised eggs of Ross 308 (160) were divided into four groups (4 × 40 eggs): without injection (control), with injection of hydrocolloid of Ag nanoparticles (Nano-Ag), with injection of hydrocolloid of adenosine triphosphate (ATP) and with injection of Nano-Ag conjugated with adenosine triphosphate (Nano-Ag/ATP). At day one of incubation, the eggs were injected into the air sac with 0.3 ml of experimental solutions. Chicken embryo morphology was evaluated according to the Hamburger-Hamilton standard stages of embryo development, furthermore, pectoral muscle was visualized by TEM. Results showed that Nano-Ag, ATP and Nano-Ag/ATP did not affect negatively growth and development. However, ultra morphology of the cross section of embryo pectoral muscles was better structured and muscle was more dense with myofibers when ATP and Ag nanoparticles were applied. The results indicate that application of Nano-Ag and ATP in ovo can affect morphology of breast muscle, but not affecting embryo growth.

Key words: silver nanoparticles, pectoral muscle, ATP, embryo, chicken, transmission electron microscope.

INTRODUCTION

Increase of genetic potential of fast growing chicken broilers to enlarge size muscles may lead to decrease quality of the pectoral muscle because of non-adequate amount of nutrient and energy, stored within the eggs, for providing rapid growth and development and for maintaining balance between hyperplasia and hypertrophy. Therefore, some authors propose additional in ovo administration of carbohydrates and/or protein to the embryos, to improve health status and muscle's development (Uni and Ferket 2003, Noy and Uni 2010).

Adenosine triphosphate (ATP) is a molecule present in cytoplasm and nucleoplasm of all cells and is an energy source for all physiological mechanisms including; synthesis of biomolecules, active transport, contraction of muscles, participation in cell division and growth of organism (Erlinge 1998, Meyer et al. 1999). However, ATP administrated to the chicken embryo, by using in ovo

method with injection to albumen or air sack, may be broken down with enzymes presented within eggs or not properly distributed. It can be supposed that applying nanoparticles as an ATP molecule delivery platform may support ATP distribution within eggs. Nanoparticles, with their smaller size, allow for penetration into the tissue and going even deeper, crossing cell membranes (Debbage and Thurner 2010, Hotowy et al. 2012, Peng et al. 2012). Some studies demonstrated that the Ag nanoparticles were non-toxic, antibacterial (Sironmani and Daniel 2011), furthermore, upregulated expression of anabolic genes – FGF2 and VEGF in chicken embryo muscles (Hotowy et al. 2012).

We hypothesized that ATP attached to Ag nanoparticles would be delivered to the muscle cells, as a promoting growth and development of embryo breast muscle. The objective of the investigation was to evaluate the effect of Ag nanoparticles, ATP and ATP conjugated with Ag nanoparticles, administrated to the chicken *in ovo*, on the morphology of pectoral muscle.

MATERIALS AND METHODS

The fertilised eggs of Ross 308 chicken were obtained from a commercial hatchery. 160 eggs were randomly divided into four groups (4×40 eggs): without injection (control), with injection of hydrocolloid of Ag nanoparticles (Nano-Ag), with injection of hydrocolloid of adenosine triphosphate (ATP) and with injection of hydrocolloid of Ag nanoparticles conjugated with adenosine triphosphate (Nano-Ag/ATP). At day 1 of incubation,

the eggs were numbered, weighed (60 ± 1.36 g) and injected into the air sac with 0.3 ml of experimental solutions using a sterile 27 gauge, 20 mm needle. Immediately after the injection, the hole was sealed with hypoallergenic tape and the eggs were placed into an incubator. The eggs were incubated for 20 days under standard conditions (temperature 37.8°C, humidity 55%, turned once per hour during the first 18 days, at a temperature of 37°C and humidity 60% from day 19). The embryos were decapitated and evaluated on day 20 of incubation. The morphological structure was compared with the standard described by Hamburger and Hamilton (1951), samples of the pectoral muscles were fixed in glutaraldehyde for electron microscopy preparation.

The hydrocolloid of Nano-Ag, at the concentration of 50 ppm and size of 2 to 35 nm was obtained from Nano-Tech (Warsaw, Poland). Pure ATP (Merck, Germany) was dissolved in distilled water at the concentration of 0.04 mg per 1 ml. ATP was conjugated with AgNano using sonification for 30 min at 15°C in an ultrasonic bath.

For observations with transmission electron microscope (TEM), tissues were cut into pieces of about 1 mm^3 and fixed (60 min) in a 3% glutaraldehyde solution (Merck) in 0.1 M sodium phosphate buffer (pH 7.2). Samples were then rinsed (2×5 min) in the same buffer and transferred to a 1% osmium tetroxide solution in 0.1 M phosphate buffer (pH 6.9) for 1 hour. Subsequently, the samples were rinsed in 0.1 M sodium phosphate buffer (5 min), dehydrated in an ethanol gradient (50–99%), and impregnated with Epon embedding resin (Merck). The blocks were cut into ultrathin sections

(50–80 nm) using an ultramicrotome (Leica, ultracut UCT) and transferred on to copper grids, 200 mesh. Subsequently, the sections were contrasted using 2% uranyl acetate dihydrate and lead citrate. The structure of the pectoral muscles were visualized by a JEM-1220 transmission electron microscope (TEM; JEOL, Japan). Morphometry of the breast muscle of chicken embryos (fiber area, cell number per 1200 μm^2 and number of nuclei per 1200 μm^2) was counting using ImageJ software.

Data analysis was carried out using one-way analysis of variance (ANOVA) test-generalized linear model (GLM) procedure in SAS 9.2 (SAS Windows, 2002–2008, version 9.2, SAS Institute Inc., Cary, NY, USA). Values differing at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Results obtained in these experiments demonstrated that Nano-Ag, ATP and Nano-Ag/ATP did not negatively affect growth and development when comparing to standard stages of Hamburger and Hamilton (1951). Also any abnormalities of morphology of the embryos and assessed organs did not point out any negative influence of the experimental treatments. This is an agreement with other results showing no harmful effects of Nano-Ag, administrated with 50 ppm in ovo (Grodzik and Sawosz 2006, Pineda et al. 2012). However, experiments in vitro demonstrated that silver nanoparticles cause death of the human fibroblasts at a concentration of more than 60 ppm (Kvitek et al. 2011).

Chicken embryo breast muscle tissues were visualized by TEM, which allows observation of the ultra structure of pectoral muscle (Figure 1). Pictures of the muscle cross section showed some differences between the groups. The areas of muscle fibers of the chicks from the control group were the smallest and fibers were placed more loosely, space between myofibers was bigger and also the number of myofibrils seen within cytoplasm was smaller. Furthermore, myofibrils were located freely and spaces between them were bigger compare to the other groups. Pictures of muscles from the control embryos may point to a non-adequate organization and an immature structure of the tissue. The muscle from treated groups were better developed, myofibers were bigger and placed more compressed. However, in embryos administrated with ATP myofibrilles were more dense and their structure was more concentrated. The quantitative measurements demonstrated that embryos from the control group had significantly the highest number of muscle cells per square unit compared to the rest of groups (Table 1). The biggest area of pectoral muscles fibers was observed in the treatment groups, especially with Nano-Ag/ATP and ATP. For the other side, the number of nuclei per quadratic surface (1200 μm^2) was at the same level in all groups. Futhermore, the number of nuclei per cell was bigger in the treatment groups, the biggest in ATP administrated embryos. In the light of obtained results it can be supposed that the better morphology of the chicken embryo muscles treated with ATP and Nano-Ag/ATP was caused because of ATP stimulating DNA synthesis, protein synthesis and also acting syner-

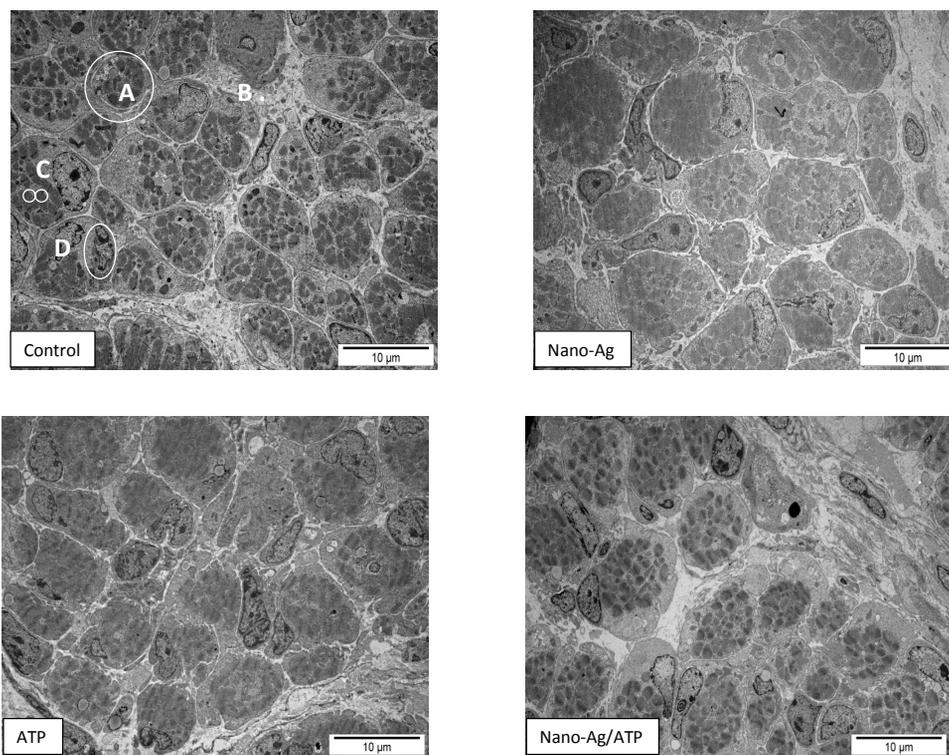


FIGURE 1. TEM image of pectoral muscle (cross-section) in the Control group and groups with treated with Ag nanoparticles (Nano-Ag), adenosine triphosphate (ATP) and in combination of Nano-Ag with ATP (Nano-Ag/ATP). (A) Single muscle fibre, (B) Endomysium, (C) Myofibrils, (D) Nucleus

TABLE 1. Morphometry and average weight of embryos and pectoral muscles of chicken

Specification	Treatment								
	Control		Nano-Ag		ATP		ATP/Nano-Ag		<i>p</i> -value
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	
Cell number	17 ^A	0.26	10 ^B	0.25	9 ^B	0.62	14 ^C	0.61	0.0001
Number of nuclei	7.3	0.67	8.2	0.53	8.9	0.60	9.0	0.41	0.1465
Fiber area	38 ^A	1.62	79 ^C	2.57	50 ^B	2.67	58 ^B	2.25	0.0001
Embryo, % e.w ¹	78 ^A	0.6	77 ^A	0.5	67 ^B	3.9	73 ^A	1.4	0.0003
Muscle, % b.w ²	0.81	0.026	0.80	0.041	0.76	0.039	0.76	0.053	0.5098
Number of nuclei per 1 cell	0.43		0.80		0.95		0.64		

¹Percentage of egg weight; ²percentage of body weight.

A, B, C – Within rows: means with different superscript differ significantly ($P < 0.05$).

gistically to genes, which are responsible for muscle development in early stages of the embryogenesis (Wang et al. 1992, Neary et al. 1994, Erlinge 1998). Consequently, the adenosine triphosphate had influence on the morphology of pectoral muscles. Nanoparticles of silver did not improve activities of ATP, however, as a gene expression activator may support the process.

CONCLUSIONS

ATP, silver nanoparticles and ATP conjugated with Ag nanoparticles, given with in ovo methods to the chicken embryo did not negatively influence growth and development. The cross section of chicken embryo pectoral muscles was better structured, matured and more dense with myofibers when ATP and Ag nanoparticles were applied. The results indicate that application of Nano-Ag and ATP in ovo can affect morphology of breast muscle furthermore, but having no influence on embryo growth.

REFERENCES

- DEBBAGE P., THURNER G.C., 2010: Nanomedicine Faces Barriers. *Pharmaceuticals*. 3 (11), 3371–3416.
- ERLINGE D., 1998: Extracellular ATP: A Growth Factor of Vascular Smooth Muscle Cells. *Gen. Pharmac.* 31 (1): 1–8.
- GRODZIK M., SAWOSZ E., 2006. The influence of silver nanoparticles on chick embryo development and bursa fabricius morphology. *J. Anim. Feed Sci.* 15 (Suppl.) 1, 111–115.
- HAMBURGER V., HAMILTON H.L., 1951: A series of normal stages in the development of the chick embryo. *Dev. Dyn.* 195: 231–272.
- HOTOWY A., SAWOSZ E., PINEDA L., SAWOSZ F., GRODZIK M., CHWALIBOG A., 2012: Silver nanoparticles administered to chicken affect VEGFA and FGF2 gene expression in breast muscle and heart. *Nanoscale Res. Lett.* 7, 418.
- KVITEK L., PANACEK A., PRUCEK R., SOUKUPOVA J., VANICKOVA M., KOLAR M., ZBORIL R., 2011: Antibacterial activity and toxicity of silver – nanosilver versus ionic silver. *Journal of Physics: Conference Series* 304 (2011) 012029.
- MEYER P.D., CLARKE J.D.W., PATEL K., TOWNSEND-NICHOLSON A., BURNSTOCK G., 1999: Selective Expression of Purinoceptor cP2Y1 Suggests a Role for Nucleotide Signaling in Development of the Chick Embryo. *Development Dynamics* 214, 152–158.
- NEARY J.T., WHITTEMORE S.R., ZHU Q., NOREMBERG M.D., 1994: Synergistic Activation of DNA Synthesis in Astrocytes by Fibroblast Growth Factor and Extracellular ATP. *Journal of Neurochemistry* 63: 490–494.
- NOY Y., UNI Z., 2010: Early nutritional strategies. *World's Poultry Science Journal* 66, 639–646.
- PENG H., ZHANG X., WEI Y., LIU W., LI S., YU G., FU X., CAO T., DENG X., 2012: Cytotoxicity of silver nanoparticles in human embryonic stem cell-derived fibroblast and an L-929 cell line. *Journal of Nanomaterials*. doi:10.1155/2012/160145.
- PINEDA L., SAWOSZ E., HOTOWYA A., ELNIFA J., SAWOSZA F., ALIA A., CHWALIBOG A., 2012: Effect of nanoparticles of silver and gold on metabolic rate and development of broiler and layer embryos. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 161, 315–319.
- SIRONMANI A., DANIEL K., 2011: Silver Nanoparticles – Universal Multifunctional Nanoparticles for Bio Sensing, Imaging for Diagnostics and Targeted

- Drug Delivery for Therapeutic Applications. (in) I.M. Kapetanović (ed.). Drug Discovery and Development – Present and Future. InTech, Rijeka – New York – Shanghai.
- UNI Z., FERKET P.R., 2003: Enhancement of development of oviparous species by *in ovo* feeding. US Patent Number 6,592,878.
- WANG D.J., HUANG M.N., HEPPEL L.A., 1992: Extracellular ATP and ADP Stimulate Proliferation of Porcine Aortic Smoot: Muscle Cells. Journal of Cellular Physiology 153, 221–233.

Streszczenie: *Wpływ nanocząstek Ag oraz ATP i biokompleksów nanocząstek Ag z ATP na morfologię mięśnia piersiowego zarodka kury. Zwiększenie potencjału genetycznego szybko rosnących ras kur w kierunku zwiększenia wielkości mięśni, może prowadzić do zmniejszenia jakości mięsa, jako następstwa względnego niedoboru składników pokarmowych i energii zgromadzonych w jajach i warunkujących optymalny rozwój. Dostarczenie zarodkowi kury dodatkowej ilości energii w postaci ATP lub ATP przyłączonego do nanocząstek srebra, jako molekule transportującej, może zapewnić właściwy rozwój mięśni piersiowych. Celem badań była ocena wpływu nanocząstek Ag i ATP, podawanych *in ovo*, na morfologię mięśni piersiowych zarodka kury. Zapłodnione jaja kur Ross 308 (180 sztuk) podzielono na 4 grupy (4 × 40 sztuk): kontrolną, poddaną iniekcji wodnego roztworu nanocząstek Ag (Nano-Ag), poddaną iniekcji wodnego roztworu ATP (ATP) i poddaną*

*iniekcji Nano-Ag z przyłączonymi cząsteczkami ATP (Nano-Ag/ATP). W pierwszym dniu inkubacji do komory powietrznej jaj wstrzykiwano 0,3 ml eksperymentalnych roztworów. Morfologia zarodków kury była oceniana według standardu Hamburgera i Hamiltona, ponadto próbki mięśnia piersiowego były oceniane za pomocą transmisyjnego mikroskopu elektronowego (TEM). Wyniki wykazały, że Nano-Ag, ATP i Nano-Ag/ATP nie wpłynęły negatywnie na wzrost i rozwój zarodków. Ocena ultra morfologii przekroju poprzecznego wycinków mięśni piersiowych wykazała lepsze wykształcenie mięśni piersiowych, większą gęstość włókien mięśniowych u zarodków, którym podawano Ag i ATP. Wyniki wykazały, że podawanie Nano-Ag i ATP *in ovo* może wpływać korzystnie na morfologię mięśni, nie działając negatywnie na wzrost zarodków.*

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Caveolin-1 localization in chicken embryo chorioallantoic membrane treated with diamond and graphite nanoparticles

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Abstract: *Caveolin-1 localization in chicken embryo chorioallantoic membrane treated with diamond and graphite nanoparticles.* Caveolin-1 is a multifunctional protein and major component of caveolae membranes that participates in regulation of signaling pathways, endocytosis and molecular transport. Caveolin-1 takes part in regulation of angiogenesis regulation signaling pathways. Diamond nanoparticle have been shown to inhibit development of blood vessel. Molecular mechanism of diamond nanoparticles anti-angiogenic activity can be related with interactions with cellular membranes. The objective of this experiment is to verify effect of carbon nanoparticles on morphology of highly vascularized chicken embryo chorioallantoic membrane (CAM) and caveolin-1 intracellular localization. In this study two types of carbon nanoparticles were used: diamond nanoparticles (ND) and graphite nanoparticles (NG), which are similar in size (3–5 nm) but different in molecular structure. At day six of chicken embryo embryonic development sterile implant of diameter 10 mm made from Waterman filter paper were placed on chicken embryo CAM. At day seven of embryonic development implants with CAM were subjected to further analyzes. CAM cross-sections were immuno-localized with anti caveolin-1 antibody and visualized by confocal microscope. Three dimensional analysis of chorion membranes show that ND, but no NG change intracellular distribution of caveolin-1. Furthermore ND decreases density of mesenchymal cells and extracellular matrix collagen fibers.

Key words: caveolin-1, angiogenesis, diamond nanoparticles, graphite nanoparticles, embryo, chicken, confocal microscope.

INTRODUCTION

Caveolin-1 is a multifunctional protein and a major component of caveolae membranes that participates in regulation of signaling pathways, endocytosis and molecular transport. Caveolin-1 is a main protein of caveolae membrane complex. Caveolae are omega-shape invagination in the cell membrane, 60 to 80 nm in size, containing besides caveolins cholesterol and sphingolipids (Stan 2005). Caveolin-1 down-regulate main pro-angiogenic factor VEGF and Akt and Stat3 signaling pathways, that are important in development of new blood vessels (Bauer et al. 2005, Li et al. 2011). Signaling capability of caveolin-1 is based on interaction between it conserved scaffolding domain (amino acids 82–101) and signaling molecules like Gα subunits and other related Src family tyrosine kinases (Li et al. 1996). However, caveolin-1 scaffolding domain also has membrane-binding activity and binding with membrane reduces interaction with signaling molecules (Epanand et al. 2005). Caveolae can provide a reservoir of caveolin molecules that are released under specific cellular conditions, for example acute mechanical stresses (Sinha et al. 2011). Cellular pool of caveolin-1, not associated with caveolae can be essential for its signaling capabilities.

Our recent reports show that diamond nanoparticles decrease mRNA level of VEGF and other pro-angiogenic factor FGF2 and decrease vesicular network density in glioblastoma tumor (Grodzik et al. 2011). Carbon nanoparticles also decrease induced by VEGF angiogenesis on chicken embryo chorioallantoic membrane (CAM) (Murugesan et al. 2007). Molecular mechanism of diamond nanoparticles anti-angiogenic activity can be related with its interactions with cellular membranes. We hypothesize that carbon nanoparticles effects cellular pool of caveolin-1, that can down-regulate cellular angiogenic signaling pathways. The objective of this experiment is to verify effect of diamond and graphite nanoparticles on morphology of well described, highly vascularized chicken embryo CAM (Hamburger and Hamilton 1951) and caveolin-1 intracellular localization.

METHODS AND MATERIALS

In this study two types of carbon nanoparticles were used: diamond nanoparticles (ND) (explosion synthesized, size: 3–4 nm, specific surface area: $\sim 282 \text{ m}^2 \cdot \text{g}^{-1}$, purity: > 95%) graphite nanoparticles (NG) (explosion synthesized, size: 3–5 nm, specific surface area: $540\text{--}650 \text{ m}^2 \cdot \text{g}^{-1}$, purity: > 93%). Nanoparticles were obtained from Skyspring Nanomaterials (Houston, USA). Nanoparticles were dispersed in demineralized water with 60 min of sonification.

Fertilized eggs from Ross line 308 hens were obtained from certified hatchery and kept for four days at 12°C . The eggs were cleaned, sterilized with UVC light and divided into three groups

(3×15 eggs). Embryos were incubated at standard conditions (temperature 37°C , humidity 60%, turned once per hour). At day six of chicken embryo embryonic development sterile implant of diameter 10 mm made from Waterman filter paper were placed on chicken embryo chorioallantoic membrane. Demineralized water (control) or hydrocolloidal nanoparticles of concentration $500 \text{ mg} \cdot \text{l}^{-1}$ were added on implants (final amount of nanoparticles on implant 0.01 mg). Paper discs were pretreated with $100 \text{ mg} \cdot \text{ml}^{-1}$ hydrocortisone sodium succinate and air dried under sterile conditions. Chicken embryos were incubated to day seven of embryonic development, when implants with CAM were prefixed with 1.5 ml of 4% paraformaldehyde. After 30 minutes of incubation in 4°C CAM with implants were cut out and fixed in 4°C 4% paraformaldehyde for 60 minutes (total fixation time 90 min). After fixation implants were gently stripped off.

For confocal immuno-fluorescent analysis CAM was frozen in Jung Tissue Freezing Medium (Leica, Wetzlar, Germany) in liquid nitrogen and cut into 5- μm -thick sections using cryostat (CM 1900, Leica, Wetzlar, Germany). The sections were attached to poly-l-lysine coated microscope slides. Tissue was washed with PBS and permeabilized with 0.5% Tween 20 (Sigma, St. Louis, USA) PBS solution for 10 min. The sections were blocked with PBS containing 2% of goat serum and 1% bovine serum albumin (Sigma, St. Louis, USA) for 30 min. Sections were incubated with primary antibody (rabbit anti Caveolin-1 antibody; Sigma, St. Louis, USA) diluted in 2% goat serum (producer recommended dilution) for 12 hrs in 4°C . After

washing sections were incubated with secondary antibody: goat anti-rabbit Atto 488 conjugate (IgG (H+L),F(ab')₂ Fragment Atto488; Cell Signaling Technology, Danvers, USA) for 2 hrs, diluted according to producer instructions. Nuclei were stain by incubation with DAPI solution for 15 min (Sigma, St. Louis, USA). After washing the coverslips were mounted on slides with Fluoromount mounting medium (Sigma, St. Louis, USA) and observed on IX 81 FV-1000 confocal microscope (Olympus Corporation, Tokyo, Japan). Image analysis in confocal mode, Nomarski interference contrast, cell counting were performed using FVIO-ASW ver. 1.7c software (Olympus Corporation, Tokyo, Japan). Three-dimensional images were assembled from 30 optical sections.

Data analysis was performed using one-way analysis of variance (ANOVA) with the Bonferroni post hoc test used for multiple comparisons. Values differing at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Analyzed CAM cross-sections show lower density of mesenchymal cells in ND treated group in comparison to control and NG treated group (Table 1). There was not statistically significant reduction of mesenchyme cells in NG treated group. Moreover analysis of Nomarski interference contrast images show that ND treated group have lower density of collagen then control and graphite treated group (Fig. 1). Consequently less developed connective tissue with low density of extracellular matrix in ND treated group can led to anti-angiogenic activities. ND

TABLE 1. Number of mesenchyme cells per 1 mm² on CAM treated with nanoparticles in comparison to control

Group	Number of mesenchyme cells per 1 mm ²
Control	5.8 ^a
ND	2.9 ^b
NG	4.8 ^a
P-value	0.002
SE-pooled	0.54

Values within rows with different superscripts are significantly different $P < 0.05$.

toxicity was demonstrated on in vivo rat model, where ND decreased the number of the phagocytizing neutrophils and increased number of cells with stimulated oxidative burst (Niemiec et al. 2011). Furthermore, ND can led to up-regulation of SOD1 expression responsible for defensive mechanisms against reactive oxygen species in human neoplastic cells cultured in vitro (Bakowicz-Mitura et al. 2007).

Confocal microscope images of CAM cross-sections from control, NG and ND treated group were analyzed for expression of caveolin-1. Mesenchyme cells, endothelium of blood vessels, chorion membrane showed expression of caveolin-1 in all analyzed groups. Chorion membranes showed the strongest expression of caveolin-1 regardless of group (Fig. 2). Three dimensional analysis of chorion membranes showed differences of caveolin-1 localization between groups. In the control and NG treated groups caveolin-1 was mainly localized on side parts of membrane (Fig. 3). In 80% of analyzed images of ND treated chorion membranes caveolin-1 was evenly distributed across cells. Intracellular caveolin-1 can down-regulate important

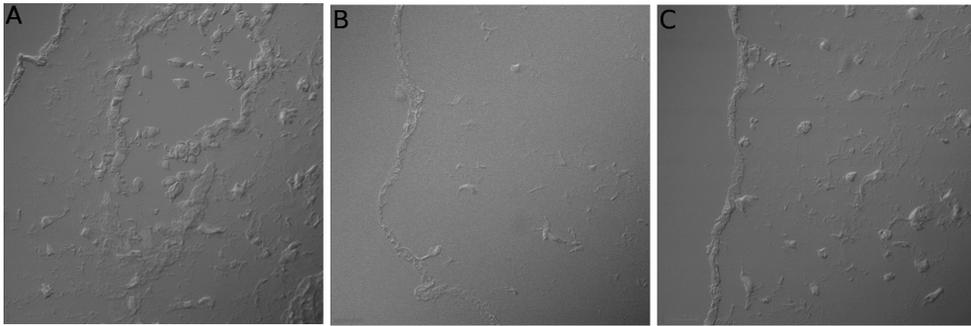


FIGURE 1. Nomarski interference contrast image of CAM tissue extracellular matrix. Nuclei are stained with DAPI A – Control, B – ND, C – NG. Scale bar 50 μm

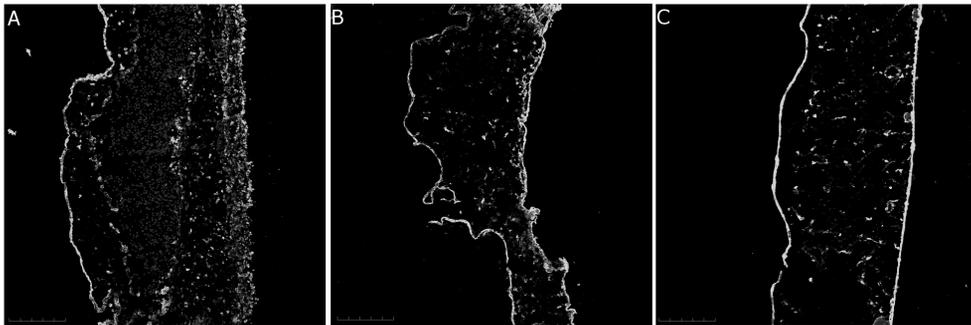


FIGURE 2. Chicken embryo CAM tissue with immune-localized caveolin-1 and stained nuclei with DAPI. A – Control, B – ND, C – NG. Scale bar 200 μm

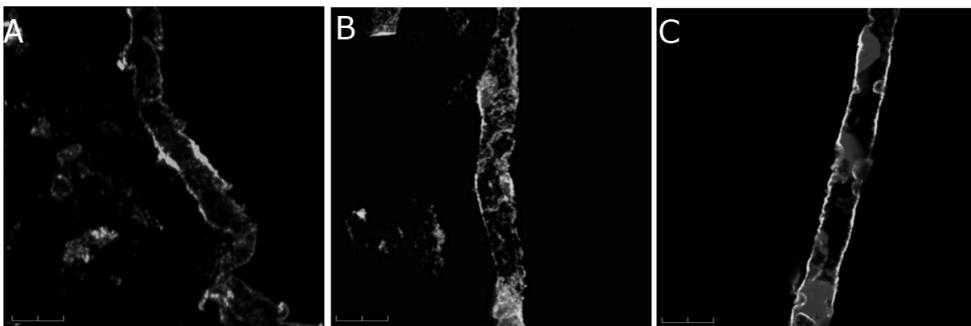


FIGURE 3. CAM chorion with immune-localized caveolin-1 and stained nuclei with DAPI. A – Control, B – ND, C – NG. Scale bar 10 μm

in angiogenesis Akt and Stat3 signaling pathways (Li et al. 2011). Caveolin-1 change of localization can be associated with interactions with proteins localized in caveolar membranes, such as receptor tyrosine kinases (receptor of VEGF – VEGFR2), Serine/Threonine, Src family kinases (Parton and Simons 2007) and caveolae-mediated endocytosis of carbon nanoparticles (Zhang et al. 2009). Moreover, ND could act by direct acute mechanism, releasing caveolin molecules from caveolae as shown by Sinha et al. (2011). Very similar in size, but different in molecular structure NG did not show distinct effects, which indicate that interaction of ND is specific for this nanoparticle. Obtained results give new insights in carbon nanoparticles interactions with biological systems. ND change structure of CAM and show inhibitory effect on connective tissue. Changes in caveolin-1 localization in ND treated group indicate potential angiogenic regulation mechanism.

CONCLUSIONS

Immuno-localization of the control, ND and NG treated chicken embryo CAM showed expression of caveolin-1. ND changed intracellular distribution of caveolin-1 in chorion membranes. Furthermore, ND decreased density of mesenchymal cells and extracellular matrix collagen fibers. The results indicate that caveolin-1 can be associated with ND anti-angiogenic properties.

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REFERENCES

- BAKOWICZ-MITURA K., BARTOSZ G., MITURA S. 2007: Influence of diamond powder particles on human gene expression. *Surf. Coatings Technol.* 201, 6131–6135.
- BAUER P.M., YU J., CHEN Y., HICKEY R., BERNATCHEZ P.N., LOOFT-WILSON R., HUANG Y., GIORDANO F., STAN R.V., SESSA W.C., 2005: Endothel-specific expression of caveolin-1 impairs microvascular permeability and angiogenesis. *Proc. Natl. Acad. Sci. USA* 102, 204–209.
- EPAND R.M., SAYER B.G., EPAND R.F. 2005: Caveolin scaffolding region and cholesterol-rich domains in membranes. *J. Mol. Biol.* 345, 339–50.
- GRODZIK M., SAWOSZ E., WIERZBICKI M., ORLOWSKI P., HOTOWY A., NIEMIEC T., SZMIDT M., MITURA K., CHWALIBOG A. 2011: Nanoparticles of carbon allotropes inhibit glioblastoma multiforme angiogenesis in ovo. *Int. J. Nanomedicine* 6, 3041–3048.
- HAMBURGER V., HAMILTON H.L., 1951: A series of normal stages in the development of the chick embryo. *Dev. Dyn.* 195, 231–272.
- LI S., COUET J., LISANTI M.P., 1996: Src tyrosine kinases, Galpha subunits, and H-Ras share a common membrane-anchored scaffolding protein, caveolin. Caveolin binding negatively regulates the auto-activation of Src tyrosine kinases. *J. Biol. Chem.* 271, 29182–29190.
- LI Y., LUO J., LAU W.M., ZHENG G., FU S., WANG T.T., ZENG H.P., SO K.F., CHUNG S.K., TONG Y., LIU K., SHEN J. 2011: Caveolin-1 plays a crucial role in inhibiting neuronal differentiation of neural stem/progenitor cells via VEGF signaling-dependent pathway. *PLoS One* 6, e22901.
- MURUGESAN S., MOUSA S.A., O'CONNOR L.J., LINCOLN D.W. 2nd, LINHARDT R.J. 2007: Carbon inhibits vascular endothelial growth factor- and

- fibroblast growth factor-promoted angiogenesis. *FEBS Lett.* 581 (6), 1157–1160.
- NIEMIEC T., SZMIDT M., SAWOSZ E., GRODZIK M., MITURA K. 2011: The Effect of Diamond Nanoparticles on Redox and Immune Parameters in Rats. *J. Nanosci. Nanotechnol.* 11, 9072–9077.
- PARTON R.G., SIMONS K. 2007: The multiple faces of caveolae. *Nat. Rev. Mol. Cell Biol.* 8, 185–194.
- SINHA B., KÖSTER D., RUEZ R., GONNORD P., BASTIANI M., ABANKWA D., STAN R.V., BUTLER-BROWNE G., VEDIE B., JOHANNES L., MORONE N., PARTON R.G., RAPOSO G., SENS P., LAMAZE C., NASSOY P. 2011: Cells respond to mechanical stress by rapid disassembly of caveolae. *Cell* 144, 402–413.
- STAN R.V. 2005: Structure of caveolae. *Biochim. Biophys. Acta* 1746, 334–348.
- ZHANG L.W., YANG J., BARRON A.R., MONTEIRO-RIVIERE N.A. 2009: Endocytic mechanisms and toxicity of a functionalized fullerene in human cells. *Toxicol. Lett.* 191, 149–157.
- Wcześniejsze badania wykazały, że nanocząstki diamentu mają zdolność hamowania rozwoju naczyń krwionośnych. Molekularny mechanizm właściwości anty-angiogennych związany jest prawdopodobnie z interakcją nanocząstek z błonami komórkowymi. Celem tego doświadczenia jest zbadanie efektów działania nanocząstek na morfologię błony kosmówkowo-omoczniowej (CAM), oraz wewnątrzkomórkową lokalizację kaweoliny-1. W badaniach wykorzystane zostały dwa rodzaje węglowych nanocząstek: nanocząstki diamentu (ND) oraz nanocząstki grafitu, charakteryzujące się podobną wielkością (3–5 nm), ale posiadające inną budowę molekularną. Szóstego dnia inkubacji zarodka kury sterylny implant o średnicy 10 mm, wykonany z papieru filtracyjnego Waterman został położony na CAM. Siódmego dnia rozwoju zarodkowego implanty razem z CAM zostały pobrane do dalszych analiz. Przekroje poprzeczne CAM zostały wyznaczone przeciwciałami anty kaweolina-1 i obserwowane pod mikroskopem konfokalnym. Analiza trójwymiarowych zdjęć błony kosmówkowej wykazała, że ND, ale nie NG zmienia wewnątrzkomórkową lokalizację kaweoliny-1. Ponadto ND zmniejsza gęstość komórek mezenchymalnych oraz ilość macierzy zewnątrzkomórkowej złożonej z włókien kolagenowych.

Streszczenie: Lokalizacja kaweoliny-1 w błonie kosmówkowo-omoczniowej zarodka kury traktowanej nanocząstkami diamentu oraz grafitu. Kaweolina-1 jest wielofunkcyjnym białkiem, będącym składnikiem błonowych kaweoli, biorącym udział w regulacji szlaków sygnałowych, endocytozy oraz transportu wewnątrzkomórkowego. Kaweolina-1 uczestniczy między innymi w regulacji szlaków sygnałowych związanych z angiogenezą.

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Effect of housing system and season of birth on the calves rearing results

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Abstract: *Effect of housing system and season of birth on the calves rearing results.* The main aim of the study was to compare rearing effect of calves kept outdoor in individual hutches and indoor in group pens. During the experiments following measurements were taken: body weight, daily and monthly gains and mortality. Study was conducted on 90 calves from 5th to 90th days of age and measures were taken in 3 seasons – spring, summer – autumn and winter. All calves were fed with the same feed and according to the same schedule. Results revealed that during first month calves from both groups achieved the same daily gains. However, older calves kept indoor characterized themselves by better gains and body weight. During the experiment only 2 calves were lost (mortality rate – 2.2%).

Key words: calves rearing, individual hutches, indoor, rising season, calves rearing parameters.

INTRODUCTION

The calves rearing plays an important role in dairy farming as healthy offspring provides the valuable replacement and affects economic efficiency of dairy producers. Typically, in Polish production systems, calves are kept in the indoor systems where the microclimate of the shed strongly depends upon the many factors, including building construction, thermal isolation as well as stocking rate. In the nineteen thirties the “cold rearing”

system has been introduced to Poland (Szewczuk et al. 2004), 30 years after has been introduced to United States (Schingoethe et al. 1986). The idea of introducing the new rearing system was to keep calves outdoor in individual hutches to prevent them from infection mainly cause by unfavorable indoor climate condition (high humidity, drafts and high concentration of toxic gases) (Grodzki 2005).

Nowadays, as shown in the survey conducted by Kowalski and Górka (2006), the large share of dairy farmers keep calves in individual hutches, mainly, made of plastic.

There are many different factors affecting the calves rearing results (Earley et al. 2004). According to Czaja et al. (2002) and Szewczuk et al. (2006) season of the calf birth strongly influenced the calves rearing. Heifers which were born at autumn and winter performed better daily gains than those born during the spring and summer.

The purpose of the study was to compare rearing parameters of calves (body weight, daily gains and mortality) kept in two different production systems: outdoor in hutches and indoor in different seasons.

MATERIAL AND METHODS

The material for analysis consisted of 90 calves-heifers of Polish Holstein-Friesian breed kept in the dairy farm located in Lubelskie Voivodship. The animals were divided into 2 equal groups according to the rearing system: indoor – in group pens (45 calves) and outdoor – in individual hutches (45 calves) and were observed between the 5th and 90th day of their life. The trial consisted of 3 seasons: 1st conducted between February and May (spring season – SS), 2nd conducted between July and August (summer – autumn season – SAS), and 3rd experience conducted between December and March (winter season – WS). In each season, the calves were treated in the same manner: up to 5th day of their life all animals were placed in the individual boxes (indoor – in the calf shed). After 5th day of age calves were moved either outdoor – to individual hutches (wooden construction) or indoor – to collective pens (5 calves a pen). Calves up to the 4th day of age were fed with colostrums (twice a day), from the 5th day of their age milk replacer was introduced to their diet (22% crude protein, 19.5% fat and 0.1% crude fiber), and at the 9th weeks milk replacer were changed into differ-

ent one designed to older calves (23.5% crude protein, 12.5% fat and 3.5% crude fiber). Colostrum and milk replacers after preparing (with accordance to the producer instruction) were fed in buckets fitted with nipples. From the second week of age all calves had unlimited access to prestarter concentrate and from 11 week of age were provided with quality hay. During the experiments calves were scaled every 2 weeks (from 5th to 115th day of their age and the daily gains (in 1st, 2nd and 3rd month) were calculated. Also the incidents of diseases occurrence were observed.

The obtained results were analyzed statistically using two factor analysis of variance by the IBM SPSS Statistics ver. 19. Calf birth season and housing system were considered.

RESULTS AND DISCUSSION

The influence of the rearing system on the monthly gains of the calves during the first 3 month of their life is presented in Table 1. Results showed that there was no influence of the rearing system on calves' monthly gains during the first month of their life. However, significant differences between both group

TABLE 1. The influence of the rearing system on the monthly gains of the calves during the first 3 months of their life (kg per month)

Month	Rearing system					
	Outdoor			Indoor		
	<i>n</i>	LSM	SE	<i>n</i>	LSM	SE
1	44	11.23	0.463	45	11.76	0.458
2	44	16.81 ^A	0.584	45	19.14 ^A	0.578
3	44	22.20 ^B	0.773	44	25.99 ^B	0.777

Significance at: a,b $P \leq 0.05$; A,B $P \leq 0.01$.

were observed at 2nd and 3rd month ($P \leq 0.01$). Calves kept indoor characterized by higher monthly gains than outdoor. Similar results obtained Adamski et al. (2004). The differences between compared groups (at 2nd and 3rd month) were probably caused by higher feed intake of indoor calves – group housing. The indoor calves intensified competition effect resulted in better prestarter consumption and thus their higher weight. Szewczuk and Kamieniecki (2003), stated that calves kept outdoor in the hutches characterized by significantly higher daily gain during the 1st, 2nd and 3rd months of their age.

The influence of the age and birth season of the studied calves on the changes in their body weight were analyzed in Table 2. There was significant difference ($P \leq 0.01$) between the 5 days old calves born in spring (SS) and winter (WS), SS heifers at 5th day of their age characterized by lower weight than those born in WS. Those results were also confirmed by Czaja et al. (2002).

The 5 days old calves born in SAS had a lower weight than those born in winter, however, the differences were not statistically significant. Similar results obtained Kuczaj (2004). However, Szewczuk et al. (2006) gained opposite results, heifers born in autumn characterized by a higher weight (43.1 kg) than those born in spring (42.5 kg), the weight of the calves born in winter and summer was similar, 41.7 and 41.8 kg respectively.

Similarly to Choroszy et al. (2003), conducted studies revealed that at the end of the rearing (90th day) the highest weight was observed in calves born in WS followed by SS and SAS.

The influence of the season of birth on the average monthly gains of calves is presented in Table 3. The monthly gains of 30 day calves were significantly different. The calves born during the spring season grew faster than animals born in other seasons which had comparable monthly gains. Szewczuk et al. (2006) stated that calves born in winter had the

TABLE 2. The influence of birth season of the calves on the changes in their body weight (kg) at different age

Day of life	Season									Total mean		
	SS			SAS			WS					
	n	LSM	SE	n	LSM	SE	n	LSM	SE	n	LSM	SE
5	40	41.68 ^A	0.977	20	44.65	1.381	29	46.27 ^A	1.147	89	44.20	0.681
19	40	47.73	1.062	20	47.75	1.501	29	49.50	1.247	89	48.33	0.741
33	40	56.43	1.101	20	54.60	1.557	29	56.05	1.294	89	55.69	0.768
47	40	65.10	1.245	20	62.45	1.761	29	64.60	1.462	89	64.05	0.868
61	40	74.47	1.436	20	72.30	2.030	29	74.22	1.687	89	73.67	1.002
75	40	85.30	1.676	20	82.95	2.370	29	85.04	1.969	89	84.43	1.169
85	40	92.28	1.867	20	91.40	2.641	29	94.77	2.193	89	92.82	1.303
90	38	97.47	2.033	20	96.33	2.800	29	99.74	2.326	87	97.85	1.390

Significance at: a, b $P \leq 0.05$; A,B $P \leq 0.01$.

TABLE 3. Influence of the season of birth on the average monthly gains (kg) of calves

Month of life	Season								
	SS			SAS			WS		
	n	LSM	SE	n	LSM	SE	n	LSM	SE
1	40	14.75 ^{A,B}	0.476	20	9.95 ^A	0.673	29	9.98 ^B	0.559
2	40	18.05	0.600	20	17.70	0.849	29	18.17	0.705
3	39	22.72	0.815	20	24.03	1.123	29	25.52	0.933

Significance at: a,b $P \leq 0.05$; A,B $P \leq 0.01$.

best daily gains over the first month of their life. The conducted studies revealed that 2 and 3 months old calves characterized by the fastest grow in comparison to other groups, however, their gains were lower than those observed by Szewczuk et al. (2006).

The weight of the calves at 47 day of age were similar in both SS and SAS seasons (Figs 1 and 2). At the age 47 to 90 days indoor heifers characterized by higher weight than those kept in the outdoor hutches, however differences between both groups were no statistically significant. Observed differences probably were caused by higher feed intake of calves kept in group pens, what also was confirmed in studies of Warnick et al. (1976), who stated that at the end of the rearing period (74–124 day) monthly

gains of calves kept in groups was by over 5 kg higher than those kept individually. Nevertheless, housing group of calves of different weight also might decrease gains of the smallest ones which are often dominated by the bigger and the strongest calves.

During the winter season calves reared in outside hutches characterized by higher weight than those kept in the calf shed (Fig. 3). Significant differences between both group were observed during the whole experiment. Calves kept outdoor in winter needed much more feed energy to face maintenance requirements e.g. for maintaining body temperature thus they characterized by a higher feed intake and better growth.

Daily gains of calves at 1st, 2nd and 3rd month of age depended upon the rear-

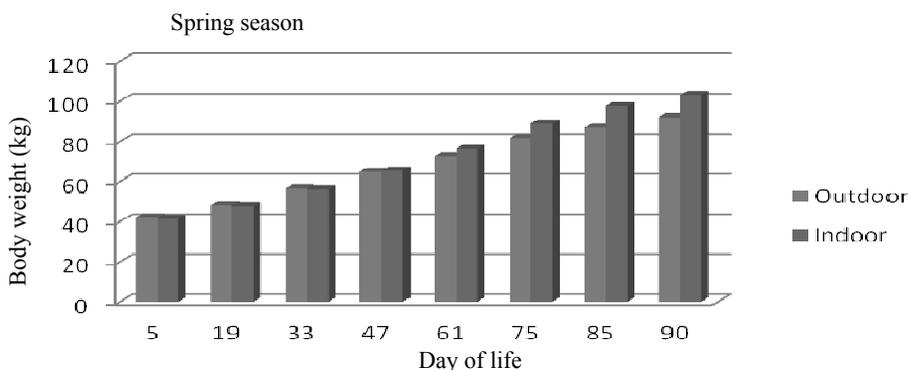


FIGURE 1. Changes in body weight of calves at age 5 to 90 days during the spring season kept in outdoor and indoor systems

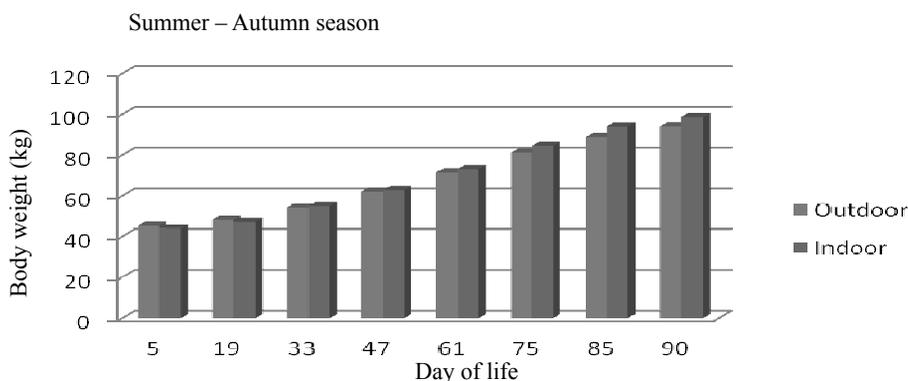


FIGURE 2. Changes in body weight of calves at age 5 to 90 days during the summer – autumn season kept in outdoor and indoor systems

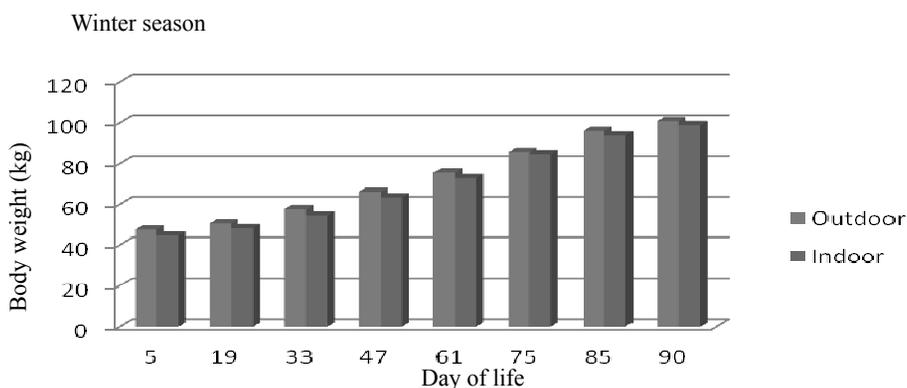


FIGURE 3. Changes in body weight of calves at age 5 to 90 days during the winter season kept in outdoor and indoor systems

ing season are presented in Figures 4–6. During the SS daily gains of 1 month old heifers were comparable in both systems, however, 2 and 3 month old calves kept indoor characterized by higher daily gains than those kept in hutches.

During the summer – autumn season heifers reared outdoors grew slower at 1st and 3rd month of age than calves kept indoors. Only during the winter season the calves at any age characterized by similar daily gains irrespectively on the rearing system.

The percentage distribution of the diseases observed during the experiments are presented at Table 4. During the spring period calves kept in the outdoor hutches were less susceptible to alimentary diseases than indoor ones. However, the 40% of outdoor calves had respiratory problems, which accounted of 10 percentage points more than for the heifers kept indoor. During the summer – autumn season frequency of diseases were similar in both systems.

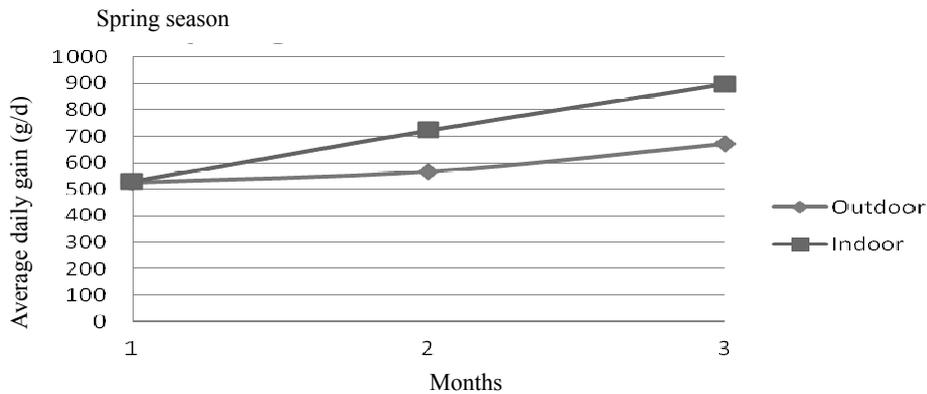


FIGURE 4. Daily gains of spring calves at 1st, 2nd and 3rd month of age reared in outdoor and indoor system

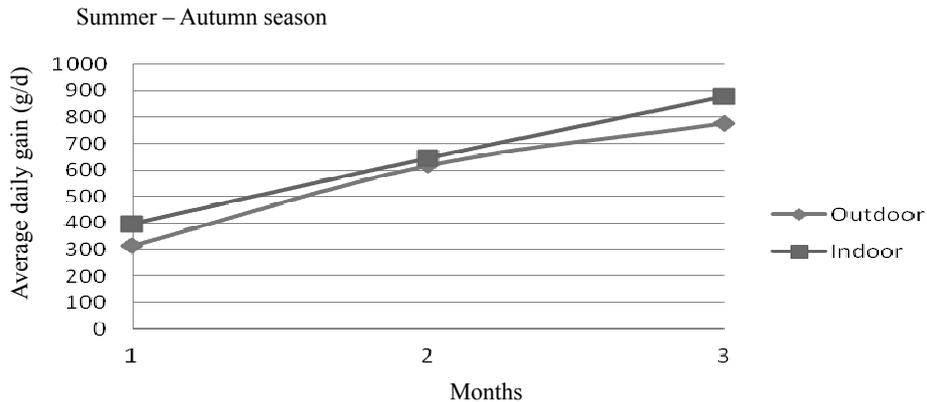


FIGURE 5. Daily gains of summer – autumn calves at 1st, 2nd and 3rd month of age reared in outdoor and indoor system

During the winter season no incidents of alimentary diseases were observed. Similar results in their studies obtained Sablik et al. (2000). Additionally, the 60% of indoor heifers had problems with respiratory system compared to 21% of calves kept outdoor. Particular attention should be paid to high rate of injuries of nasal mucosa (others) observed in calves kept in group pens, which was not the case in individual hutches. Studies of Szewczuk and Kamieniecki (2003), re-

vealed that calves kept indoor were more prone to respiratory diseases than those kept outdoor in the hutches. Only 5% of outdoor calves had significant problems with alimentary tract while the 15% of indoor calves had repeatable problems with diarrhea. The increased rate of incidents of alimentary problems in indoor calves was caused by a higher concentration of microbes in their environment (dump bedding and wasted feed).

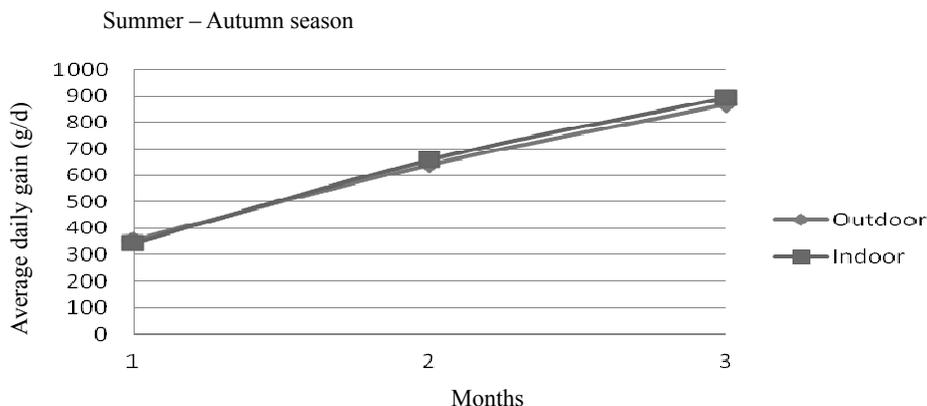


FIGURE 6. Daily gains of winter calves at 1st, 2nd and 3rd month of age reared in outdoor and indoor system

TABLE 4. Percentage distribution of the diseases observed during the experiments.

Diseases	Unit	Season					
		SS		SAS		WS	
		out	in	out	in	out	in
Diarrhea	%	10	35	10	10	0	0
Pneumonia	%	40	30	40	40	21	60
Others	%	0	15	0	10	0	0
Sum	%	50	65	50	60	21	60

During the all experiments 2 heifers were lost, 1 in the spring (indoor) and 1 in the winter season (outdoor). Total mortality rate stood at 2.2%.

prestarter concentrates and competition within the group significantly intensify feed intake rate and thus accelerates daily gains.

CONCLUSIONS

Irrespectively to rearing system during the first month of their life calves should be kept individually as their growing rate is comparable to calves kept indoor. Moreover, individual calf handling positively affects its health status.

Since second month of age group housing is the most preferable. At this age calves start to intensively consume

REFERENCES

- ADAMSKI M., KUPCZYŃSKI R., ZACHWIEJA A., 2004: Efektywność odchowu cieląt w zależności od systemu utrzymania. Zeszyty Naukowe AR we Wrocławiu Zootechnika 52, 19–26.
- CHOROSZY B., CZAJA H., CHOROSZY Z., MAZUR M., 2003: Wyniki odchowu cieląt rasy simentalskiej w zależności od sezonu urodzenia. Zeszyty Naukowe Przegląd Hodowlan 67, 99–104.

- CZAJA H., CHOROSZY B., KORZONEK H., 2002: Wpływ wybranych czynników na wyniki odchowu cieląt rasy czarnobiałej. *Zeszyty Naukowe Przegląd Hodowlany* 62, 281–302.
- EARLEY B., MURRAY M., FARRELL J.A., NOLAN M., 2004: Rearing calves outdoors with and without calf jackets compared with indoor housing on calf health and live-weight performance. *Irish J. Agric. Food Res.* 43, 59–67.
- GRODZKI H., 2005: Wychów cieląt i młodego bydła. (in): Z. Liwińczuk, T. Szulc (eds). *Hodowla i użytkowanie bydła*. Państw. Wyd. Rol. i Leśne, Warszawa.
- KOWALSKI Z. M., GÓRKA P., 2006: Jak odchowujemy. *Hoduj z głową*, 3/4, 36–40.
- KUCZAJ M., 2004: Wyniki odchowu cieląt w okresie żywienia siarą w zależności od sezonu wycielenia i długości zasuszenia krów. *Zeszyty Naukowe AR we Wrocławiu Zootechnika* 51, 137–141.
- SABLIK P., GRADOMSKA M., MALINOWSKI E., 2000: Porównanie dwóch systemów odchowu cieląt w gospodarstwie rolnym o wielkostadnej technologii produkcji. *Folia Universitatis Agriculturae Stetinensis, Zootechnica*, 39, 153–158.
- SCHINGOETHE D.J., CASPER D.P., DDRACKLEY J.K., LUDENS F.C., 1986: Increased solids intake and feeding frequency for calves in hutches during cold winter. *J. Dairy Sci.* 69, 1063–1069.
- SZEW CZUK M., CZERNIAWSKA-PIĄTKOWSKA E., KAMIENIECKI H., 2004: Porównanie różnych systemów odchowu cieląt. [In] *Racjonalny odchów cieląt i młodzieży warunkiem uzyskania zdrowych i wysokoprodukcyjnych krów*. Konferencja naukowa, Lubiana, 17–18.06.2004, 78–87.
- SZEW CZUK M., KAMIENIECKI H., 2003: Ocena przebiegu wzrostu i rozwoju cieląt w zależności od systemu utrzymania. *Zeszyty Naukowe Pol. Tow. Zootech. Przegląd Hodowlany* 69, 153–158.
- SZEW CZUK M., KAMIENIECKI H., CZERNIAWSKA-PIĄTKOWSKA E., SZATKOWSKA I., 2006: Wpływ sezonu urodzenia na zdrowotność i wyniki odchowu cieląt. *Acta Sci. Pol., Zootechnica* 5 (2), 119–124.
- WARNICK V.D., ARAVE C.W., MICKELSEN C.H., 1976: Effects of group, individual, and isolated rearing of calves on weight gain and behavior. *J. Dairy Sci.* Vol. 60, 6, 947–953.

Streszczenie: Porównanie efektów wychowu cieląt utrzymywanych w budkach i w cielętniku. Celem pracy było porównanie efektów odchowu cieląt utrzymywanych w budkach indywidualnych na zewnątrz budynku i w cielętniku w tym samym gospodarstwie, mierzonych masą ciała, przyrostami i zachorowalnością. Badania przeprowadzono na 90 cieliczkach w okresie od 5. do 90. dnia życia, a obserwacje prowadzono w 3 sezonach: wiosennym, letnio-jesiennym i zimowym. Cielęta żywiono takimi samymi paszami i według tego samego schematu. Stwierdzono, że cielęta w 1. miesiącu życia osiągały podobne parametry odchowu w budkach indywidualnych i w cielętniku, natomiast w dalszym okresie odchowu jałówki z cielętnika odznaczały się większymi masami ciała i przyrostami. W okresie prowadzonych badań zaobserwowano zdecydowanie mniej przypadków zachorowań u cieląt utrzymywanych w budkach indywidualnych (36,7%) niż w cielętniku (63,3%).

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