

# Annals of Warsaw University of Life Sciences – SGGW

Animal Science No 53  
Warsaw 2014

---

## Contents

- BOJKO P., REKIEL A. Evaluation of productivity of imported crossbred pigs (Landrace × Yorkshire × Duroc) 5
- DAMAZIAK K., MICHALCZUK M., KUREK A. Effect of rearing system on the microbiological quality of Pekin P44 and Muscovy MR71 ducks bowel 13
- KRÓLEWSKA B., REKIEL A., WIĘCEK J. Effect of birth body weight of piglets on their rearing up to the age of 10 weeks 21
- KUCHARSKA K., PEZOWICZ E., TUMIALIS D., KUCHARSKI D., ZAJDEL B. Nanoparticles of copper and entomopathogenic nematodes *Steinernema feltiae* (Filipjev, 1934) in reducing the number of the lesser mealworm beetle *Alphitobius diaperinus* (Panzer, 1797) 29
- KULESZA D., MOZGA K., NIŻNIKOWSKI R., STRZELEC E., ŚWIĄTEK M., ŚLĘZAK M. Evaluation of thickness and color of wool in primiparas of Żelaźnieńska and Corriedale Sheep 37
- NIŻNIKOWSKI R., CZUB G., KAMIŃSKI J., NIERADKO M., ŚWIĄTEK M., GŁOWACZ K., ŚLĘZAK M. Polymorphism of insulin-like growth factor

- (*IGF-1*) gene in Polish Lowland Sheep from Podlaskie Voivodeship 43
- NIŻNIKOWSKI R., OPRZADEK A., GŁOWACZ K., STRZELEC E., CZUB G., ŚWIĄTEK M., ŚLĘZAK M. The application of ultrasonography (USG) technique for assessment of muscularity of Berrichon du Cher and Polish Merino lambs at the age of 70 days 47
- PRZYSUCHA T., STEFANIUK M., GOŁĘBIEWSKI M., SŁÓSZARZ J., WNEK K., KUNOWSKA-SŁÓSZARZ M. Analysis of fattening results of Polish Holstein-Friesian bulls and PHF × Belgian Blue crossbreds bulls 55
- REKIEL A., WIĘCEK J., CICHOWICZ M., BIELECKI W., WIESZCZY P. The effect of fibre level in the mixture on the state of intestinal epithelium of fatteners 61
- WNUK A., ŁUKASIEWICZ M., MROCZEK-SOSNOWSKA N., NIEMIEC J., POPCZYK B., BALCERAK M. Effect of sex on results of slaughter analysis of grey partridge *Perdix perdix* 67
- ZAJDEL B., GĄBKA J., KUCHARSKA K., KUCHARSKI D. The role of vestibulum in the nests of the red mason bee *Osmia bicornis* L. 73
- BIELA I., KOŹLIŃSKA M., SOSNOWSKA P., JĘDRUCHÓW D., ŁUKASIEWICZ M. Behaviors of the corvids towards common buzzard *Buteo buteo* on urban and extra-urban areas of the Mazowieckie Province 79
- DROBIK W., MARTYNIUK E. Factors affecting prolificacy and lambs rearing results in Olkuska sheep population 85
- DZIERŻANOWSKA-GÓRYŃ D., BRZOZOWSKI M., GÓRAL-RADZISZEWSKA K. Young chinchillas weight gain, depending on their body mass at birth 95
- HALIK G.D., ŁOZICKI A., KOZIORZĘBSKA A., DYMNIKA M., ARKUSZEWSKA E. Effect of ensiling pumpkin *Cucurbita maxima* with the addition of inoculant or without it on chemical composition and quality of silages 103
- HOŁDA K., GŁOGOWSKI R. A survey of Deoxynivalenol and Zearalenone content in commercial dry foods for growing dogs 111
- JESION I., LEONTOWICZ M., LEONTOWICZ H., GRALAK M.A., KMIĘĆ H., GORINSTEIN S., HARUENKIT R. The effect of different ripening stages of durian *Durio zibethinus* fruit on zinc content in liver of rats loaded with cholesterol 119
- JESION I., LEONTOWICZ M., LEONTOWICZ H., KOŁNIERZAK M., GRALAK M.A., PARK Y.-S., GORINSTEIN S. Diets supplemented with *Mytilus galloprovincialis* from polluted and non-polluted waters and their influence on zinc content in liver of rats loaded with cholesterol 127

MROCZEK-SOSNOWSKA N., ŁUKASIEWICZ M., WNUK A., SAWOSZ E., NIEMIEC J. Effect of copper nanoparticles and copper sulfate administered *in ovo* on copper content in breast muscle, liver and spleen of broiler chickens  
135

TOBOLOVÁ R., WIETROW P., KO-SIARZ P., GŁOWACKA A., ŁUKASIEWICZ M. Study on the relation between an accipiter bird and man  
143

WNUK A., ŁUKASIEWICZ M., MROCZEK-SOSNOWSKA N., NIEMIEC J., POPCZYK B., BALCERAK M., ADAMEK D., KAMASZEWSKI M. Characteristics of muscle fibers of breast and leg muscles of grey partridges *Perdix perdix*  
151

#### List of Reviewers 2014

Apostol Apostolov, Justyna Batkowska, Teresa Bombik, Danuta Borkowska, Bronisław Borys, Anna Czech, André Chwalibóg, Marta Gajewska, Vasfi H. Gencer, Karol Giejdasz, Zygmunt Giżewski, Henryk Grodzki, Andrzej Gugolek, Hanna Jankowiak, Alina Janocha, Zenon Jastrzębski, Magdalena Jaworska, Anna Kasprzyk, Nikolaj V. Kazarovets, Renata Klebaniuk, Krzysztof Kozłowski, Józef Krzyżewski, Dariusz Lisiak, Stoycho Metodiev, Zenon Nogalski, Sławomir Nowicki, Katarzyna Ognik, Anna Pecio, Beata Prusak, Małgorzata Remiszewska, Ferdinand Ringdorfer, Katarzyna Ropka-Molik, Anna Sawa, Beata Seremak, Stanisław Socha, Robert Soliva-Fortuny, Bogdan Szostak, Tomasz Szwaczkowski, Svetlin Tanchev, Leon Tarasewicz, Krzysztof Tereszkiwicz, Alexandra Trbolová, Leszek Tymczyna, Beata Wasilewska-Nascimento, Stanisław Wężyk, Anna Winnicka, Jerzy Wójcik, Jacek Wójtowski, Marina Zemser

#### SERIES EDITORIAL BOARD

Editor-in-Chief  
Anna Rekiel

*Animal Science* series Secretary  
Katarzyna Góral-Radziszewska

Address of Editorial Office  
Wydział Nauk o Zwierzętach SGGW  
ul. Ciszewskiego 8, 02-786 Warszawa  
Poland

#### EDITORS

Statistics editor – Wojciech Hyb  
English language consultant – Natalia Filipczak  
Polish language consultant – Agata Kropiwić

#### THEME EDITOR

Genetics and animal breeding – Elżbieta Michalska  
Biology and ecology – Elżbieta Pezowicz  
Animal nutrition and feedstuffs – Iwona Kosieradzka  
Behaviour and welfare of animal – Tadeusz Kaleta  
Animal husbandry and production technology – Justyna Więcek

#### SERIES EDITOR

Anna Rekiel

The Editorial Board (Office) of “Annals of Warsaw University of Life Sciences – SGGW. Animal Science” informs that the printed version of the journal is the original version.

Redakcja „Annals of Warsaw University of Life Sciences – SGGW. Animal Science” informuje, że wersja drukowana czasopisma jest wersją pierwotną (referencyjną).

Covered in: AGRO, Index Copernicus (5.85), CAB Direct, CEON, ARIANTA, e PNP.

Bazy: AGRO, Index Copernicus (5.85), CAB Direct, CEON – Biblioteka Nauki, ARIANTA, e-Publikacje Nauki Polskiej.

#### SERIES EDITORIAL ADVISORY COUNCIL

Prof. DSc. Andrzej Chwalibóg (Denmark)  
Prof. DSc. Konrad Dąbrowski (U.S.A.)  
Prof. DSc. Ondrej Debréćeni (Slovakia)  
Prof. Ewgienij Dobruk (Belarus)  
Prof. dr hab. Robert J. Eckert (Poland)  
Prof. Dr. Sophie Ermidou-Pollet (Greece)  
Prof. dr hab. Grażyna Garbaczewska (Poland)  
Prof. DSc. Luis L. Gosálvez (Spain)  
Prof. DSc. Adrian Harrison (Denmark)  
Prof. dr hab. Jarosław O. Horbańczuk (Poland)  
Prof. dr hab. Marta Kamionek (Poland)  
Prof. Dr Drago Kompan (Slovenia)  
Prof. Dr Sándor Kukovics (Hungary)  
Prof. Dr Stoycho Metodiev (Bulgarian)  
Prof. DSc Francois K. Siebrits (R.S.A.)  
Prof. dr hab. Jacek Skomial (Poland)  
Prof. dr hab. Romuald Zabielski (Poland)

 WARSAW UNIVERSITY OF LIFE SCIENCES PRESS  
e-mail: [wydawnictwo@sggw.pl](mailto:wydawnictwo@sggw.pl)

ISSN 1898-8830

#### EDITORIAL STAFF

Anna Dołomisiewicz  
Violetta Kaska  
Laura Szczepańska

Edition: 150 prints  
PRINT: Agencja Reklamowo-Wydawnicza A. Grzegorzcyk  
[www.grzeg.com.pl](http://www.grzeg.com.pl)

## Evaluation of productivity of imported crossbred pigs (Landrace × Yorkshire × Duroc)

PAULINA BOJKO, ANNA REKIEL

Department of Pigs Breeding, Warsaw University of Life Sciences – SGGW

**Abstract:** *Evaluation of productivity of imported crossbred pigs (Landrace × Yorkshire × Duroc).*

The aim of the conducted studies was to determine the run of fattening and slaughter value of imported hybrid pigs: L × Y × D. The uniform nutrition and management was employed; the pigs were fattened from body weight 30 to 115–120 kg. For 4433 animals, who completed fattening and were slaughtered, very good production results were obtained: mean daily body gains – 989 g, feed conversion – 2.88 kg/kg; meatiness – 58.86%; participation of carcasses in classes S and E – 92.9% in total. The obtained results indicate a very good, and, first of all, repeatable quality of the examined hybrid fatteners in respect of traits which are economically important for meat producers and meat industry. They are also the evidence of high and stabilized genetic potential of purebred populations of animals, employed in three-breed commercial crossbreeding, serving for production of piglets for fattening.

*Key words:* crossbred fatteners, production parameters

### INTRODUCTION

In pig management, good quality of animal material is the condition for obtaining satisfying economic results. Its improvement is based on measurements of live animals and accurate post-slaughter evaluation (Blicharski 1999). The selection-breeding work, oriented to the betterment of utility traits of pigs,

including meatiness of carcasses, has caused that at present, the animals differ in utility, conformation and growth rate as compared to those ones who were at the disposal of breeders several years ago (Różycki 2004). The breeding work and high import of pigs for domestic breeding have brought about the change of in relationships between the particular slaughter traits and meatiness (Żak et al. 2008). The animals, imported to Poland, were bred within the frames of various programmes. Their assumptions differed often each other because the level of utility traits of the animals in the particular countries was different as well as the expectations of the receivers of breeding material, live pork and of the consumers (Różycki and Żak 2001). The genetic breed and environmental (feeding) factors have an impact on the carcass fat content and distribution as well as its composition – fatty acids' profile (Scheeder 2004, De Smet et al. 2004, Wood et al. 2008). The quality of carcass is also dependent on the meat content (Orzechowska et al. 2012).

After the entrance of Poland to the EU structures and opening of the European market, the national producers of live

pork began to buy material for fattening not only from Polish piggeries but also from abroad. It was supported by the offer of delivering greater, uniform and healthy lots of animals at competitive prices.

The reached considerable progress in meatiness of domestic and imported animals, the changes in the systems of feeding the pigs, abbreviating fattening period and the introduced prophylactic programmes are favourable for obtaining good results (Różycki and Żak 2001). From the economic point of view, at the present level of live pork purchasing prices, the parity income may be obtained when selling at least several hundreds (300–500) fatteners during a year.

The aim of the work was to evaluate productivity and repeatability of the

result of imported hybrid pigs, fattened in private farm in the standardized (uniform) conditions of nutrition and management.

## MATERIAL AND METHODS

The crossbred piglets (Landrace × Yorkshire × Duroc) were intended for fattening at body weight of 30.5 ± 0.5 kg. The pigs were *ad libitum* fed in the two-stage system. Initially, a ready granulated feed for weaners was used and then, the feed for fatteners and/or the own mixture, containing cereals and concentrate was administered (Table 1). Fattening of pigs was conducted in the building with the area of 360 m<sup>2</sup> (30 × 12 m) in 14 pens on a deep litter, with maximum density of 450 head (one

TABLE 1. Composition of raw materials and nutritional value of mixtures

Specification	Grower (BW 20–35 kg)	Fatteners (BW 35–70 / 80 kg)	Concentrate weaner / fattener (20 / 17%)
Raw material composition	Cereal meals: corn, wheat, barley, triticale, animal pork fat, mineral-vitamin and enzymatic additives, tryptophan		Vegetal by-products of spirit products' manufacture, products of pork blood, mineral-vitamin and enzymatic additives
	Extraction soy and rape meal, wheat brans, wheat protein		
Nutritional value of 1 kg of mixture (g)			
Specification	Grower (20–35 kg m.c.)	Fatteners (35–70 / 80 kg)	Concentrate weaner / fattener (20 / 17%)
Crude ash	44.60	43.32	126.23
Crude protein	165.00	160.00	399.98
Crude fat	37.65	29.70	35.99
Crude fibre	39.88	43.07	56.90
Calcium	7.00	6.50	33.00
Phosphorus	5.80	5.70	15.80
Sodium	2.00	2.00	10.00
Lysine	11.30	10.00	40.00
Methionine	3.71	2.77	10.75

lot). The animals were kept in standard management and nutrition conditions. After completion of fattening of a lot of pigs, manure was removed and the premises were cleaned, washed and disinfected, using jodosol preparation and lime milk. During the observation period, the requirements concerning animal welfare were satisfied (EU 1999, EU 2001). In ten successively fattened groups, 4433 animals were produced in total. After reaching body weight of ca. 115–120 kg, the pigs were slaughtered according to the procedures, obligatory in the meat industry. The mean daily body gains and feed conversion per 1 kg of body weight gain were determined. Meatiness of carcasses was assessed using choirometer (Sydel CGM) and then, they were qualified into appropriate classes in EUROP system. The results were given in tables and figures as the weighed means.

RESULTS AND DISCUSSION

The obtained fattening parameters are given in Table 2. They are found within the ranges established by the receiver of live pork – ANIMEX Group SA and the mentioned limits should be found below 900 g/day, with feed conversion on the level of 2.99 kg/kg of body weight gain. Better results were obtained in own studies; daily gains were higher by 89 g (+10.99%), feed conversion was lower by 0.11 kg/kg of body weight gain (Figs 1 and 2). It means saving of ca. 10 kg of feed per fattener in relation to threshold value, established by the receiver. It may be stated that Danish hybrids have good body weight gains as well as they utilize feed well in the appropriate management conditions; they had such conditions ensured during the monitored fattening period. Their meatiness was very high (Table 3). Classes S and E included 92.9% of carcasses in total.

TABLE 2. The selected indicators of fattening and slaughter value (10 groups of fatteners; n = 4433)

Group of fatteners	Quantity in the group	Period of fattening (days)	Body weight of fatteners at slaughter (kg)	Dressing percentage (%)	Mean meatiness (%)
1	414	94.68	119.17	79.35	59.39
2	442	85.23	119.15	79.41	58.87
3	441	93.83	120.91	77.64	57.65
4	440	85.00	118.70	79.03	59.08
5	432	87.85	119.54	79.29	59.56
6	475	92.14	124.21	78.77	59.09
7	458	97.34	124.29	79.07	58.30
8	458	92.89	119.53	78.70	59.02
9	443	96.84	124.05	79.07	59.02
10	435	85.82	116.60	79.39	58.64
Mean	443.3	91.30	120.81	79.05	58.86

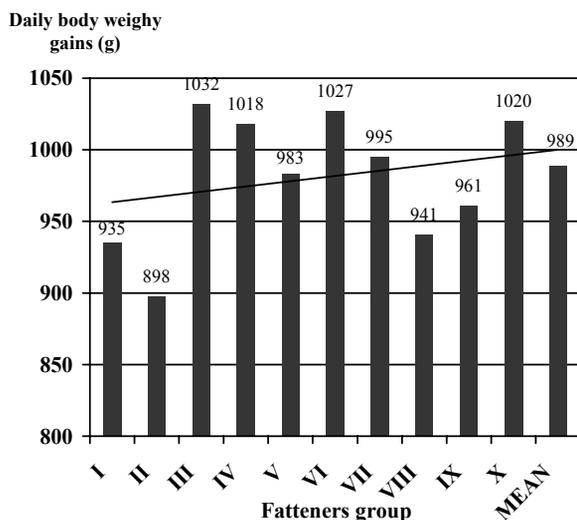


FIGURE 1. Daily body weight gains of fatteners in groups

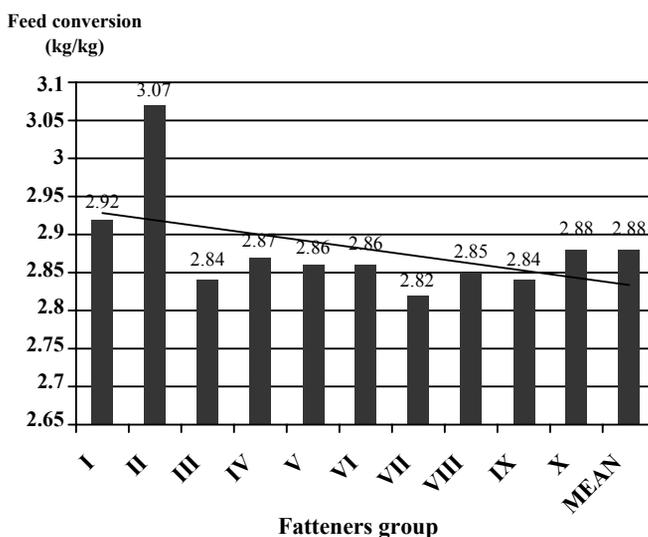


FIGURE 2. Feed conversion by fatteners in groups

The mean meatiness of the fatteners from 10 groups was comparable with the results of Siczowska et al. (2009) and Moczowski (2010).

The comparison of results of the study of Różycki and Żak (2001) versus own analysis indicates to differences

in meat percentage in carcass and feed conversion per 1 kg of body weight gain (PIC – 58.06% and 3.09 kg/kg, HYPOR – 53.27% and 2.97 kg/kg, PENAR LAN – 54.30% and 2.96 kg/kg), in favour of own study (58.86% and 2.88 kg/kg). The maximal difference in meat in carcass

TABLE 3. Evaluation of carcasses according to EUROP scale (10 groups of fatteners; n = 4433)

Class of fatteners	Number of fatteners in class (heads)	Number of heads in class (%)	Mean meatiness (%)	Mean weight of carcass in class (kg)
S	1606	36.2	61.35	95.59
E	2512	56.7	57.97	93.43
U	302	6.8	53.51	93.99
R	13	0.3	47.89	89.70
Total	4433	100	58.86	94.24

amounted to 5.59 percentage points. Significant time difference between the two studies has to be, however noted.

Meatiness of fatteners was by 3% higher as compared to the threshold value, established by meat factories and higher than the national mean for mass population, evaluated in the years 2011–2012. In case of a very good meatiness, feed conversion per 1 kg of gain was equal to 2.88 kg what affected positively the effectiveness of production. If we compare the consumption of feeds in the own studies and the consumption of the mixtures by the animals PEN AR LAN, we will find a difference – 0.08 kg/kg of gain. In case of fattening, lasting 91.3 days, it means saving of feed on the level of 7.22 kg per animal (with assumption adopted by Animex SA group, it is 9.94 kg/head). In case of the group of fattened pigs equal to 450 heads (one lot), it means demand on feed versus PEN AR LAN, as being lower by 4 t. In relation to the assumptions of Animex SA group, the demand on the feeds for the whole quantity of fatteners (n = 4433 heads) amounted to more than 44 t (44,064 kg) what gave a guarantee of good fattening effectiveness. Moczowski (2010)

analyzed a group of crossbred pigs produced on the grounds of the breeds, managed in Poland (PLW × PL) × Duroc and (PLW × PL) × Pietrain and Danish and Dutch breeds – (Landrace × Yorkshire) × Duroc. In case of production of more than 6.3 thousand animals, deaths in experimental fattening period were as follows: Polish (P) – 3.1%; Dutch (H) – 6.3%; and Danish (D) – 2.5%. In the structure of the sold pigs, the participation of fatteners P, H and D was 37.07, 26.07 and 36.86%, respectively. The participation of carcasses in classes S and E was as follows: Polish fatteners – 25 and 59%, Dutch fatteners – 14 and 46% and Danish fatteners – 46 and 49%, respectively. The results, obtained for Polish fatteners were considered by Moczowski (2010) as satisfying, however, the relatively low participation of the pigs in class S decreased the incomes. In evaluation of the cited author, a low meatiness of Dutch fatteners resulted, first of all, from the state of induction of immunological system. A very good meatiness of Danish fatteners confirmed their high genetic potential in respect of slaughter traits. Siczowska et al. (2009) emphasize

that the Danish pigs are characterized by a very good quality and technological suitability with preservation of high, 58%-percentage-meatiness. The meatiness on the level of 58–59% for a big group of Danish fatteners was also obtained in the studies of Moczowski (2010). Sieczkowska et al. (2009) recognize the mentioned level of meatiness as being good. The results of Różycki and Żak (2001), Eckert and Orzechowska (2002), Rybarczyk et al. (2004), Borzuta et al. (2008), Strzelecki et al. (2008) and Grzeškowiak et al. (2010) also confirm a good quality of slaughter hybrid pigs. High meatiness of fatteners in the own studies is, *inter alia*, the effect of a very good nutrition. The animals with a high growth potential require the mixtures with a high concentration of energy and nutrients (Rekiel and Olejniczak 2009). *Ad libitum* nutrition is the simplest method for intensive fattening; the excessive fatness of carcass is its defect. It may be prevented when limiting the quantity of the consumed feed by the animals for the period of a few weeks before slaughter and, as it was revealed in the studies of Affentranger et al. (1996), by a decrease of the quantities of energy in the mixture during the final stage of fattening. It lowers the costs of fattening and causes depositing of by 2–3% greater quantity of lean meat in the carcass.

## CONCLUSIONS

The obtained results indicate a very good and, first of all, repeatable quality of the examined hybrid fatteners in respect of

the traits, being economically important for meat producers and meat industry. They are also an evidence of a high and stabilized genetic potential of initial, purebred populations of animals, used in three-breed commercial crossbreeding.

## REFERENCES

- AFFENTRANGER P., GERWIG C., SEEWER G.J.F., SCHWORER D., KUNZI N., 1996: Growth and carcass characteristics as well as meat and fat quality of three types of pigs under different feeding regimens. *Livest. Prod. Sci.* 45(10), 187–196.
- BLICHARSKI T., 1999: Genetyczne uwarunkowania wzrostu mięsności świń w Polsce. II Międzynarodowa Konferencja „Rola klasyfikacji EUROP jako czynnika poprawy jakości surowca wieprzowego”, Poznań 7-8.12.1999, 1–17.
- BORZUTA K., STRZELECKI J., DZIADEK K., GRZEŠKOWIAK E., LISIAK D., JANISZEWSKI P., 2008: Analiza porównawcza wartości rzeźnej i jakości mięsa świń hybrydowych linii PEN-AR LAN oraz 990. *Roczn. Nauk. Zoot.* 35 (1), 63–73.
- De SMET S., RAES K., DEMEYER D., 2004: Meat fatty acid composition as affected by fatness and genetic factors: a review. *Anim. Res.* 53, 81–98.
- ECKERT R., ORZECHOWSKA B., 2002: Mięsność tusz loszek i kastratów od mieszańców loch wbp × pbz, po knurach rasy duroc bądź pietrain. *Prace i Mater. Zoot. Zeszyt Specjalny* 13, 37–41.
- EU, 1999. EU Council Regulation no 1804/1999 of 19 July supplementing Regulation (EEC) no. 2092/91 on organic production of agricultural products and indications referring there to on agricultural products and foodstuffs to include livestock production.
- EU, 2001. EU Council Regulation no. 2001/88 of 23 October 2001 amending Directive 91/630

- EEC laying down minimum standards for the protection of pigs.
- GRZEŚKOWIAK E., BORZUTA K., LISIAK D., JANISZEWSKI P., STRZELECKI J., 2010: Przydatność kulinarna mięsa świń raz białych oraz mieszańców z udziałem knurów rasy Duroc i Pietrain. *Nauk. Przyr. Techn.* 4 (5), # 58, 1–11.
- MOCZKOWSKI R., 2010: Analiza wpływu wartości zwierząt hybrydowych pochodzących z ferm polskich, duńskich i holenderskich na wyniki tuczu i wartość rzeźną w ujednoczonych warunkach tuczu. Pr. mgr WNZ SGGW, 1–67.
- ORZECZOWSKA B., TYRA M., MUCHA A., ŻAK G., 2012: Jakość tusz świń ras wbp i pbz ze szczególnym uwzględnieniem zawartości tłuszczu śródmięśniowego (IMF) w zależności od poziomu mięsności. *Rocz. Nauk. Zoot.* 39 (1), 77–85.
- REKIEL A., OLEJNICZAK D., 2009: The influence of the mixture type on the productive and economic results of fattening of hybrid pigs. *Rocz. Nauk. PTZ* 5 (3), 53–61.
- RÓŻYCKI M., 2004: Zmiany genetyczne świń i ich wpływ na kierunki użytkowania. *Prace i Mat. Zoot.* 15, 9–18.
- RÓŻYCKI M., ŻAK G., 2001: Wyniki poubojowego testu świń pochodzących z firm hybrydowych. *Trz. Chlew.* 39 (10), 28–30.
- RYBARCZYK A., KORTZ J., SZARUGA R., NATALCZYK-SZYMKOWSKA W., 2004: Jakość mięsa tusz tuczników hybrydowych PEN AR LAN sklasyfikowanych w klasach systemu EUROP z uwzględnieniem płci. *Rocz. Inst. Przem. Mięś. Tłuszcz.* 11, 75–83.
- SCHEEDER M.R.L., 2004: The fatty acid composition of meat: manipulation and relevance for human nutrition. *Proc. Brit. Soc. Anim. Sci.* 14–17.
- SIECZKOWSKA H., KOĆWIN-PODSIADŁA M., KRZĘCIO E., ANTOSIK K., ZYBERT A., WŁOSZEK E., 2009: Mięśność i jakość mięsa mieszańców (landrace × yorkshire) × × duroc oraz (landrace × yorkshire) × Hampshire. *Rocz. Nauk. PTZ* 5 (4), 209–216.
- STRZELECKI J., BORZUTA K., LISIAK D., BORYS A., GRZEŚKOWIAK E., JANISZEWSKI P., 2008: Wpływ masy tuczników linii PEN-AR-LAN na wartość rzeźną i jakość mięsa. *Rocz. Inst. Przem. Mięś. Tłuszcz.* XLVI (3), 73–81.
- WOOD J.D., ENSER M., FISHER A.V., NUTE G.R., SHEARD P.R., RICHARDSON R.I., HUGHES S.I., WHITTINGTON F.M., 2008: Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci.* 78 (4), 343–358.
- ŻAK G., ECKERT R., BERETA A., KRUK M., 2008: Przydatność wskaźników rzeźnych uzyskiwanych poubojowo do określania mięsności tusz świń rasy polskiej białej zwisłouchej. *Rocz. Nauk. PTZ* 4 (3), 311–319.

**Streszczenie:** Ocena produktywności importowanych świń hybrydowych landrace × yorkshire × duroc. Celem przeprowadzonych badań było określenie przebiegu tuczu i wartości rzeźnej importowanych świń mieszańców L × Y × D. Stosując ujednoczone żywienie i utrzymanie, świnię tuczono od masy 30 do 115–120 kg. Dla 4433 zwierząt, które ukończyły tucz i były ubite, uzyskano bardzo dobre wskaźniki produkcyjne; średnie przyrosty dobowe – 989 g, wykorzystanie paszy – 2,88 kg/kg, mięsność – 58,86%, udział tusz w klasach S i E – łącznie 92,9%. Uzyskane wyniki wskazują na bardzo dobrą, a przede wszystkim powtarzalną jakość badanych tuczników hybrydowych w zakresie cech ważnych gospodarzo dla producentów i przemysłu mięsnego. Dowodzą też wysokiego i ustabilizowanego potencjału genetycznego czystorasowych populacji zwierząt używanych w 3. rasowym krzyżowaniu towarowym, służącym produkcji prosiąt do tuczu.

*MS. received in November 2014*

**Author's address:**

Anna Rekiel  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Trzody Chlewnej  
ul. Ciszewskiego 8, 02-786 Warszawa  
Poland  
e-mail: anna\_rekiel@sggw.pl



## Effect of rearing system on the microbiological quality of Pekin P44 and Muscovy MR71 ducks bowel

KRZYSZTOF DAMAZIAK<sup>1</sup>, MONIKA MICHALCZUK<sup>1</sup>, ANNA KUREK<sup>2</sup>

<sup>1</sup>Department of Poultry Breeding, <sup>2</sup>Department of Horses Breeding

Warsaw University of Live Science – SGGW

**Abstract:** *Effect of rearing system on the microbiological quality of Pekin P44 and Muscovy MR71 ducks bowel.* In this study, we analyzed the effect of rearing system of ducks on the quantitative and qualitative composition of their gut microflora. 180 ducks and 180 drakes of Pekin 44 and Muscovy MR71 lines were kept in the intensive system on litter; the same number of birds was kept in the semi-intensive system. In the latter, starting from the 3rd week of life the birds were allowed to use free ranges. After slaughter of 15 ducks and 15 drakes from each group, their intestinal digesta was subjected to microbiological analysis. The possibility of using the free ranges had a positive effect on the quantitative and qualitative composition of intestinal microflora of these birds. The count of aerobic mesophilic heterotrophs in the intestinal digesta of ducks was higher in the case of birds kept in the semi-intensive system. More beneficial results were achieved in the case of Muscovy ducks considering mainly the coli/lacto ratio which was the lowest (0.08) in this group for both sexes kept on free range. Noteworthy is also that no pathogenic bacteria of the genera: *Salmonella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Clostridium perfringens* and *Escherichia coli* were found in any of the analyzed samples.

*Key words:* Pekin ducks, Muscovy ducks, bowel, microbiological quality, rearing system

### INTRODUCTION

Owing to the ban on the use of antibiotic growth stimulants in poultry feeding imposed on the 1 January 2006 in the EU

Member States, the maintenance of the appropriate microbiological medium of the gastrointestinal tract is a prerequisite of the production cycle. Intestinal bacteria have a great impact on effective utilization of nutrients, functions of the immune system and, thereby, on the development of the whole body of birds (Kohl 2012). Irrespective of species, it is assumed that chicks hatch with sterile gastrointestinal tract that is next colonized by microorganisms inhabiting their environment. According to Cook et al. (2005), Peralta-Sa´nchez et al. (2010) and Ruiz-De-Castan˜eda et al. (2011), under natural conditions a great role is ascribed in this process to hatching behavior of parents and their microbiological status. In the production process, however, the role of adult birds was limited only to the production of hatching eggs. As a consequence, the microbiological status of chicks is influenced mainly by conditions occurring in the hatching incubator and in the rearing facility, meaning indirectly by man's actions.

Development of methods for the intensive production of poultry, with

the main assumption of as maximal as possible isolation of the area the birds are kept at (constant keeping the birds indoors) from impacts of the external environment as well as dissemination of the so-called principles of bio-protection, have in part created the possibility of controlling the development of the appropriate microflora in the gastrointestinal tract of poultry. Today, production of this type is most frequently condemned by animal rights defenders and consumers as it diminishes the welfare of birds (Fanatico et al. 2008) and deteriorates the quality of finished products (Berri et al. 2005). The rearing of birds in semi-intensive, ecological systems which allow the birds to use free ranges for at least 1/3 of their life span (Council Directive No 2092/91/EEC on 24 June 1991/Rozporządzenie Rady nr 2092/91/EWG z dnia 24 czerwca 1991 roku) is becoming an alternative to this system. A question then arises whether birds produced via artificial incubation and kept indoors at least in the initial period of life are capable to cope with and yield effective production results when allowed to use free ranges. It needs to be emphasized that even upon the greatest caution of man, it is impossible to isolate slaughter poultry from wild fowl and to control microclimatic conditions, as well as to avoid changes in the total administered feed as affected by ingestion of fresh vegetation by birds at the pasture.

This study was aimed at analyzing the effect of rearing system (intensive – IS, semi-intensive – OS) on the microbiological quality of bowels of two types of functional ducks in Poland: Pekin P44 and Muscovy MR71.

## MATERIAL AND METHODS

Experimental procedures were approved by the Ethical Commission (approval no. 27/2009 of 16 April 2009).

The study was conducted with Muscovy ducks (Grimaud, Rossy, France) of R71 line (MR71) (Grimaud Frères 2012) and Polish Pekin ducks of P44 line (P44) (Kokoszyński et al. 2010). Till the 3<sup>rd</sup> week of life, 360 ducks from each group (MR71: 180 ♂♂ and 180 ♀♀; P44: 180 ♂♂ and 180 ♀♀) were kept in the intensive system on litter in the same building (2.9 bird/m<sup>2</sup>), afterwards half of ducks and drakes randomly selected from each group were allowed to use free ranges (0.08 bird/m<sup>2</sup>) – Figure 1. Both sexes of P44 ducks were reared till the 7<sup>th</sup> week of life, whereas MR71 ducks till the 10<sup>th</sup> and MR71 drakes till the 12<sup>th</sup> week of life, however chick inclusions were made at various terms in order to synchronize slaughter time. Detailed data related to the characteristics of free ranges, weather conditions occurring when the birds were using free ranges, nutritive values of feed mixtures and basic production results including: growth, feed conversion ratio and mortality, were published in our earlier work (Damaziak et al. 2014).

Muscovy MR71 M (n=180) 2.9 bird/m <sup>2</sup> Outdoor system	Muscovy MR71 M (n=180) 2.9 bird/m <sup>2</sup> Intensive system	Muscovy MR71 F (n=180) 2.9 bird/m <sup>2</sup> Outdoor system	Muscovy MR71 F (n=180) 2.9 bird/m <sup>2</sup> Intensive system	Pekin P44 M (n=180) 2.9 bird/m <sup>2</sup> Outdoor system	Pekin P44 M (n=180) 2.9 bird/m <sup>2</sup> Intensive system	Pekin P44 F (n=180) 2.9 bird/m <sup>2</sup> Outdoor system	Pekin P44 F (n=180) 2.9 bird/m <sup>2</sup> Intensive system
Ducks-run 0.08 bird/m <sup>2</sup>		Ducks-run 0.08 bird/m <sup>2</sup>		Ducks-run 0.08 bird/m <sup>2</sup>		Ducks-run 0.08 bird/m <sup>2</sup>	

FIGURE 1. Experimental scheme showing the location of pens (—) and runs (.....) for experimental birds

After slaughter, samples of small intestine digesta were collected from 15 ducks and 15 drakes from each groups (in total: 120 birds) for microbiological analyses. Determinations were carried out for: ammonifying heterotrophic bacteria (nutrient agent without/with the addition of 5% defibrinated ram blood at 37°C); bacteria (lac<sup>+</sup> and lac<sup>-</sup>) of the family Enterobacteriaceae (culture medium according to McConkey' (at 37°C); bacteria of the genus *Pseudomonas* – culture medium according to King B (28°C); microscopic filamentous fungi and yeast – culture medium according to Martin and Sabouraud with the addition of streptomycin (28°C); lactic fermentation bacilli of the family *Lactobacillus* – solid culture medium according to Eijkman with glucose, lactose and bromocresole purple and Sabouraud's medium (without antibiotics) (37°C); and sulfate-reducing *Clostridium perfringens* bacteria – liquid medium and solid medium according to Wilson-Blair (37°C). The count of bacteria was determined with the plate method and test tube method (MPN – most probable number).

Microorganisms were identified based on macroscopic observations of liquid and solid cultures and microscopic preparations of life bacteria in a flat droplet, stained with the Gram's method and with acridine orange.

The statistical analysis of results was conducted using statistical package SPSS 21.0 (IBM SPSS 2012). Normality of variables distribution was checked with Kolmogorow-Smirnow test. The contribution of ammonifying heterotrophs and Enterobacteriaceae family bacteria (lac<sup>+</sup>) showed normal distribution after logarithmic transformation (decimal logarithm from the variable + 1). The Lac – variable did not show normal distribution, even after transformation, hence non-parametric Mann-Whitney test was used in its case. The effect of factors on other parameters, which showed normal distribution, was estimated with analysis of variance (GLM procedure). The model of variance analyses for variables possessing normal distribution was as follows:

$$Y_{ijmk} = \mu + P_i + U_j + R_m + (PU)ij + (PR)im + (UR)jm + (PUR)ijm + e_{ijmk}$$

where:

- $Y_{ijmk}$  – trait;  
 $\mu$  – general mean;  
 $P_i$  – effect of  $i$ -th sex,  $i = 1, 2$ ;  
 $U_j$  – effect of  $j$ -th housing system;  
 $R_m$  – effect of  $m$ -th genotype,  $m = 1, 2$ ;  
 $(PU)ij$  – effect of interaction between sex and housing system;  
 $(PR)im$  – effect of interaction between sex and genotype;  
 $(UR)jm$  – effect of interaction between housing system and genotype;  
 $(PUR)ijm$  – effect of interaction between sex, housing system and genotype;  
 $e_{ijmk}$  – random error.

## RESULTS AND DISCUSSION

Based on study results presented in Table 1, it was stated that the count of aerobic mesophilic heterotrophic bacteria in small intestinal digesta of ducks was significantly ( $P \leq 0.01$ ) higher in the case of birds kept in the OS system, compared to the IS system. This was, probably, linked with the possibility of ingesting green vegetation by birds. Gajewska et al. (2009) demonstrated earlier the feeding chickens mixtures with the addition of plant preparations containing mixtures of various herbs significantly increased the count of aerobic microorganisms. In addition, a reduction was observed in the count of bacteria of the

family Enterobacteriaceae, grown on McConkey's medium, in both types of ducks produced in the OS system (Table 1). The use of Sabouraud's medium and Eijkman's medium for the culture of lactic fermentation bacteria did not cause any significant differences in bacterial counts between ducks kept in different rearing systems. A higher count of *Lactobacillus* bacteria was determined only in the case of MR71 ducks reared in the OS system, but the significance was confirmed at  $P \leq 0.05$  (Table 1). It is known that the basic microorganisms for birds are facultative and strict anaerobic bifidobacteria, *Lactobacillus* and lactate-fermentation bacteria, and *Bacteroides* (Yaghobfar et al. 2006).

No pathogenic bacteria of the genera: *Salmonella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Clostridium perfringens* and *Escherichia coli* were found in any of the analyzed samples. No filamentous fungi and yeast were either detected with the use of Martin's medium, irrespective of the rearing system, origin and sex of birds (Table 1). According to Ziółkowska and Tokarzewski (2007), an increase of infection rate with *Salmonella* spp., *Pasteurella* spp., *Campylobacter* spp. and *Listeria* spp., was observed in geese as a result of progressing intensification of the production process and increasing stock density of birds. The lack of these microorganisms in digesta of ducks in this study could be due to rearing conditions (OS groups), as well as

TABLE 1. Total count of aerobic heterotrophs, filamentous fungi and yeast (LSM; SEM) in digesta of small intestines of Pekin P44 and Muscovy MR71 ducks (1 g d.m.), reared on litter (IS) and on free ranges (OS)

Housing system	Geno-type	Sex	Sabouraud <i>Lactobacillus</i>	Eijkman <i>Lactobacillus</i>	Nutrient agar <sup>a</sup> Endo_lac+/ /lac-	McConkey Enterobacteria- ceae	Martin <sup>b</sup> fungi and yeast
OS	P44	M	$2.48 \times 10^5$	$1.11 \times 10^5$	$3.09 \times 10^7$	$3.21 \times 10^4$	$<1.0 \times 10^2$
		F	$3.17 \times 10^5$	$2.00 \times 10^5$	$3.27 \times 10^7$	$3.00 \times 10^4$	$<1.0 \times 10^2$
	MR71	M	$2.33 \times 10^5$	$1.02 \times 10^5$	$0.12 \times 10^7$	$5.51 \times 10^3$	$<1.0 \times 10^2$
		F	$3.01 \times 10^5$	$1.00 \times 10^5$	$2.21 \times 10^7$	$4.89 \times 10^3$	$<1.0 \times 10^2$
IS	P44	M	$2.20 \times 10^5$	$1.87 \times 10^4$	$2.25 \times 10^6$	$2.52 \times 10^5$	$<1.0 \times 10^2$
		F	$0.66 \times 10^5$	$0.36 \times 10^4$	$6.67 \times 10^5$	$2.40 \times 10^5$	$<1.0 \times 10^2$
	MR71	M	$2.05 \times 10^3$	$4.3 \times 10^5$	$3.34 \times 10^6$	$1.93 \times 10^5$	$<1.0 \times 10^2$
		F	$1.19 \times 10^4$	$3.9 \times 10^5$	$3.23 \times 10^6$	$2.20 \times 10^5$	$<1.0 \times 10^2$
SEM			0.20	0.05	0.49	0.75	0.00
Main effects							
housing system			*	NS	**	**	NS
genotype			*	*	**	**	NS
sex			*	NS	NS	*	NS
housing system × × genotype			NS	*	**	**	NS
housing system × × sex			*	NS	**	**	NS
genotype × sex			NS	NS	**	**	NS
housing system × × genotype × sex			NS	NS	**	**	NS

<sup>a</sup> Nutrient agar with the addition of ram blood; <sup>b</sup> filamentous fungi and yeast; NS:  $P > 0.05$ , \* $P \leq 0.05$ , \*\* $P \leq 0.01$ ; SEM – standard error of the mean.

to low stock density and relatively low number of birds in experimental flocks.

The housing system was observed to have a significant effect on changes in the coli/lacto ratio computed based on the ratio of total count of Enterobacteriaceae family bacteria to the count of LAF bacteria (Table 2). In the case of both sexes of MR71 ducks, a more favorable, lower (ca. 8 times lower) value of the ratio was noted for ducks from the OS system, compared to birds produced

in the IS system. In the case of P44 ducks, values of the coli/lacto ratio did not differ significantly between birds reared in the IS and OS systems. Similar values were noted for birds of both sexes. Probably, the lowest values of the coli/lacto ratio determined for MR71 ducks produced in the OS system are linked with longer rearing period of these birds and, thereby, with longer period when the birds could use free ranges, compared to P44 birds.

TABLE 2. Values of coli/lacto ratio determined based on counts of Enterobacteriaceae family bacteria and LAF bacteria (LSM; SEM), present in digesta of small intestines of Pekin 44 and MR71 Muscovy ducks, reared on litter (IS) and on free range (OS)

Housing system	Genotype	Sex	Endo_lac+	Endo_lac-	Lactobacillaceae	Coli/lacto
OS	P44	M	$5.51 \times 10^6$	$0.01 \times 10^5$	$2.30 \times 10^6$	0.52
		F	$1.40 \times 10^7$	$0.11 \times 10^6$	$3.12 \times 10^7$	0.65
	MR71	M	$2.15 \times 10^6$	$0.00 \times 10^5$	$2.30 \times 10^6$	0.08
		F	$2.20 \times 10^6$	$0.00 \times 10^5$	$2.79 \times 10^6$	0.08
IS	P44	M	$9.66 \times 10^6$	$0.01 \times 10^5$	$2.12 \times 10^7$	0.66
		F	$3.36 \times 10^6$	$0.01 \times 10^5$	$0.57 \times 10^6$	0.74
	MR71	M	$2.70 \times 10^6$	$0.31 \times 10^5$	$1.84 \times 10^7$	0.59
		F	$6.64 \times 10^6$	$0.24 \times 10^6$	$1.16 \times 10^7$	0.54
SEM			0.17	0.00	0.58	0.05
Main effects						
housing system			**	**	*	**
genotype			**	NS	NS	*
sex			NS	NS	NS	NS
housing system $\times$ genotype			**	**	NS	**
housing system $\times$ sex			**	**	*	**
genotype $\times$ sex			**	*	NS	**
housing system $\times$ genotype $\times$ sex			**	NS	NS	NS

NS:  $P > 0.05$ , \* $P \leq 0.05$ , \*\* $P \leq 0.01$ ; SEM – standard error of the mean.

The housing system was observed to have a significant effect on changes in the coli/lacto ratio computed based on the ratio of total count of Enterobacteriaceae family bacteria to the count of LAF bacteria (Table 2). In the case of both sexes of MR71 ducks, a more favorable, lower (ca. 8 times lower) value of the ratio was noted for ducks from the OS system, compared to birds produced in the IS system. In the case of P44 ducks, values of the coli/lacto ratio did not differ significantly between birds reared in the IS and OS systems. Similar values were noted for birds of both sexes. Prob-

ably, the lowest values of the coli/lacto ratio determined for MR71 ducks produced in the OS system are linked with longer rearing period of these birds and, thereby, with longer period when the birds could use free ranges, compared to P44 birds.

In summary, the results obtained indicate that allowing ducks to use free ranges had a positive effect on the qualitative and quantitative composition of their gut microflora. Of the two genetic groups of ducks (P44 and Muscovy), better microbiological parameters were noted in the case of MR71 OS group,

which could be due to the longer period of rearing and using free ranges. In the future, results of microbiological assays of intestinal digesta should be confronted with results of parasitological analyses, since the risk posed by internal parasites is one of the major causes postulating for reduced application of the semi-intensive methods of poultry rearing.

## REFERENCES

- BERRI C., Le BIHAN-DUVAL E., BAËZA E., CHARTRIN P., PICGIRARD L., JEHL N., QUENTIN M., PICCARD M., DUCLOS J., 2005: Further processing characteristics of breast and leg meat from fast-, medium- and slow-growing commercial chickens. *Anim. Res.* 54, 123–134.
- COOK M.I., BEISSINGER S.R., TORANZOS G.A., ARENDT W.J., 2005: Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection. *Ecol. Lett.* 8, 532–537.
- DAMAZIAK K., MICHALCZUK M., ADAMEK D., CZAPLIŃSKI M., NIEMIEC J., GORYL A., PIETRZAK D., 2014: Influence of housing system on the growth and histological structure of duck muscle. *SAJAS* 44, 97–109.
- FANATICO A.C., PILLAI P.B., HESTER P.Y., FALCONE C., MECH J.A., OWENS C.M., EMMERT J.L., 2008: Performance, livability, and carcass yield of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. *Poult. Sci.* 87, 1012–1021.
- GAJEWSKA J., BUCKA J., ŻABIK A., RIEDEL J., MICHALCZUK M., 2009: Wpływ naturalnych preparatów roślinnych na stan mikroflory jelitowej brojlerów kurzych. *Ochrona Środowiska i Zasobów Naturalnych* 41, 302–309.
- Grimaud Frères, 2012: <http://www.grimaud.com>. Accessed March 2013.
- IBM Corp. Released 2012: IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY.
- KOHL K.D., 2012: Diversity and function of the avian gut microbiota. *J. Comp. Physiol. B.* 182, 591–602.
- KOKOSZYŃSKI D., KORYTKOWSKA H., KORYTKOWSKI B., 2010: Comparison of some meat traits of ducks from P44 and P55 flocks. *Acta Sci. Pol. Zootech.* 9, 21–28.
- PERALTA-SANCHEZ J.M., MØLLER A.P., MARTIN-PLATERO A.M., SOLER J.J., 2010: Number and colour composition of nest lining feathers predict eggshell bacterial community in barn swallow nests: an experimental study. *Funt. Ecol.* 24, 426–433.
- Rozporządzenie Rady nr 2092/91/EWG z dnia 24 czerwca 1991 roku w sprawie produkcji ekologicznej produktów rolnych oraz znakowania produktów rolnych i środków spożywczych (Dz.U. L 198, 22.7.1991).
- RUIZ-De-CASTANEDA R., VELA A.I., LOBATO E., BRIONES V., MORENO J., 2011: Bacterial loads on eggshells of the pied flycatcher: environmental and maternal factors. *Condor* 113, 200–208.
- YAGHOBFAR A., REZAIAN M., ASHRAFI-HELAN M., BARIN J., FAZAELI H., SAYED DAVOD S.H., 2006: The effect of hull-less barley dietary on the activity of gut microflora and morphology small intestinal of layer hens. *Pak. J. Biol. Sci.* 9, 659–666.
- ZIÓLKOWSKA G., TOKARZEWSKI S., 2007: Occurrence of moulds in reproductive goose flocks in southern-eastern Poland. *Bull. Vet. Inst. Puławy* 51, 553–561.

**Streszczenie:** *Wpływ systemu utrzymania na jakość mikrobiologiczną jelit kaczek Pekin P44 i piżmowych MR71.* W badaniach analizowano wpływ systemu utrzymania kaczek na ilościowy i jakościowy skład mikroflory jelitowej. Po 180 kaczek i 180 kaczorów Pekin P44 i piżmowych MR71 utrzymywano w systemie intensywnym na ściółce i taką samą ilość ptaków w systemie półintensywnym. W systemie półintensywnym ptaki od 3. tygodnia życia mogły korzystać z ograniczonych wybiegów. Po uboju 15 kaczek i 15 kaczorów z każdej grupy wykonano analizę mikrobiologiczną treści ich jelit cienkich. Możliwość korzystania z wybiegów miało pozytywny wpływ na jakościowy i ilościowy skład ich mikroflory jelitowej. Liczebność tlenowych mezofilnych bakterii heterotroficznych, w treści jelita

cienkiego kaczek, była wyższa dla ptaków utrzymywanych w systemie półintensywnym. Korzystniejsze wyniki stwierdzono w przypadku kaczek piźmowych, głównie odnośnie współczynnika coli/lacto, który był najniższy (0,08) u tej grupy dla obu płci utrzymywanych na wybiegu. Na szczególną uwagę zasługuje również fakt, iż nie stwierdzono obecności bakterii chorobotwórczych z rodzaju *Salmonella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Clostridium perfringens* i *Escherichia coli* w żadnej z badanych prób.

*MS. received in November 2014*

**Authors' address:**

Monika Michalczuk  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Drobiu  
ul. Ciszewskiego 8, 02-786 Warszawa  
Poland  
e-mail: monika\_michalczuk@sggw.pl

## Effect of birth body weight of piglets on their rearing up to the age of 10 weeks

BARBARA KRÓLEWSKA, ANNA REKIEL, JUSTYNA WIĘCEK  
Department of Pigs Breeding, Warsaw University of Life Sciences – SGGW

**Abstract:** *The effect of birth body weight of piglets on their rearing up to the age of 10 weeks.* The aim of the paper was to evaluate the effect of the birth body weight of the piglets on their productivity and survivability up to the age of 10 weeks. The observations covered the piglets born by 11 sows F1 of Polish Large White × Polish Landrace, inseminated by the semen of the boras (Duroc × Pietrain). The piglets were weighed on 1st, 21st, 35th and 70th day of life and the feed intake (per litter) was controlled. Depending on their birth body weight, the piglets were classified into two groups: light (L) <1.5 kg (n = 52), heavy (H) ≥1.5 kg (n = 60). The mean general body weight of the piglets on 1st, 21st, 35th and 70th day was equal to 1.45, 5.09, 7.70 and 14.18 kg, respectively. In the groups, it was as follows: L – 1.17, 4.58, 6.91 and 13.00 kg; H – 1.70, 5.51, 8.29 and 15.12 kg, respectively (L–H, P ≤ 0.001). The deaths of the piglets in groups L and H amounted to 15.4 and 6.7%, respectively. In the situation of a free access of the progeny to mother's feed and solid feedstuff, the obtained results indicate that the worse productivity, as expressed by lower feed conversion (by 10.5–16%), slower growth rate (by 12–20%) and lowered survivability (by 8.7 percentage points) of the piglets from groups L vs. group H, were the effect of considerably lower body weight of the newborn piglets as compared to their mean body weight.

*Key words:* piglets, body weight, growth rate, survivability

### INTRODUCTION

In spite of the improvement of management techniques and intensive studies concerning nutritional requirements of

mammals during the recent half of the century, low body weight still becomes a serious problem in animal production and the knowledge on the effect of nutrition on mechanisms, regulating the growth of foetus, has become incomplete (Wu et al. 2006, Rekiel et al. 2014a).

The growth and development of foetuses is affected by genetic and environment (Redmer et al. 2004). Due to incidence of IUGR (intrauterine growth retardation), we state the occurrence of considerable losses of foetuses in farm animals, in the initial as well as in the middle and final period of gestation (Wu et al. 2006). The environmental factors, favourable for IUGR and low body weight include; nutrition of mother (low or high consumption of feed and lack of balance of nutrients). The effect is also affected by temperature of environment and stress, and bad management conditions (Redmer et al. 2004, Wu et al. 2004a).

Insufficient volume of uterus and inadequate (compared to the requirements) nutrition are the main reasons for retardation of foetus growth and low body weight (Redmer et al. 2004). The environment of uterus may affect the size of foetuses; it was revealed in

various pig breeds. The available data indicate that the prenatal growth of all higher mammals (placenta mammals) is dependent on the direct and indirect effect of mother's nutrition and uterus volume (Robinson et al. 1999, Allen et al. 2002, cited by Rekiel and Królewska 2014, Ferguson 2005).

As compared to the offspring with a high or normal birth body weight, the newborns with light body weight, i.e. lambs, piglets or foals often die, and they need more time as to adapt themselves to postnatal life. At the moment of birth, the heavier young animals are more vital and they adapt more quickly to extrauterine environment (Wu et al. 2004a, b). From among domestic animals, most frequently in the case of pigs, light body weight and intrauterine growth retardation (IUGR) occur naturally what is connected with the high fertility of the mentioned species. In the case of the piglets with light body weight and IUGR, we find structural and functional changes, including the changed structure of the whole body, muscles and types of muscle fibres, higher content of intra-muscular fat, less skeletal muscles, weakened growth of the whole body since the birth until slaughter, weaker feed conversion as compared to the piglets with high body weight at birth, worse quality of slaughter raw material and pork meat (Gondret et al. 2005, Karunaratne et al. 2005, Rehfeldt et al. 2008, Rekiel et al. 2013a, b, Rekiel et al. 2014a, b).

The aim of the work was to examine the effect of the body weight of the piglets at birth on their rate of growth, feed conversion and survivability until 70th day of life.

## MATERIAL AND METHODS

The piglets for the studies derived from eleven sows F1 of Polish Large White × Polish Landrace, inseminated with the semen of crossbred boar Duroc × Pietrain. The pregnant females (after 3 week of gestation) were kept in group pen and one week before the parturition, they were transferred to parturition pens; they stayed there until weaning of the piglets (5 weeks). The study covered 112 piglets from birth until 70th day of life.

The pregnant and suckling sows were individually fed the mixture of barley and wheat meal and protein concentrate (Table 1). The participation of cereal

TABLE 1. Energy content and feeding value of concentrate for sows

Specification	Concentrate
Metabolic energy (MJ)	11.8
Crude protein (%)	32
Crude fat (%)	2.60
Crude ash (%)	16.50
Phosphorus (%)	1.20
Lysine (%)	2.94
Crude fibre (%)	5.50
Vitamin A (i.u./kg)	71 500
Vitamin D <sub>3</sub> (i.u./kg)	14 300
Vitamin E (i.u./kg)	371
6-Phytase EC (FTU/kg)	4 290
Copper (mg/kg)	57.2

meals and concentrate was as follows: mixture LP – 82.5 and 17.5% and LK 80 and 20%, respectively. In low and high gestation, the rate of 2.4 kg/animal/day and 3.0 kg/animal/day was employed. During lactation period, the rate was dependent on the number of fed piglets (Standards of Pig Nutrition 1993). The piglets were additionally fed the mixture of prestarter type and after weaning – with starter mixture (Table 2).

TABLE 2. Energy content and feeding value of mixture for piglets

Specification	Prestarter	Starter
Metabolic energy (MJ)	13.6	13.4
Crude protein (%)	18.00	17.00
Crude fat (%)	5.10	3.00
Crude ash (%)	5.20	4.50
Phosphorus (%)	0.53	0.35
Lysine (%)	1.13	1.11
Crude fibre (%)	3.60	3.80
Vitamin A (i.u./kg)	12 000	12 000
Vitamin D <sub>3</sub> (i.u./kg)	2 000	2 000
Vitamin E (i.u./kg)	100	50
Phytase (FYT/kg)	603	750
Copper (mg/kg)	160	15

The newborn piglets were subject to basic zootechnical treatment; they were marked at birth; they received iron preparation (Ferrovet) on 3rd day of life; they received ear rings on 21st day. The body weight of the piglets, their deaths and feed intake by the litter were controlled on 1st, 21st, 35th and 70th day of life. After 35 days of rearing, the piglets were transferred to pens without the change of the structure of the group and they were reared up to the age of 10

weeks. The analysis of the rearing results was conducted after classification of the piglets into two groups, differing in body weight; light piglets (L) < 1.5 kg BW, n = 52 (mean BW in the group was 1.17 kg; the number of boar piglets 29 heads; gilts 23 heads); heavy piglets (H) > 1.50 kg BW, n = 60 (mean BW in group 1.70 kg; boar piglets 30 heads, gilts 30 heads) (L–H, P ≤ 0.001).

The results have been statistically tested using SPSS Statistics 21 software. Normality of results has been checked using the Shapiro-Wilk test. The differences between the groups have been analysed using the t-Student test (for traits with normal distribution, i.e. body weight on 21st and 35th day of life) or the U Mann-Whitney test (for all other traits).

## RESULTS AND DISCUSSION

The mean body weight of all examined piglets was average, typical of the species and it amounted to 1.45 kg at birth. The number of the piglets born in the litter was equal in average to 10 animals (8–14 piglets/litter), sex ratio – male : female equals 1.1 : 1), deviating from the standard equal to 1 : 1 but it was confirmed at various animal species (Rekiel et al. 2010).

The piglets from groups L and H revealed the increase of their body weight during the successive weightings (Table 3). Body weight of the piglets at birth in group L vs. group H was lower; it amounted to 68.8% of BW in group C

TABLE 3. Body weight of light (L) and heavy (H) piglets during rearing period

Item	Group L < 1.5 kg	Group H ≥ 1.5 kg	P
Body weight at birth (kg)	1.17	1.70	0.001
Body weight on 21st day of life (kg)	4.58	5.51	0.001
Body weight on 35th day of life (kg)	6.93	8.30	0.001
Body weight on 70th day of life (kg)	13.00	15.11	0.001

and on 70th day of rearing it was equal to 86.7%. The resulting change may indicate the compensation of growth in the piglets of group L. In the opinion of the researchers, it is possible (Rehfeldt et al. 2011, 2012a, 2012b, cited by Rekiel et al. 2014a); in the specified case, it has a limited extent. It seems, however, that in the own studies the discussed difference could result in a greater degree from the fact of the increased deaths of the piglets to be weaned in group L as compared to group H. The losses concerned very light piglets: in group L, 8 piglets died and their mean birth weight was equal to 0.92 kg; in group H, 4 piglets died and their mean birth body weight amounted to 1.70 kg. The differences between the groups of the piglets L and H were also characterized by body weight of the heaviest individuals on 70th day of life: in groups L and H, it was 16.85 and 20.15 kg, respectively (difference of 3.30 kg in favour of group H vs. group L, i.e. 19.58%). The progress of body weight of the piglets was lower

until the moment of weaning in group L vs. group H. The changes in the groups were as follows: 1–21 day – 3.41 and 3.81 kg, respectively; 21–35 day – 2.33 and 2.78 kg, 35–70 day – 6.09 and 6.03 kg, respectively; the difference in the further successive periods in group H vs. group L was: +11.73, +19.31 and –0.99%. The recent result confirms the mentioned earlier compensation of growth. Lack of capacity of increasing the protein synthesis in tissues explains the incomplete compensation of growth during the postnatal period. The longer is the period of intrauterine limitation of nutrients, the lower are the capabilities of the IUGR pigs to restore their correct condition (Rehfeldt et al. 2011, 2012a, 2012b, cited by Rekiel et al. 2014a).

In the group of the piglets L since birth until 21st day of rearing, 6 piglets died what constituted 75% of losses; the remaining 25% occurred during the period after weaning. In the group of the piglets H, also 75% of deaths had place up to 21st day, but there were only 3 animals; the successive 25% concerned the period before weaning. The level of losses until 10th week of life was higher in group L vs. group H and it amounted to 15.4 and 6.7%, respectively Herpin et al. (2002) and Wolter et al. (2002), cited by Rekiel et al. (2013b), Gondret et al. (2005), Rehfeldt et al. (2008) state that a low birth body weight is relating to the lowered survivability of the piglets. In the groups of the piglets L, the deaths after weaning could be caused by weaning

stress and weaker development of internal organs, *inter alia*, of gastrointestinal system (Wang et al. 2005) in 2 animals, the birth body weight of which was low and amounted to 0.99 and 1.09 kg, respectively. During the whole rearing period, the mortality of boar piglets was higher than that of gilts; in total, 7 males and 5 females died (deaths in group L – 4/4, in group H – 3/1). In the studies of Bocian et al. (2011), the mortality of the piglets was the highest up to 21st day and concerned mainly the individuals, the birth body weight of which was lower than 1.2 kg. The losses in the discussed group were equal to 24.49%; on the other hand, among the piglets with body weight of 1.2–1.6 kg, it amounted to 6.78%. Bocian et al. (2011) recorded the lowest mortality among the piglets weighing at birth beyond 1.6 kg (only 4%). Dysfunctions of intestines and respiratory system are the main factors, predisposing the newborns to higher

mortality rate before weaning (Wu et al. 2004b).

The higher level of losses and slower growth rate of the piglets lighter at birth and the disorders in postnatal development of muscle fibres and skeletal muscles are explained by the results of the studies and monographic descriptions, published in scientific foreign and national periodicals (Wu et al. 2004b, Rekiel et al. 2013a, 2013b, Rekiel et al. 2014c). Small intestine plays an important role in final digestion and absorption of nutrients and postnatal growth of animals (Wang et al. 2005). Naturally occurring or experimentally induced IUGR is related with the incorrect morphology of alimentary tract and stomach-intestinal disturbances what contributes to decrease of utilization of nutrients coming from feed (Wu et al. 2004a). The mean intake and conversion of feed has been given in Table 4. The conversion of feed, as expressed by the degree of changes

TABLE 4. Feed intake and conversion in the successive periods of rearing the piglets

Period of rearing (days)	Mean feed intake by the litter (kg)	Mean feed intake by one piglet (kg)	Piglets light at birth – 1.17 kg (L)	Piglets heavy at birth – 1.70 kg (H)	Ratio of changes in feed conversion by the piglets, H vs. L (%)
			Feed conversion (kg/kg of body weight gain of one piglet)		
Since 1st up to 21st day	2.56	0.25	0.074	0.066	–10.55
Since 21st up to 35th day	6.75	0.72	0.309	0.259	–16.16
Since 35th up to 70th day	6.30	0.69	0.114	0.102	–10.81
Since 1st up to 35th day	9.31	1.00	0.173	0.151	–12.87
Since 1st up to 70th day	15.62	0.25	0.145	0.128	–11.72

in group H vs. group L occurred to be better by ca. 10.5–16%. It had also the influence on growth rate which was revealed to be higher in the case of the piglets heavier at birth as compared to the lighter animals. The ratio of the changes depending on the rearing period was equal to ca. 12–20% (Table 5). The highest body weight gains were reached by piglets H vs. L since 21st up to 35th day of life; the difference amounted to 33 g.

body weight of the newborn animals, the indicators of growth in the postnatal period until reaching the slaughter weight, are lowered.

The conducted own studies as well as the selected examples of the studies carried out in various scientific centres by different research teams (Wallace et al. 2005, Wu et al. 2006, Rehfeldt et al. 2008, Bocian et al. 2011, Van Vliet et al. 2013) indicate the meaningful effect of

TABLE 5. Growth rate of the piglets

Period of rearing (days)	Piglets light (L) at birth with mean body weight of 1.17 kg		Piglets heavy (H) at birth with mean body weight of 1.70 kg		Ratio of changes in daily body weight gain for piglets H vs. L (%)
	Mean body weight gain of piglet (kg)	Mean daily gain (g)	Mean body weight gain of piglet (kg)	Mean daily gain (g)	
Since 1st up to 21st day	3.41	162	3.81	181	11.73
Since 21st up to 35th day	2.33	166	2.78	199	19.88
Since 35th up to 70th day	6.09	174	6.83	195	12.07
Since 1st up to 35th day	5.74	164	6.59	188	14.63
Since 1st up to 70th day	11.83	169	13.42	192	13.61

Bocian et al. (2011) confirmed a significant effect of body weight at birth on the growth rate of the piglets during rearing period. The piglets with the lower body weight had slower weight gains during the discussed period and they also reached weaker fattening results. Gondret et al. (2005), Rehfeldt et al. (2008) and Herpin et al. (2002) and Wolter et al. (2002), cited by Rekiel et al. (2013b) state that in the case of low

the body weight of the newborn animals at birth on production results, including their rate of growth and survivability. The low birth body weight is connected with IUGR syndrome. IUGR occurs in economically important utility animals, including pigs (Rekiel and Królewska 2014). In spite of the improvement of management techniques and intensive studies in respect of the nutritional requirements of mammals, IUGR still re-

mains the important problem in breeding due to the incomplete knowledge on the effect of nutrition on mechanisms, regulating the foetuses' growth (Wu et al. 2004a, Murphy et al. 2006). Finding the solution for the discussed problem will have a meaningful influence on the improvement of the rearing results and by this, profitability of production.

## CONCLUSIONS

In the compared groups and periods, the average rate of growth of the examined piglets was found; it did not exceed 200 g. It was revealed that the piglets with the lower body weight at birth (group L) as compared to heavier piglets (group H) were characterized by slower growth rate ( $P \leq 0.001$ ). The differences in the gains between the groups during the research periods, i.e. since 1st up to 21st, 21st to 35th and since 35th up to 70th day of life amounted to 19 g (11.73%), 33 g (15.0%) and 21 g (12.07%), respectively. From birth until weaning, the difference was equal to 24 g (14.63%) and up to the age of 10 weeks – 23 g (13.61%). The mean feed intake by the litter amounted to 15.62 kg, including the period after weaning when it was only 6.30 kg what may be considered as the basic reason for weak gains of the piglets from the both groups for the period of 5 weeks after weaning. The ratio of the changes for feed conversion and growth rate of the piglets H vs. L indicates the better by 10.5–16% utilization of the mixtures and by 12–20%

better growth rate of the piglets H vs. L. The deaths in groups L and H amounted to 15.44 and 6.7%, respectively, what indicates the unfavourable effect of the lowered body weight at birth on survivability of the piglets.

## REFERENCES

- BOCIAN M., JANKOWIAK H., GRAJEWSKA S., KAPELAŃSKA J., WŁODARSKI W., 2011: Wpływ masy ciała prosiąt przy urodzeniu na efekty ich odchowu i wyniki tuczu. *Rocz. Nauk. Zoot.* 38 (2), 189–195.
- FERGUSON J.D., 2005: Nutrition and reproduction in dairy herds. *Vet. Clin. Food Anim.* 21, 325–347.
- GONDRET F., LEFAUCHEUR L., LOUVEAU I., LEBRET B., 2005: The long-term influence of birth weight on muscle characteristics and eating meat quality in pigs individually reared and fed during fattening. *Arch. Tierz. Dummerstorf.* 48, 68–73.
- KARUNARATNE J.F., ASHTON C.J., STICKLAND N.C., 2005: Fetal programming of fat and collagen in porcine skeletal muscles. *J. Anat.* 207, 763–768.
- MURPHY V.E., SMITH R., GILES W.B., CLIFTON V.L., 2006: Endocrine regulation of human fetal growth: The role of the mother, placenta, and fetus. *Endocr. Rev.* 27, 141–169.
- REDMER D.A., WALLACE J.M., REYNOLDS L.P., 2004: Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. *Domest. Anim. Endocrinol.* 27, 199–217.
- REHFELDT C., TUCHSCHERER A., HARTUNG M., KUHN G., 2008: A second look at the influence of birth weight on carcass and meat quality in pigs. *Meat Sci.* 78, 170–175.
- REKIEL A., BARTOSIK J., WIĘCEK J., BATORSKA M., KUCZYŃSKA B., ŁOJEK A., 2014b: Effect of piglet birth weight on selected characteristics of pork. *Ann. Anim. Sci.* 14 (4), 967–975.
- REKIELA., KRÓLEWSKA B., 2014: Wewnątrzmaciczne zahamowanie wzrostu u zwierząt – syndrom IUGR. *Przegl. Hod.* 5, 10–12.

- REKIEL A., WIĘCEK J., BATORSKA M., KULISIEWICZ J., 2013a: Wpływ masy ciała prosiąt przy urodzeniu na wartość rzeźną tuczników i jakość wieprzowiny. *Przegl. Hod.* 5, 23–25.
- REKIEL A., WIĘCEK J., BATORSKA M., KULISIEWICZ J., 2014a: Effect of sow prolificacy and nutrition on pre and postnatal growth of progeny – a review. *Ann. Anim. Sci.* 14 (1), 3–15.
- REKIEL A., WIĘCEK J., BATORSKA M., KULISIEWICZ J., 2014c: Effect of piglet birth weight on carcass muscle and fat content and pork quality – a review. *Ann. Anim. Sci.* (in press).
- REKIEL A., WIĘCEK J., KULISIEWICZ J., BATORSKA M., 2013b: Wybrane czynniki wpływające na masę ciała prosiąt przy urodzeniu i jej związek z cechami wzrostu młodych świń. *Przegl. Hod.* 4, 11–14.
- REKIEL A., WIĘCEK J., WOJTASIK M., KULISIEWICZ J., BATORSKA M., 2010: Środowisko wewnętrzne a reprodukcja u gatunków wielopłodowych. *Roczn. Nauk. Zoot. Monogr. i Rozpr.* 44, 79–88.
- ROBINSON J.J., SINCLAIR K.D., MCEVOY T.G., 1999: Nutritional effects on foetal growth. *Anim. Sci.* 68, 315–331.
- VLIET Van E., EIARCH E., ILLA M., ARBAT-PLANA A., GONZÁLEZ-TENDERO A., HOGBERG H.T., ZHAO L., HARTUNG T., GRATACOS E., 2013: Metabolomics Reveals Metabolic Alterations by Intrauterine Growth Restriction in the Fetal Rabbit Brain. *PLOSone*. DOI: 10.1371/journal.pone.0064545.
- WALLACE J., MATSUZAKI M., MILNE J., AITKEN R., 2005: The effect of maternal growth hormone treatment on fetal growth and adiposity in rapidly growing adolescent sheep. *Pediatr. Res.* 58, 1030.
- WANG T., HUO Y.J., SHI F.X., XU R.J., HUTZ R.J., 2005: Effects of intrauterine growth retardation on development of the gastrointestinal tract in neonatal pigs. *Biol. Neonate* 88, 66–72.
- WU G., BAZER F.W., CUDD T.A., MEININGER C.J., SPENCER T.E., 2004a: Maternal nutrition and fetal development. *J. Nutr.* 134, 2169–2172.
- WU G., BAZER F.W., WALLACE J.M., SPENCER T.E., 2006: Intrauterine growth retardation: Implications for the animal science. *J. Anim. Sci.* 84, 2316–2337.
- WU G., KNABE D.A., KIM S.W., 2004b: Arginine nutrition in neonatal pigs. *J. Nutr.* 134, 2783S–2790S.

**Streszczenie:** *Wpływ masy ciała prosiąt przy urodzeniu na ich odchów do wieku 10 tygodni.* Celem pracy była ocena wpływu masy ciała prosiąt przy urodzeniu na ich produkcyjność i przeżywalność do wieku 10 tygodni. Obserwacjami objęto prosięta urodzone przez 11 loch F1 rasy wielka biała polska × polska biała zwiśloucha inseminowanych nasieniem knurów (duroc × pietrain). Prosięta ważono w 1., 21., 35. i 70. dniu, kontrolowano pobranie paszy (na miot). Prosięta w zależności od masy ciała przy urodzeniu podzielono na grupy: lekkie (L) <1,5 kg (n = 52), ciężkie (H) ≥1,5 kg (n = 60). Średnia masa ciała wszystkich prosiąt w 1., 21., 35., i 70. dniu wyniosła: 1,45; 5,09; 7,70; 14,18 kg, a w grupach odpowiednio: L – 1,17; 4,58; 6,91; 13,00 kg oraz H – 1–70; 5–51; 8–29; 15–12 kg (L–H, P ≤ 0,001). Upadki prosiąt grup L i H wyniosły 15–4 i 6–7%. Przy swobodnym dostępie potomstwa do pokarmu matki i paszy stałej uzyskane wyniki wskazują, że gorsza produkcyjność wyrażona słabszym o 10,5–16% wykorzystaniem paszy, wolniejszym o 12–20% tempem wzrostu oraz obniżoną o 8,7 punktu procentowego przeżywalnością prosiąt z grupy L względem grupy H, były efektem znacząco mniejszej w stosunku do średniej masy ciała nowonarodzonych prosiąt.

*MS. received in November 2014*

**Author's address:**

Anna Rekiel  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Trzody Chlewnej  
ul. Ciszewskiego 8, 02-786 Warszawa  
Poland  
e-mail: anna\_rekiel@sggw.pl

## **Nanoparticles of copper and entomopathogenic nematodes *Steinernema feltiae* (Filipjev, 1934) in reducing the number of the lesser mealworm beetle *Alphitobius diaperinus* (Panzer, 1797)**

KORNELIA KUCHARSKA<sup>1</sup>, ELŻBIETA PEZOWICZ<sup>1</sup>, DOROTA TUMIALIS<sup>1</sup>,  
DARIUSZ KUCHARSKI<sup>2</sup>, BARBARA ZAJDEL<sup>3</sup>

<sup>1</sup>Department of Zoology, Warsaw University of Life Sciences – SGGW

<sup>2</sup>Department of Ecology, Warsaw University

<sup>3</sup>Bee Division, Warsaw University of Life Sciences – SGGW

**Abstract:** *Nanoparticles of copper and entomopathogenic nematodes *Steinernema feltiae* (Filipjev, 1934) in reducing the number of the lesser mealworm beetle *Alphitobius diaperinus* (Panzer, 1797).* Antibacterial properties of metal nanoparticles are well documented and known, but its potential use in agriculture as an anti pest agents – not. Possible negative or positive reactions with popular integrated pest management (IPM) methods need to be checked and verified. The effect of copper nanoparticles on the mortality of entomopathogenic nematodes (EPNs) *Steinernema feltiae* from Owinema biopreparation was tested. This biopreparation is being used against serious agricultural pests. It was found that mortality of nematodes depends on nano-Cu concentrations and on the length of contact of *Steinernema* larvae with nano-Cu solution. In this study the effect of different concentrations of nanoparticles on pathogenic properties of entomopathogenic nematodes – mortality of pest beetle *Alphitobius diaperinus* (Panzer, 1797) infected by EPNs and extensiveness of infection, was also studied. It showed that the high concentrations of Cu may decrease abilities of EPNs to enter, grow and proliferate inside the host body.

**Key words:** entomopathogenic nematodes, *Steinernema feltiae*, Owinema, *Alphitobius diaperinus*, lesser mealworm beetle, copper nanoparticles

## INTRODUCTION

Entomopathogenic nematodes (EPNs) are different nematode species, that during their development display pathogenicity against insects (Brzeski and Sandner 1974). Two nematode families (Steinernematidae and Heterorhabditidae) are being used as biological control agents on a commercial scale (Kowalska 2006). Invasive larvae of EPNs live together, in mutualistic relationship, with bacteria from the Enterobacteriaceae family. These microorganisms use nematodes as vectors, that inject them into the insect bodies. Finally, bacteria kill the new host (Martens et al. 2003). EPNs are used in poultry houses to control the lesser mealworm beetle *Alphitobius diaperinus* (Tenebrionidae). It is a pest of bred birds and a vector of many diseases, example Marek's, Gumboro and Newcastle diseases (Lancaster and Simco 1967, De la Casas et al. 1972, De la Casas et al. 1976, Geden et al. 1987,

Avancini and Ueta 1990, Despins et al. 1994, Goodwin and Waltman 1996, Steelman 1996, Pezowicz 2005, Ignatowicz 2008, Ignatowicz 2009, Chernaki-Leffer et al. 2010, Walldorf et al. 2012).

History of nanotechnology starts in 1950s. With use of nanoparticle materials it is possible to create, for example, new biopreparations with different biochemical properties. "Nano" scale in connection with huge reactive surface of nanoparticles causes that they are characterized by high biochemical reactivity, even at low concentrations (Myczko 2006). Nanocolloidal copper is being used in cosmetics, household, industry, medicine and in agriculture (www.nano-tech.pl).

## MATERIAL AND METHODS

The effect of copper nanoparticles (firm Nano-tech Polska sp. z o.o.) on the mortality and pathogenic properties of entomopathogenic nematodes *Steinernema feltiae* (Filipjev, 1934) (Owinema made by the firm OWIPLANT in Owińska) was studied in experimental conditions. Copper nanoparticles suspended in deionized water in concentrations of 5, 2 and 0.5 ppm were used in the experiments.

The experiment was carried out during 5 days under laboratory conditions at a temperature of  $25 \pm 1^\circ\text{C}$ . Larvae of the 3rd invasive growth stage (IJs) were placed in water solutions containing the appropriate concentration of nano-Cu. The control group consisted of larvae

kept in distilled water. Samples of solution were taken and nematodes mortality was estimated every day. Tests were performed in 5 repetitions. After 5 days the nematodes that survived the contact with nano-Cu were separated by sedimentation. The sedimentation did not, however, allow for complete removing of chemical compounds from the sample. Live nematodes obtained in that way and the residues of chemical substances were used to infect the four-week larvae and the adult insects of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae).

Next experiment was performed in Petri dishes of a diameter of 9 cm lined with filter paper in which 10 insects were placed. Five hundred invasive larvae (IJs) were added to each dish, which made 50 IJs/insect. Tests were made in 3 repetitions. Mortality was controlled during 5 days. Dead insects were transferred to empty dishes and placed in the incubation chamber for 48 h. Then the insects were dissected to check whether nematodes were the cause of their death. The control consisted of insects in the respective growth stage infected by nematodes which had no contact with nano-Cu. The mortality and the extensiveness of infection of *A. diaperinus* were analyzed.

The obtained results were statistically processed with the SPSS 15.0 software (Chi<sup>2</sup> test). Statistical significance was tested at  $p < 0.05$ .

## RESULTS AND DISCUSSION

With increasing concentrations of nano-Cu, higher mortality of entomopathogenic nematodes was observed (Fig. 1). The highest concentration of nanoparticles (5 ppm) caused 48% mortality in *S. feltiae* in the fifth day of experiment. The lowest concentration (0.5 ppm) caused much lower death rate – 10%. Mortality in the control group, in the last day of experiment, averaged 15%. Wang et al. (2009) showed that the nano- $\text{Al}_2\text{O}_3$ , nano-ZnO and nano- $\text{TiO}_2$  affected reduction of body length of *C. elegans* and the number of eggs produced by nematodes. In contrast, Oberdörster et al. (2005), Jones et al. (2008) and Ma et al. (2011) found high toxicity of nano-ZnO and nano- $\text{TiO}_2$ . The above-mentioned high concentration of nanopar-

ticles and hydroxylated fullerenes also negatively affect nematode survival (Wang et al. 2009, Cha et al. 2012). In Poland, the impact of nanocolloidal silver and copper on EPNs was studied by Kuźniar (2009). He found that nano-Ag in concentrations ranging from 5 to 50 mg/dm<sup>3</sup> were highly toxic to nematodes *S. feltiae* from biopreparations: Owinema, Nemasys, Nemaplus. After 2 h of direct contact of IJs with different concentrations of nano-Ag he reported almost 100% mortality. A different situation was related to nano-Cu, in nematodes from biopreparations Owinema and Nemasys. In these nematodes 100% survival was observed even at the highest concentration of compound (50 mg/dm<sup>3</sup>). However, in the case of nematodes from Nemaplus bioprepara-

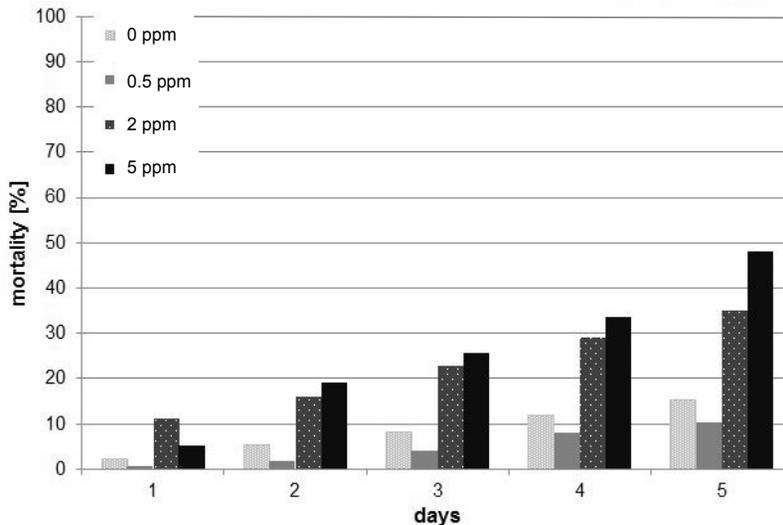


FIGURE 1. The effect of nano-Cu on the mortality of the IJs of *Steinernema feltiae* (test  $\chi^2$  refers to the last day of experiment –  $\chi^2 = 2002.2$ ,  $df = 3$ ,  $p < 0.001$ )

tion, at the highest concentration, larval mortality was approximately at 29%.

Different concentrations of nano-Cu solutions (5, 2, 0.5 ppm), with which IJs had contact, did not influence their abilities to kill larvae of *A. diaperinus*. This may give evidence that nematode

In both cases it reached 53%. The extensivity (13%) was lower after the contact of EPNs with nano-Cu. Mortality and extensivity of infection of adult beetles was the highest in control group (36 and 33% respectively). After the contact of EPNs with nanoparticles, death rate averaged

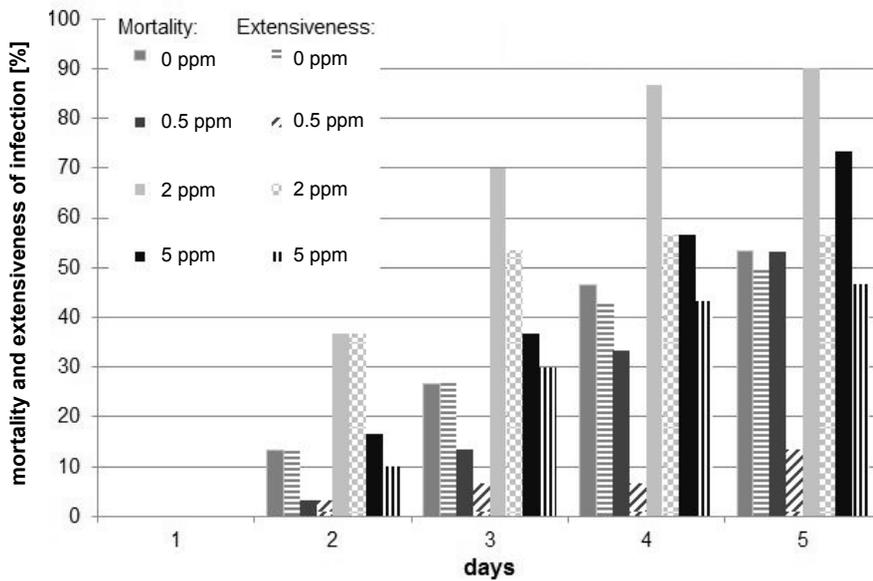


FIGURE 2. The effect of nanoparticles on pathogenic properties of the nematodes *Steinernema feltiae* exposed for 5 days to nano-Cu solutions. Tests  $\chi^2$  of mortality ( $\chi^2 = 1.3$ ,  $df = 3$ ,  $p > 0.05$ ) and extensiveness of infection ( $\chi^2 = 6.1$ ,  $df = 3$ ,  $p > 0.05$ ) of the *Alphitobius diaperinus* larvae

mutualistic bacteria are not vulnerable to nano-Cu (Fig. 2). Mortality and extensivity of infection, after the contact of EPNs with nano-Cu (2 ppm) in the last day of experiment, averaged 90 and 56% respectively. Lower death rate was observed in lower concentrations of the solution (0.5 ppm) and in a control group.

3 and 10% and extensivity of infection – 0 and 3% (Fig. 3). Kuźniar (2009) found that nematodes *S. feltiae* from Owinema biopreparation, that have been exposed to solutions of nano-Cu concentrations ranging from 1 to 50 mg/dm<sup>3</sup>, caused mortality of larvae *Tenebrio molitor* ranging from 40 to 80%.

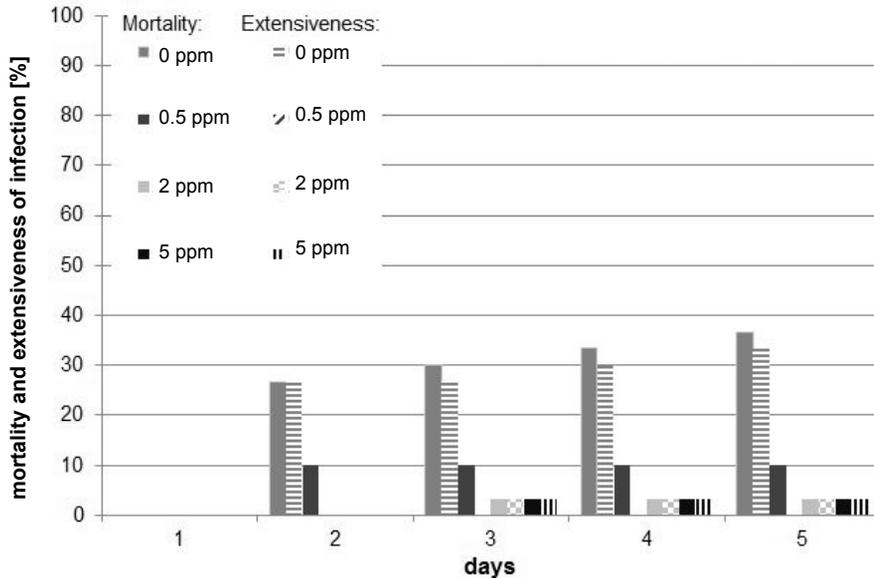


FIGURE 3. The effect of nanoparticles on pathogenic properties of the nematodes *Steinernema feltiae* exposed for 5 days to nano-Cu solutions. Tests  $\chi^2$  of mortality ( $\chi^2 = 14.03$ ,  $df = 3$ ,  $p < 0.01$ ) and extensiveness of infection ( $\chi^2 = 12.22$ ,  $df = 3$ ,  $p < 0.01$ ) of the *Alphitobius diaperinus* imagines

## CONCLUSIONS

1. Mortality of invasive larvae of *Steinernema feltiae* depends on concentrations of nano-Cu and on the time of exposition.
2. Mortality of *Alphitobius diaperinus* larvae after the contact of nematodes with nano-Cu is higher than in the control.
3. The extensity of infection of *A. diaperinus* larvae after the contact of nematodes with nano-Cu is the highest at 2 ppm (56%), and the lowest at a concentration of 0.5 ppm (13%).
4. Invasive larvae of nematodes treated with various concentrations of nano-Cu cause low mortality (3 and 10%)

and extensity of infection (0 and 3%) in adult beetles. In the control group mortality and extensiveness of infection are 37 and 33%.

## REFERENCES

- AVANCINI R.M.P, UETA M.T., 1990: Manure breeding insects (Diptera and Coleoptera) responsible for cestoidosis in caged layer hens. *J. Appl. Entomol.* 110, 307–312.
- BRZESKI M., SANDNER H., 1974: *Zarys nematologii*. PWN, Warszawa.
- CHA Y.J., LEE J., CHOI S.S., 2012: Apoptosis – mediated in vivo toxicity of hydroxylated fullerene nanoparticles in soil nematode *Caenorhabditis elegans*. *Chemosphere.* 87, 49–54.
- CHERNAKI-LEFFER A.M., KUTTEL J., MARTINS L.M., PEDROSO A.C., ASTOLFI-FERREIRA C.S., FERREIRA F., FERREIRA A.J., 2010: Vectorial competence of

- larvae and adults of *Alphitobius diaperinus* in the transmission of *Salmonella* Enteritidis in poultry. *Vector Borne and Zoonotic Diseases* 10 (5), 481–487.
- De La CASAS E., HAREIN P.K., DESHMUKH D.R., POMEROY B.S., 1976: Relationship between the lesser mealworm, fowl pox, and Newcastle disease virus in poultry. *J. Econ. Entomol.* 69 (6), 775–779.
- De La CASAS., POMEROY B.S., HAREIN P.K., 1972: Infection and quantitative recovery of *Salmonella typhimurium* and *Escherichia coli* from within the lesser mealworm, *Alphitobius diaperinus* (Panzer). *Poult. Sci.* 47 (6), 1871–1875.
- DESPINS J.L., AXTELL R.C., RIVES D.V., GUY J.S., FICKEN M.D., 1994: Transmission of enteric pathogens of turkeys by darkling beetle larvae (*Alphitobius diaperinus*). *J. Appl. Poultry Res.* 3, 61–65.
- GEDEN C.J., ARENDS J.J., AXTELL R.C., 1987: Field trials of *Steinernema feltiae* (Nematoda: Steinernematidae) for control of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in commercial broiler and turkey houses. *J. Econ. Entomol.* 80 (1), 136–141.
- GOODWIN M.A., WALTMAN W.D., 1996: Transmission of *Eimeria*, viruses, and bacteria to chicks: Darkling beetles (*Alphitobius diaperinus*) as vector of pathogens. *J. Appl. Poult. Res.* 5, 51–55.
- IGNATOWICZ S., 2008: Pleśniakowiec lśniący w fermach drobiarskich i jego zwalczanie. *Polskie Drobiarstwo* 12, 42–44.
- IGNATOWICZ S., 2009: Pleśniakowiec lśniący jego szkodliwość i zwalczanie. *Bydło* 6, 78–81.
- JONES N., RAY B., RANJIT K.T., MANNA A.C., 2008: Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS Microbiol. Lett.* 279, 71–76.
- KOWALSKA J., 2006: Wzajemne powiązania pomiędzy nicieniami owadobójczymi, owadami i bakteriami oraz ich wykorzystanie w praktyce. *Wiadomości Parazytologiczne* 52 (2), 93–98.
- KUŹNIAR T. 2009: Wpływ nanocząsteczkowej miedzi i srebra na żywotność i patogeniczność owadobójczych nicieni. Monografia 2009: Wielokierunkowość badań w rolnictwie i leśnictwie. Wyd. Uniwersytetu Rolniczego w Krakowie 1, 347–354.
- LANCASTER J.L., SIMCO J.S., 1967: Biology of the lesser mealworm, a suspected reservoir of avian leucosis. Report series (University of Arkansas (Fayetteville campus). Agricultural Experiment Station). Fayetteville.
- MA H., KABENGI N.J., BERTSCH P.M., UNRINE J.M., GLENN T.C., WILLIAMS P.L., 2011: Comparative phototoxicity of nanoparticulate and bulk ZnO to a free – living nematode *Caenorhabditis elegans*: The importance of illumination mode and primary particle size. *Environ. Pollut.* 159, 1473–1480.
- MARTENS E.C., HEUNGENS K., GOODRICH-BLAIR H., 2003: Early colonization events in the mutualistic association between *Steinernema carpocapsae* nematodes and *Xenorhabdus namatophila* bacteria. *J. Bacteriol.* 185, 3147–3154.
- MYCZKO A., 2006: Zastosowanie nanotechnologii w praktyce rolniczej. *Inż. Rol.* 2, 45–50.
- OBERDÖRSTER G., OBERDÖRSTER E., OBERDÖRSTER J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.* 113 (7), 823–839.
- PEZOWICZ E., 2005: Nicienie owadobójcze jako czynnik zmniejszający liczebność populacji pleśniakowca lśniącego (*Alphitobius diaperinus* Panzer) w brojlerniach. *Rozprawy Naukowe i Monografie*. Wyd. SGGW w Warszawie, Warszawa, 91.
- STEELMAN D., 1996: Darkling beetles are costly pests. *Poul. Digest.* 55, 22–23.
- WALLDORF V., MEHLHORN H., AI-QU-RAISHY S., AI-RASHEID K.A.S., ABDEL-GHAFFAR F., MEHLHORN J., 2012: Treatment with a neem seed extract (MiteStop®) of beetle larvae parasitizing the plumage of poultry. *Parasitol. Res.* 110, 623–627.

WANG H., WICK R. L., XING B., 2009: Toxicity of nanoparticulate and bulk ZnO, Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> to the nematode *Caenorhabditis elegans*. Environ. Pollut. 157 (4), 1171–1177.

**Streszczenie:** Nanocząstki miedzi i nicienie entomopatogeniczne *Steinernema feltiae* (Filipjev, 1934) w ograniczaniu liczebności pleśniakowca lśniącego *Alphitobius diaperinus* (Panzer, 1797). Właściwości antybakteryjne nanocząsteczek metali są dość dobrze znane, jednak ich możliwe wykorzystanie w rolnictwie, do zwalczania szkodliwych bezkręgowców, już nie. Pozytywne i negatywne interakcje z popularnymi, zintegrowanymi metodami zwalczania szkodników (IPM) powinny zostać sprawdzone i zweryfikowane. Zbadano wpływ nanocząsteczek miedzi na śmiertelność nicieni entomopatogenicznych *Steinernema feltiae* pochodzących z biopreparatu Owinema. Stwierdzono, że śmiertelność ich zależy od stężenia nanocząsteczek miedzi oraz czasu kontaktu larw z tymi roztworami. Zbadano również wpływ różnych stężeń nano-Cu na patogeniczność nicieni – śmiertelność zainfekowanych chrząszczy *Alphitobius diaperinus* (Panzer, 1797), ekstensywność infekcji. Wykazano, że

w wyższych stężeniach Cu nicienie mogą mieć mniejszą zdolność do penetrowania, wzrostu i rozmnażania się w ciele gospodarza.

*MS. received in November 2014*

**Authors' address:**

Kornelia Kucharska, Elżbieta Pezowicz,  
Dorota Tumialis  
Wydział Nauk o Zwierzętach SGGW  
Katedra Biologii Środowiska Zwierząt  
Zakład Zoologii  
02-787 Warszawa, ul. Ciszewskiego 8  
Poland

Dariusz Kucharski  
Uniwersytet Warszawski  
Wydział Biologii  
Instytut Zoologii  
Zakład Ekologii  
02-097 Warszawa, ul. Żwirki i Wigury 101  
Poland

Barbara Zajdel  
Wydział Nauk o Zwierzętach SGGW  
Pracownia Pszczelnictwa  
02-787 Warszawa, ul. Nowoursynowska 166  
Poland  
e-mail: kornelia.kucharska@op.pl



## Evaluation of thickness and color of wool in primiparas of Żelaźnieńska and Corriedale Sheep

DOROTA KULESZA, KATARZYNA MOZGA, ROMAN NIŻNIKOWSKI,  
EWA STRZELEC, MARCIN ŚWIĄTEK, MAGDALENA ŚLĘZAK  
Department of Animal Breeding and Production, Warsaw University of Life Sciences – SGGW

**Abstract:** *Evaluation of thickness and color of wool in primiparas of Żelaźnieńska and Corriedale Sheep.* The study was conducted on 19 samples of wool of Żelaźnieńska Sheep and 28 samples of wool of Corriedale Sheep. The thickness of fibre and rendement were examined. The color of wool were measured by colorimetric technique. Basis of studies found a wider range of thickness fibre of Corriedale Sheep and narrow range of thickness fibre of Żelaźnieńska Sheep compared with the standards. Particular importance is to eliminate from the breeding primiparas Corriedale Sheep characterized by nominal thickness of fibers 25  $\mu\text{m}$  and less. Average nominal thickness of the fibers were consistent with the cited standards. In Żelaźnieńska Sheep breed obtained, positive and highly significant correlation coefficients of rendement and thickness to brightness ( $L^*$ ) and found a significant correlation coefficient between rendement and the share of yellow color ( $b^*$ ). In Corriedale Sheep found positive and significant statistical correlation coefficient between the brightness ( $L^*$ ) and nominal thickness. Correlations between traits characterizing the color of wool, and in particular the measurement of brightness ( $L^*$ ), to features such as the nominal thickness of the fibers, indicate different trends occurring in Żelaźnieńska Sheep and Corriedale Sheep.

*Key words:* sheep, wool, thickness of fiber, color

### INTRODUCTION

Sheep's wool had a large role in the production, in times of the planned economy (GUS database from 2013).

Currently, due to a significant decrease in its economic meaning, there is no pay attention to quality of wool in breeding work. Due to the fact that in genetic resources program many breeds of sheep are protected, it is interesting to check the thickness of its wool and compare the obtained results with the standards described by Wójcikowska-Soroczyńska et al. (1993). That could be useful to track possible changes of wool. The objective aim of protecting native breeds of sheep is to keep quantitative and qualitative usability of animals (Krupiński 2012). That helps to compare possible changes and allows the adjustment of direction of breeding work.

Therefore, it was decided to investigate the thickness, color and rendement of the wool of Corriedale Sheep and Żelaźnieńska Sheep. Due to need for free interpretation of the results, the samples of wool were collected during the shearing from 2 years old ewes, before their first birth Wójcikowska-Soroczyńska et al. (1993).

### MATERIAL AND METHODS

The research material consisted of samples of wool from primiparas of Żelaźnieńska

Sheep (n = 19) and Corriedale Sheep (n = 28). The sheep were from a private farm located near Poddebice and from Research Farm of Sheep and Goats – WULS-SGGW in Żelazna, both farms located in Łódź Voivodeship. The main subject of studies was the wool taken from primiparas of both breed during shearing. It took place in January four weeks before lambing. Wool samples were collected from the side part of the animal (center of the last rib) and used to determine the thickness and color of the fibers. Then the wool has been washed with a detergent in water at a temperature not higher than 40°C. The wool was thoroughly dried and removed from it all impurities. The next steps were done in laboratory. Loosening of fibers was performed on samples weighing 2.5 g derived from each of the tested sheep.

Measurement of content of impurities consisted of weighting the wool samples before washing and after washing. During washing all impurities (plants, sand, dust and lanolin) were removed.

The rendement and level of impurities were determined. Both parameters were expressed as percentage. Measuring the thickness of the wool fiber was carried out using the method of Air-Flow (SGS 2011). The device used for measuring was the production of CLM Components LTD, and the measurement has been done according to following standard: IWTO-6-98 (2010). The results were expressed in nominal value ( $\mu\text{m}$ ). The results of thickness measurements of wool were divided into groups ( $\mu\text{m}$ ):

- Żelaźnieńska Sheep: 24–26.9; 27.0–28.9; 29.0–30.9; 31.0–32.9; 33.0–34.9; 35.0–36.9;
- Corriedale Sheep: 23.0–24.9; 25.0–26.9; 27.0–28.9; 29.0–30.9; 31.0–32.9; 33.0–34.9.

The color of wool was examined using device Chroma Mater CR-40 (Konica Minolta Ltd.). The color was measured using the system of color space  $L^*a^*b^*$  ( $L^*$  – brightness,  $a^*$  – share of the red color,  $b^*$  – share of the yellow color). The results were statistically analyzed within each breed separately using the IBM SPSS Statistics 21 software. The statistical model included the group of thickness of wool. Correlation coefficients between the rendement and nominal thickness of fibers and wool color measurement were calculated. Results of studies are presented in charts and tables.

## RESULTS AND DISCUSSION

The results of measurement of wool for both breeds are presented in Table 1. In both breeds the average thickness of wool were consistent with the model described by Wójcikowska-Soroczyńska et al. (1993). However, the rendement in Żelaźnieńska Sheep was higher and the content of impurities was lower compared to the model. In case of Corriedale Sheep rendement and the content of impurities consisted with described model. Range of thickness of wool is presented in Figures 1 and 2. In both breeds, the class of thickness of wool showed a highly significant effect on the

TABLE 1. Wool measurements of primiparas Żelaźnieńska Sheep and Corriedale Sheep

Specification		Żelaźnieńska Sheep Primiparas	Corriedale Sheep Primiparas
n		19	28
Nominal thickness of fiber (μm)	$\bar{x}$	30.95	29.96
	SE	0.75	0.45
Rendement (%)	$\bar{x}$	84.57	66.48
	SE	2.14	1.03
Impurities (%)	$\bar{x}$	15.43	33.52
	SE	2.14	1.03
Color of wool			
L*	$\bar{x}$	82.46	79.16
	SE	0.48	0.43
a*	$\bar{x}$	-0.23	-1.42
	SE	0.11	0.11
b*	$\bar{x}$	14.29	12.54
	SE	0.51	0.72

nominal thickness traits (Niżnikowski and Rant 1997, Niżnikowski et al. 1997, 1998, 2005). The range of the size nominal thickness of wool showed the highest number of samples in the range of 29.0–30.9 in Żelaźnieńska Sheep (Fig. 1). However, the nominal thickness value range was much narrower than that described in the standard (Wójcikowska-Soroczyńska et al. 1993). That indicates to conduct breeding work towards expanding the range of fluctuations of the nominal thickness, even at the expense, thinning of that thickness of fiber. The distribution of the occurrence of a nominal thickness of the fibers had upward trend for the frequency of fibers in the higher thickness class for Corriedale Sheep. In Corriedale Sheep breed correction of nominal thickness value should be done with keeping the average values on

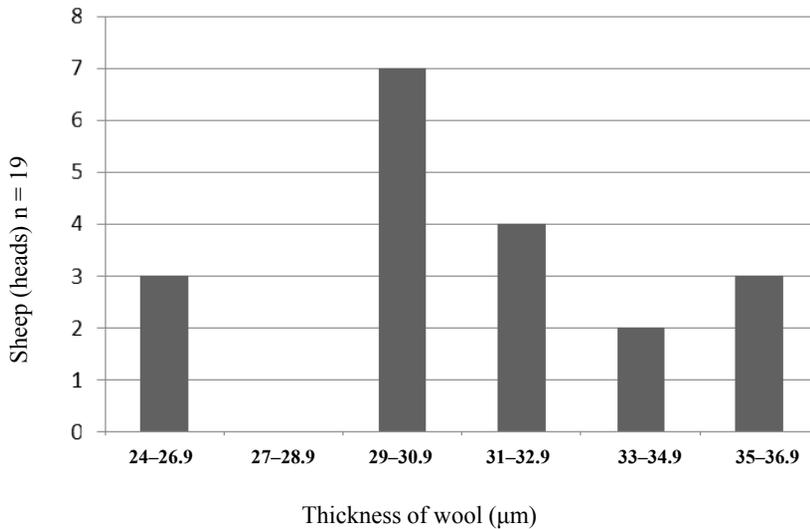


FIGURE 1. The number of Żelaźnieńska Sheep in fiber thickness ranges ( $P \leq 0.01$ )

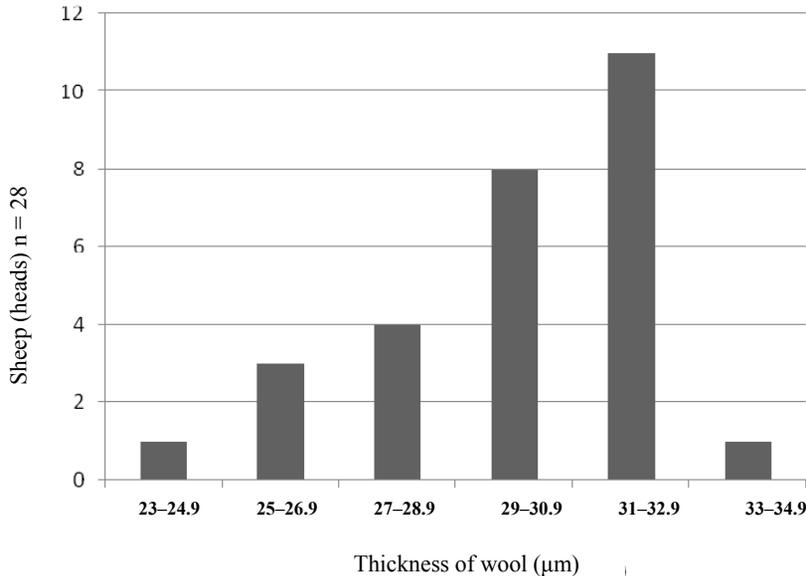


FIGURE 2. The number of Corriedale Sheep in fiber thickness ranges ( $P \leq 0.01$ )

the current level. Animals that were characterized by very low values at the nominal thickness of 25 µm and less should be eliminated. The results of the assessment of color wool measured colorimetric technique were very interesting (Table 1). In Żelaźnieńska Sheep noted the high level of brightness ( $L^*$ ) at small values of green color ( $a^*$ ) and high values for the yellow color ( $b^*$ ). Corriedale Sheep have also high level of brightness of the color, but the share of green color was higher but yellow color was lower.

It is difficult to comment on these results due to the fact that the colorimetric technique so far in the national survey was not used before. Table 2 presents the correlation coefficients between the technological features of wool and color within breed. Particularly impor-

tant were highly statistically significant correlations between the brightness of the color ( $L^*$ ) and rendement, as well as between the brightness of the color and the nominal thickness in Żelaźnieńska Sheep. There were positive and achieve high value (Niżnikowski and Rant 1997, Niżnikowski et al. 1997, 1998, 2005)

The correlation coefficient between rendement and yellow color was statistically significant but the value was lower and negative. The obtained results indicate the possibility of using colorimetric techniques in the evaluation of the quality characteristics of wool from Żelaźnieńska Sheep. In Corriedale Sheep obtained only a significant and negative correlation between the nominal thickness and the brightness ( $L^*$ ). That indicates the different

TABLE 2. The correlations between the measurements of the color and the wool traits in Żelaźnieńska Sheep and Corriedale Sheep

Specification	Żelaźnieńska Sheep (n = 19)			Corriedale Sheep (n = 28)		
	L*	a*	b*	L*	a*	b*
Rendement (%)	0.970 <sup>b</sup>	-0.270	-0.490 <sup>a</sup>	-0.013	0.086	0.207
Nominal thickness of fiber (µm)	0.950 <sup>b</sup>	-0.120	-0.410	0.434 <sup>a</sup>	-0.060	-0.069

<sup>a</sup>  $p \leq 0.05$ , <sup>b</sup>  $p \leq 0.01$ .

trends in the relationship of color wool with a nominal thickness in comparison with the values of this indicator identified in Żelaźnieńska Sheep. Summing up the correlation coefficients and the level of statistical significance can be observed the possibility of using a colorimetric technique in the assessment of the quality characteristics of wool from Żelaźnieńska Sheep inspired by these results requires further research in this area.

## CONCLUSIONS

The obtained results lead up to following statements and conclusions:

1. A wider range of nominal thickness in Corriedale Sheep, and too narrow in Żelaźnieńska Sheep compared with the standards described by Wójcikowska-Soroczyńska et al. (1993) which requires correction by the further breeding work. Elimination of the breeding primiparas Corriedale Sheep characterized by a nominal thickness of fiber of 25 µm and less, should be regarded as particularly important. The average nominal thickness of the fibers was within the cited standards.

2. Very high values for the brightness of the color measured by the colorimetric method in both breeds.
3. Positive and highly significant correlation coefficients of rendement and thickness to brightness (L\*) and found a significant correlation coefficient between rendement and the share of yellow color (b\*) in Żelaźnieńska Sheep.
4. Positive and significant statistical correlation coefficient between the brightness (L\*) and nominal thickness in Corriedale Sheep.
5. Correlations between traits characterizing the color of wool, and in particular the measurement of brightness (L\*) to features such as the nominal thickness of the fibers, indicate different trends occurring in Żelaźnieńska Sheep and Corriedale Sheep. The correlations should be used to assessing the productive value and developed through further research, separately for each breed.

## REFERENCES

- IWTO-6-98, 2010. Retrieved from location [www.iwto.org/uploaded/publications/rb\\_web\\_index\\_2010.pdf](http://www.iwto.org/uploaded/publications/rb_web_index_2010.pdf).
- KRUPIŃSKI J. (red.), 2012: Polskie rasy zachowawcze – atlas zwierząt gospodarskich

- objętych programem ochrony w Polsce. Wyd. Inst. Zoot. – PIB, Kraków.
- NIŻNIKOWSKI R., RANT W., 1997: Prace twórcze zmierzające do wytworzenia polskich owiec nizinnych odmiany żelaźnieńskiej o użytkowości wełnisto-mięsnej. Rola i znaczenie hodowlane chronionych przed wyginięciem ras i odmian owiec. Fundacja „Rozwój SGGW”, Warszawa, 131–144.
- NIŻNIKOWSKI R., JANIKOWSKI W.T., RANT W., HABER M., BOLIMOWSKI R., 1997: Wpływ genotypu i typu urodzenia na wybrane cechy użytkowości maciorek, uzyskanych w trakcie prac zmierzających do wytworzenia owiec typu corriedale. Rola i znaczenie hodowlane chronionych przed wyginięciem ras i odmian owiec. Fundacja „Rozwój SGGW”, Warszawa, 145–151.
- NIŻNIKOWSKI R., KUŹNICKA E., RANT W., 1998: Poziom produkcji wełny u polskich owiec nizinnych odmiany żelaźnieńskiej, doskonalonych w kierunku poprawy cech rozrodu, w zależności od roku i typu urodzenia. Zesz. Nauk. ZHOiK KSHZ SGGW 2, 107–115.
- NIŻNIKOWSKI R., GOSŁAWSKI W., POPIELARCZYK D., STRZELEC E., PIECHO K., 2005: Poziom wybranych cech użytkowych w stadach owiec typu corriedale utrzymywanych w ramach programu hodowli zachowawczej. Rocz. Nauk Zoot. 21, Suplement, 33–36.
- WÓJCIKOWSKA-SOROCZYŃSKA M., RADZIK-RANT A., SZTYCH D., 1993: Owce występujące w kraju i ich wełna. Wyd. SGGW, Izba Wełny w Gdyni, Warszawa.
- Société Générale de Surveillance (SGS) in New Zealand, 2011: Zasada metody Airflow. Retrieved from location [www.nz.sgs.com/info-bulletin3.3-2.pdf](http://www.nz.sgs.com/info-bulletin3.3-2.pdf)
- Streszczenie:** Ocena grubości i barwy wełny przystępek owcy żelaźnieńskiej i corriedale. Badania przeprowadzono na 19 próbach wełny owcy żelaźnieńskiej i 28 corriedale pobranych podczas strzyży wykonanej na przystękach przeznaczonych do dalszej hodowli. Ocenie poddano cechy grubości włókien i rendement oraz barwy wełny mierzonej techniką kolorymetryczną. Na podstawie przeprowadzonych badań stwierdzono szerszy przedział grubości nominalnych u owiec corriedale oraz zbyt wąski u owiec żelaźnieńskich w porównaniu z przyjętym wzorcem, co wymaga korekty poprzez pracę hodowlaną. Za szczególnie ważne uznać należy wyeliminowanie z hodowli przystępek corriedale charakteryzujących się grubością nominalną włókien 25  $\mu\text{m}$  i mniej. Średnie grubości nominalne włókien mieściły się w standardach zgodnych z cytowanym wzorcem. Uzyskano również wysokie, dodatnie i wysoko istotne współczynniki korelacji rendement i grubości nominalnej z pomiarem stopnia jasności ( $L^*$ ) oraz istotny współczynnik pomiędzy rendement a udziałem barwy żółtej ( $b^*$ ) u owiec żelaźnieńskich oraz dodatni i istotny statystyczny współczynnik korelacji pomiędzy pomiarem stopnia jasności ( $L^*$ ) a grubością nominalną u owiec corriedale. Związki korelacyjne pomiędzy cechami charakteryzującymi barwę wełny, a w szczególności pomiarem stopnia jasności ( $L$ ) a cechami np. grubości nominalnej włókien, wskazują na odmienne tendencje występujące u owiec żelaźnieńskich i corriedale.

*MS. received in November 2014*

**Author's address:**

Roman Niżnikowski  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Owiec i Kóz  
ul. Ciszewskiego 8, 02-786 Warszawa  
Poland  
e-mail: roman\_niznikowski@sggw.pl

## Polymorphism of insulin-like growth factor (*IGF-1*) gene in Polish Lowland Sheep from Podlaskie Voivodeship

ROMAN NIŻNIKOWSKI<sup>1</sup>, GRZEGORZ CZUB<sup>1</sup>, JERZY KAMIŃSKI<sup>2</sup>,  
MARIOLA NIERADKO<sup>2</sup>, MARCIN ŚWIĄTEK<sup>1</sup>, KRZYSZTOF GŁOWACZ<sup>1</sup>,  
MAGDALENA ŚLĘZAK<sup>1</sup>

<sup>1</sup> Department of Animal Breeding and Production, Warsaw University of Life Sciences – SGGW

<sup>2</sup> Polish Union of Sheep-Farmers in Białystok

**Abstract:** *Polymorphism of insulin-like growth factor (IGF-1) gene in Polish Lowland Sheep from Podlaskie Voivodeship.* Research was carried out on 432 Polish Lowland Sheep (405 ♀ and 27♂) of three varieties: Corriedale, Żelaźnieńska Sheep and Polish Lowland Sheep of Podlasie region. All animals were subjected to gene identification factor insulin – *IGF-1*, in the assessment of alleles C and T. In conclusion it should be noted that in the three examined varieties of Polish Lowland Sheep showed no polymorphism in exon 3 of the insulin-like growth factor (*IGF-1*) gene, limiting its scope to determine the allele C, respectively genotype CC. This result indicates the need for further research in this area in “culture” sheep imported and adapted to Polish conditions and the production environment.

*Key words:* Polish Lowland sheep, *IGF-1* gene, genetic polymorphism

### INTRODUCTION

Protein *IGF-1* is one of the key components of the pathway of growth hormone (Franco et al. 2005). *IGF-1* is produced in the liver and is responsible for cell growth and body treatments.

It is believed that the effect of growth hormone occurs in the tissue in coopera-

tion with local somatomedin e.g. *IGF-1* and stimulates the secretion of hypothalamic somatostatin inhibits secretion of growth hormone (Krzyszowski – Ed. 1998). Insulin factor gene is exchanged among the conditions which were useful to the identification of races, as demonstrated in the Mediterranean countries (Pariset et al. 2006). Niżnikowski et al. (2013) presents a different view in relation to the research carried out on domestic sheep breeds compared to the ancestor of the sheep – European mouflon *Ovis aries musimon*. Taking into account the fact reported in the cited work conditions influence the incidence of insulin-like factor gene *IGF-1*, it was decided to examine its frequency of occurrence in three varieties of Polish Lowland Sheep: Żelaźnieńska, Corriedale and Polish Lowland Sheep of the Podlasie region (PZO 2013).

### MATERIAL AND METHODS

The studies were conducted on Polish Lowland Sheep: Corriedale Sheep (2 herds), Żelaźnieńska Sheep (2 herds)

and Polish Lowland Sheep of Podlasie region (3 herds). Ewes and rams were aged 2 to 11 years (Table 1). Herds were randomly selected for sampling. For the isolation of genomic DNA blood samples were obtained of animals from *vein jugularis* into tubes containing anticoagulant EDTA. The study was carried out assessment of the polymorphic region (exon 3) in insulin-like growth factor (*IGF-1*) gene.

TABLE 1. Experimental material used in the study

Sheep Breeds	Number of ewes and rams		
	Total ♀	Total ♂	Herd sampling
Corriedale	108	6	Herd 1 – 98 ♀, 6 ♂ Herd 2 – 10 ♀
Polish Lowland Sheep of the Podlasie region	185	14	Herd 1 – 25 ♀, 4 ♂ Herd 2 – 57 ♀, 4 ♂ Herd 3 – 93 ♀, 6 ♂
Żelaznieńska	112	7	Herd 1 – 96 ♀, 6 ♂ Herd 2 – 16 ♀, 1 ♂
Total within gender	405	27	×
Total	432		

TABLE 2. The name of *locus* primer sequence and SNP of the polymorphic region in *IGF-1* gene

<i>Locus</i>	Name	Primers sequences 3'–5' (forward/reverse)	SNP	Localization
<i>IGF-1</i>	insuline-like factor	CACACACCTTGTTGCACTCC/ /GCTGAGTTGGTTGGATGCTCT	AY737509: 211 C > T <sup>a</sup>	Exon 3

<sup>a</sup> Pariset et al. (2006).

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

modification by removal of heme-compounds lysis of erythrocytes products.

DNA is isolated from leukocytes by chromatography on mini-columns of silicate A&A Biotechnology. The fraction thus obtained was used as template DNA for amplification of polymorphic gene allele fragment. Sample genotyping was performed with KASPar® system (www.kbioscience.co.uk), which use the single nucleotide polymorphism (SNP) based on primers listed in Table 2.

The genotyping of DNA samples within the ewes and rams shows distributions of alleles and genotypes frequency separately for each varieties of studied sheep. Allele and genotype frequencies were compared depending on the varieties of sheep using  $\chi^2$  test and SPSS v. 21 software, with was assessed range of alleles and genotypes frequency between varieties, sexes, and the differences between the sexes in terms of individual alleles and genotypes.

## RESULTS AND DISCUSSION

Studies have shown that for factor *IGF-1* gene were found only the C allele. Analyses of 432 samples collected from the rated sheep did not show the presence of

the T allele in any case. In comparison with the results of Pariset et al. (2006), mainly describing sheep found in the region of the Mediterranean Sea, Black Sea and Germany, sheep tested in Poland showed no polymorphism here. That was confirmed by the results achieved by Niżnikowski et al. (2013) in studies conducted in sheep breeds in Poland. It is expected that the distribution of the conditions occurring in sheep breeds in Poland was typical for this region of the world and different from the observed trends in other European countries (Pariset et al. 2006). Perhaps it has to do with another course of life processes of growth and development, which wrote Krzymowski – Ed. (1998). In this situation, can be a useful series of studies on sheep imported into Polish, polymorphism alleles and genotypes of insulin-like factor gene subject to change due to processes of adaptation – which requires further work in this area.

Generally, it should be noted that the summing up of the three examined varieties of Polish Lowland Sheep showed no polymorphism alleles and genotypes of factor *IGF-1*, limiting its scope to determine is the C allele and genotype CC. This result indicates the need for further research in this area in “culture” sheep imported and adapted to Polish conditions and the production environment.

## REFERENCES

- FRANCO M.M., ANTUNES R.C., SILVA H.D., GOULARDT L.R., 2005: Association of PIT1, GH and GHRH polymorphisms with performance and carcass traits in Landrace pigs. *Journal of Applied Genetics* 46 (2), 195–200.
- KRZYMOWSKI T. (red.), 1998: *Fizjologia zwierząt*. PWRiL, Warszawa, 143–200.
- NIŻNIKOWSKI R., CZUB G., GŁOWACZ K., ŚWIĄTEK M., ŚLĘZAK M., 2013: Polymorphism of insulin like growth factor *IGF-1* in position 211 in national sheep breeds with carpeted wool compared to Polish Merino and European Muflon (*Ovis aries musimon*). *Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci.* 52, 157–160.
- PARISET L., CAPPUCIO I., AJMONE-MAR-SAN P., BRUFORD M., DUNNER S., CORTES O., ERHARDT G., PRINZENBERG E.-M., GUTSCHER K., JOOST S., PINTO-JUMA G., NIJMAN I.J., LENSTRA J.A., PEREZ T., VALENTINI A., Econogene Consortium, 2006: Characterization of 37 Breed-Specific Single-Nucleotide Polymorphisms in Sheep. *Journal of Heredity* 97 (5), 531–534.
- PZO, 2013: *Hodowla Owiec i Kóz w Polsce w 2012 roku*. Wyd. PZO, Warszawa.

**Streszczenie:** Polimorfizm genu czynnika insulinopodobnego *IGF-1* u polskich owiec nizinnych utrzymywanych w województwie podlaskim. Badania przeprowadzono na materiale 432 polskich owiec nizinnych (405 ♀ i 27 ♂) trzech odmian: corriedale, owcy żelaźnieńskiej i polskiej owcy nizinnej regionu podlaskiego. Wszystkie zwierzęta były poddane identyfikacji genu czynnika insulinopodobnego *IGF-1*, w zakresie oceny występowania alleli C i T. Podsumowując, należy stwierdzić, iż u badanych trzech odmian polskich owiec nizinnych nie wykazano polimorfizmu występowania alleli i genotypów genu czynnika insulinopodobnego *IGF-1*, ograniczając jego zakres do ustalenie jedynie do allelu C i genotypu CC. Wynik ten wskazuje na potrzeby przeprowadzenia dalszych badań z tego zakresu u owiec kulturowych pochodzących z importu i adaptowanych do polskich warunkach środowiska produkcyjnego.

*MS. received in November 2014*

**Author's address:**

Roman Niżnikowski  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Owiec i Kóz  
ul. Ciszewskiego 8, 02-786 Warszawa  
Poland  
e-mail: roman\_niznikowski@sggw.pl

## The application of ultrasonography (USG) technique for assessment of muscularity of Berrichon du Cher and Polish Merino lambs at the age of 70 days

ROMAN NIŻNIKOWSKI<sup>1</sup>, ARTUR OPRZADEK<sup>2</sup>, KRZYSZTOF GŁOWACZ<sup>1</sup>,  
EWA STRZELEC<sup>1</sup>, GRZEGORZ CZUB<sup>1</sup>, MARCIN ŚWIĄTEK<sup>1</sup>, MAGDALENA  
ŚLEZAK<sup>1</sup>

<sup>1</sup> Department of Animal Breeding and Production, Warsaw University of Life Sciences – SGGW

<sup>2</sup> The Agricultural Property Agency

**Abstract:** *The application of ultrasonography (USG) technique for assessment of muscularity of Berrichon du Cher and Polish Merino lambs at the age of 70 days.* The research was conducted on 106 Polish Merino lambs in 2010–2013 and 221 Berrichon du Cher lambs in 2008–2013 (except 2009). The research took place in GRH Żydowo near Gniezno. Animal muscularity was tested by USG technique on *mld* muscle cross section. Based on obtained results, the effect of type of birth, sex, father origin and class of scrapie resistance and double-factor interactions were statistically insignificant impact (except interactions sex × year of birth on round of “eye” of loin) on muscularity traits of Polish Merino and Berrichon du Cher lambs, highly statistical significant effect of the year of birth on USG measurement traits  $p \leq 0.01$  (except spread width of “eye” of loin in Polish Merino breed). No effect of scrapie resistance class for muscularity traits indicates the possibility of freely conduct breeding work towards increasing resistant to scrapie genetics, without a negative impact on muscularity of lambs of both breeds. Strongly influence of year of the research as an environmental factor on the level of muscularity of lambs of both breeds at the age of 70 days has been shown, rather than the type of birth, sex, father origin or class of genetic resistance to scrapie.

**Key words:** sheep, ultrasonography technique, “eye” of the loin, muscle measurements

## INTRODUCTION

Ultrasonography technique can be used to estimate the level of muscularity of the lambs (Przybylak et al. 2008, Knapik et al. 2009, Niżnikowski et al. 2010a, b, Knapik 2011). Thanks that reason the assessment of muscularity of lambs could be done with high repeatability of results. This technique was also used to compare muscularity between genetic combinations of lambs and giving the possibility to use this method for assessing the level body conformation (Niżnikowski et al. 2010a, b).

Considering the use of USG technique, it was decided to take measurements *mld* muscle at age of 70 day in Polish Merino lambs and Berrichon du Cher lambs. The aim was to examine the effect of some important factors taken into breeding work on the tested muscularity traits using ultrasonography technique.

## MATERIAL AND METHODS

The research was conducted on 106 Polish Merino lambs in 2010–2013 and 221 Berrichon du Cher lambs in 2008–2013 (except 2009). The research took place in GRH Żydowo near Gniezno. The animals were selected for the renovation of the herd during the selection work carried out in both sexes. Both ewes and rams were born as singles, twins or triplets. The animals were kept in building during whole year and fed according to the norms (Osikowski et al. 1993). Mating season was conducted in period of May – June for Polish Merino and for Berrichon du Cher the mating season was conducted in period August – September. Basing on the breeding books the data of origin of father was collected: in case of Polish Merino Sheep (Polish or German) as well as Berrichon du Cher (Polish or France). In 2004 the German Mutton Merino rams were imported to Poland and included to Polish Merino breed genotype at no more than 50%. In 2005 and 2012 Berrichon du Cher rams were imported from France.

In both breed's offspring the genotype of prion protein was examined according to the method by Niżnikowski et al. (2013) and the class of frequency resistant to scrapie were specified (DEFRA 2006).

In Polish Merino breed following genotypes were observed: in class G1 (ALRR/ALRR), in class G2 (ALRR/AFRQ, ALRR/ALHQ, ALRR/ALRQ), in class G3 (ALRQ/ALHQ, ALRQ/

/ALRH, ALRQ/ALRQ), in class G4 (VLRQ/ALRR) and in class G5 (VLRQ/AFRQ, VLRQ/ALRQ).

In Berrichon du Cher breed following genotypes were observed: in class G1 (ALRR/ALRR), in class G2 (ALRR/ALHQ, ALRR/ALRH, ALRR/ALRQ), in class G3 (ALRQ/ALRQ, ALRQ/ALHQ) and in class G5 (VLRQ/ALHQ).

The USG measurement (using Honda 2000 device) were done on the *mld* muscle on live lambs at 70 day of age. The USG measurement allowed to establish the live parameters of muscularity: height, spread width, round and area of “eye” of loin, as well as the fat over the “eye” of the loin.

The statistical analysis was depended on the breed according to the statistical model included following variation sources: the year of birth, sex, type of birth, the origin of father, the class of genetic resistance to scrapie and selected double-factor interactions. The Duncan test was used to evaluate the effect of the year on examined traits (Ruszczyc 1981). The statistical calculations were done in SPSS software (version 21 for Windows). The results are shown in tables.

## RESULTS AND DISCUSSION

The effect of chosen factors and interactions on muscularity traits of Polish Merino breed was presented in Table 1. The effect of the year of birth was observed in majority USG measurement traits ( $p \leq 0.01$ ) (except spread of “eye” of loin). The effect of interaction (sex  $\times$  birth of

TABLE 1. The effect of factors and interactions on measurement traits on *mld* muscle cross section by USG technique at the last thoracic vertebra on Polish Merino lambs at the age of 70 days (n = 106)

Traits	Factors					Interactions			$\bar{x}$	SE
	year of birth	type of birth	sex	origin of father	scrapie genotype class	sex × type of birth	year of birth × type of birth	sex × year of birth		
<i>mld</i> "eye" height (mm)	NS	NS	NS	NS	NS	NS	NS	NS	21.475	0.476
<i>mld</i> "eye" width (mm)	**	NS	NS	NS	NS	NS	NS	NS	54.390	0.765
fat over the "eye" of the loin (mm)	**	NS	NS	NS	NS	NS	NS	NS	1.257	0.067
<i>mld</i> "eye" round (mm)	**	NS	NS	NS	NS	NS	NS	*	136.930	1.882
<i>mld</i> "eye" area (cm <sup>2</sup> )	**	NS	NS	NS	NS	NS	NS	NS	8.824	0.241

Statistical significance at: \*\* p ≤ 0.01; \* p ≤ 0.05; NS – no significance effect.

year) was observed for round of „eye” of loin (p ≤ 0.05). The statistical significant effect of the rest of variation sources on the USG measurement was not observed. The effect of chosen factors and interactions on muscularity traits of Berrichon du Cher was presented in Table 2. In this case only the effect of the year of birth was observed in all USG measurement traits (p ≤ 0.01).

The effect of year of birth on USG measurement traits was presented in Tables 3 and 4. In 2012 year were the best conditions for both breed. The greatest values of measurement were reported in that year. This indicates a significant influence of environmental conditions on the body composition of both examined breeds. This study confirms the results of Niżnikowski et al. (2010a, b) made in various breeds of sheep also using USG techniques.

Due to the fact that USG measurement has a great relationship with slaughter value its very helpful to estimate live body conformation during a selection (Przybylak et al. 2008, Knapik et al. 2009, Knapik 2011).

No effect of type of birth on muscularity was not a characteristic trend for lambs (Niżnikowski et al. 2010a, b). It is known phenomenon catching body weight in lambs from twins compared to single. Everything points to the fact that the achievement of this level of body conformation was at the age of 70 days for lambs from twins in environmental conditions GHR Żydowo, as well as

TABLE 2. The effect of factors and interactions on measurement traits on *mld* muscle cross section by USG technique at the last thoracic vertebra on Berrichon du Cher lambs at the age of 70 days (n = 106)

Traits	Factors					Interactions			$\bar{x}$	SE
	year of birth	type of birth	sex	origin of father	scrapie genotype class	sex × type of birth	year of birth × type of birth	sex × year of birth		
<i>mld</i> “eye” height (mm)	**	NS	NS	NS	NS	NS	NS	NS	18.795	0.329
<i>mld</i> “eye” width (mm)	**	NS	NS	NS	NS	NS	NS	NS	54.916	0.614
fat over the “eye” of the loin (mm)	**	NS	NS	NS	NS	NS	NS	NS	1.611	0.055
<i>mld</i> “eye” round (mm)	**	NS	NS	NS	NS	NS	NS	NS	134.497	1.49
<i>mld</i> “eye” area (cm <sup>2</sup> )	**	NS	NS	NS	NS	NS	NS	NS	7.969	0.194

Statistical significance at: \*\* p ≤ 0.01; \* p ≤ 0.05; NS – no significance effect.

sustainable impact of these conditions on the development of muscle in lambs from different sexes.

The aim of import Merino Mutton rams from Germany and Berrichon du Cher rams from France was to indicate relatively low genetic distance of both breed breeding in our country. No effect of origin of father on the live body composition was inconsistent with the results of Niznikowski et al. (2010a). The author reported that the lambs coming from the cross with Berrichon du Cher rams imported from France had lower thickness of fat over the “eye” of the loin. It should be noted that these results only applied to one year of research and a smaller number of animals used in combination with long-term test results described in this paper.

The impact of class resistance to scrapie on the muscularity showed no statistically significant effect in both breeds. That results was inconsistent with results of Wiśniewska et al. (2009), moreover it was very positive in case of examined sheep.

Both breeds are selected to increase the frequency of resistant to scrapie.

No effect of genotype of the prion protein gene PRNP on the muscularity points to the possibility of breeding work in this direction without having a negative impact on the muscularity. Generally, muscularity of lambs of both sexes and breeds was more influenced by environmental factors than the type of birth, sex, father’s genotype or class resistance to scrapie.

TABLE 3. The effect of year of birth on measurement traits on *mld* muscle cross section by USG technique at the last thoracic vertebra on Polish Merino lambs at the age of 70 days

Traits	Duncan test	Year of birth			
		2010	2011	2012	2013
		Number of lambs (n)			
		26	28	25	27
<i>mld</i> “eye” height (mm)	LSM	20.585	20.683	22.537	23.334
	SE	1.164	0.742	0.720	0.667
<i>mld</i> “eye” spread (mm)	LSM	52.376 <sup>A</sup>	53.828 <sup>B</sup>	63.579 <sup>A,B,C</sup>	49.348 <sup>B,C</sup>
	SE	1.870	1.191	1.157	1.071
fat over the “eye” of the loin (mm)	LSM	1.166 <sup>D</sup>	1.093 <sup>E</sup>	1.248 <sup>F</sup>	1.730 <sup>D,E,F</sup>
	SE	0.165	0.105	0.102	0.094
<i>mld</i> “eye” round (mm)	LSM	132.702 <sup>G</sup>	135.845 <sup>H</sup>	152.340 <sup>G,H,I</sup>	130.030 <sup>I</sup>
	SE	4.601	2.931	2.846	2.634
<i>mld</i> “eye” area (cm <sup>2</sup> )	LSM	7.693 <sup>J</sup>	8.592 <sup>K</sup>	10.787 <sup>J,K,L</sup>	9.022 <sup>L</sup>
	SE	0.589	0.375	0.364	0.337

A–L – statistical significance at  $p \leq 0.01$ .

TABLE 4. The effect of year of birth on measurement traits on *mld* muscle cross section by USG technique at the last thoracic vertebra on Berrichon du Cher lambs at the age of 70 days

Traits	Duncan test	Year of birth				
		2008	2010	2011	2012	2013
		Number of lambs (n)				
		50	72	25	32	42
<i>mld</i> “eye” height (mm)	LSM	17.645 <sup>A</sup>	17.485 <sup>B</sup>	17.025 <sup>C</sup>	23.854 <sup>A,B,C</sup>	20.177 <sup>A,B,C</sup>
	SE	0.557	0.809	0.814	0.785	0.532
<i>mld</i> „eye” spread (mm)	LSM	54.907 <sup>D</sup>	58.156 <sup>E</sup>	51.992 <sup>E,F,a</sup>	62.197 <sup>D,F,G</sup>	48.192 <sup>D,E,a,G</sup>
	SE	1.040	1.512	1.520	1.466	0.994
fat over the “eye” of the loin (mm)	LSM	1.479 <sup>H</sup>	1.487 <sup>I,b</sup>	1.321 <sup>J</sup>	2.104 <sup>H,I,J</sup>	1.865 <sup>H,b,J</sup>
	SE	0.093	0.135	0.135	0.130	0.088
<i>mld</i> “eye” round (mm)	LSM	134.525 <sup>K</sup>	141.209 <sup>c,L</sup>	128.325 <sup>c,M</sup>	150.596 <sup>K,M,N</sup>	119.919 <sup>K,L,N</sup>
	SE	2.522	3.668	3.686	3.556	2.412
<i>mld</i> “eye” area (cm <sup>2</sup> )	LSM	7.677 <sup>O</sup>	7.981 <sup>d,P</sup>	6.582 <sup>d,R</sup>	10.832 <sup>O,P,R,Q</sup>	7.588 <sup>Q</sup>
	SE	0.329	0.478	0.480	0.463	0.314

A–Q – statistical significance at  $p \leq 0.01$ , a–d – statistical significance at  $p \leq 0.05$ .

## CONCLUSIONS

The obtained results led up to following statements and conclusions:

1. The effect of type of birth, sex, father origin and class of scrapie resistance and double-factor interactions were statistically insignificant impact (except interactions sex x year of birth on round of "eye" of loin) on muscularity traits of Polish Merino and Berrichon du Cher lambs.
2. The effect of the year of birth affected USG measurement traits  $p \leq 0.01$  (except spread width of "eye" of loin).
3. No effect on scrapie resistance class for muscularity traits indicates the possibility of freely conduct breeding work towards increasing resistant to scrapie genetics, without a negative impact on muscularity of lambs of both breeds.
4. Strongly influence of year of the research as an environmental factor on the level of muscularity of lambs of both breeds at the age of 70 days has been shown, rather than the type of birth, sex, father origin or class of genetic resistance to scrapie.

## REFERENCES

- DEFRA, The Department for Environment, Food and Rural Affairs in National Scrapie Plan for Great Britain, 2006.
- KNAPIK J., JUNKUSZEW A., MENDEL G., 2009: Bewertung der Faktoren, die Wiederholbarkeit bei Ultraschallmessungen des Musculus longissimus bei Lämmern beeinflussen. Bayerische Schafhalter 4, 13–15.
- KNAPIK J., 2011: Zastosowanie metody SOT w kwalifikacji tryków do produkcji jagniąt rzeźnych. Wiadomości Zootechniczne XLIX, 1, 39–45.
- NIŻNIKOWSKI R., OPRZĄDEK A., STRZELLEC E., POPIELARCZYK D., GŁOWACZ K., 2010a: Level of reproduction performance and body conformation of Berrichonne du Cher sheep bred in Poland. Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 47, 135–142.
- NIŻNIKOWSKI R., OPRZĄDEK A., STRZELLEC E., POPIELARCZYK D., GŁOWACZ K., 2010b: Comparison of reproduction level and body conformation of Suffolk and Charollais sheep breed in Poland. Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 47, 143–148.
- NIŻNIKOWSKI R., CZUB G., GŁOWACZ K., ŚLĘŻAK M., ŚWIĄTEK M., 2013: Ocena frekwencji występowania uwarunkowań białka prionowego *PRNP* u merynosa polskiego starego typu na przykładzie regionu mazowieckiego. Zesz. Nauk. PTZ 9, 21–32.
- OSIKOWSKI M., PORĘBSKA W., KORMAN K., 1993: Normy żywienia owiec. Normy żywienia bydła i owiec systemem tradycyjnym. Instytut Zootechniki, Kraków, 29–57.
- PRZYBYŁAK A., BONIECKI P., KOZŁOWSKI R.J., ŚLÓSZARZ P., 2008: Neuronowa analiza zdjęć ultrasonograficznych w procesie identyfikacji poziomu zawartości tłuszczu – badania wstępne. Inżynieria Rolnicza 6 (104), 159–165.
- RUSZCZYC Z., 1981: Metodyka doświadczeń zootechnicznych. PWRiL, Warszawa.
- SPSS for Windows version 21.0, IBM Inc.
- WIŚNIEWSKA E., PORTOLANO B., MROCKOWSKI S., 2009: Związek polimorfizmu genu białka prionowego z wybranymi cechami produkcyjnymi owiec. Materiały konferencyjne „Pasażowalne gąbczaste encefalopatie u zwierząt gospodarskich – diagnostyka i zapobieganie”, Balice 2009, 63–64.

**Streszczenie:** Wykorzystanie techniki USG w ocenie umięśnienia jagniąt rasy merynos polski i Berrichon du Cher w 70. dniu życia. Badaniami objęto 106 jagniąt rasy merynos polski w latach 2010–

–2013 oraz 221 jagniąt rasy Berrichon du Cher w latach 2008–2013 (z wyłączeniem 2009 roku, w którym prac nie prowadzono) w GRH Żydo-wo pod Gnieznem. Zwierzęta poddano ocenie umięśnienia techniką USG, wykonując pomiary na przekroju poprzecznym mięśnia *mld*. Na podstawie przeprowadzonych prac badawczych stwierdzono: nieistotny wpływ typu urodzenia, płci i pochodzenia po ojcu oraz klasy oporności na trzęsawkę i interakcji dwuczynnikowych (z wyłączeniem wpływu interakcji płęć  $\times$  rok urodzenia na pomiar obwodu przekroju poprzecznego mięśnia *mld*) na cechy umięśnienia jagniąt rasy merynos polski i Berrichon du Cher mierzone techniką USG; bardzo istotny wpływ roku doświadczenia na cechy umięśnienia mierzone techniką USG (za wyjątkiem wysokości „oka” połędwicy u rasy merynos polski); brak wpływu klasy oporności na trzęsawkę na cechy umięśnienia wskazuje na możliwość swobodnego prowadzenia pracy hodowlanej w kierunku zwiększenia

opornych na trzęsawkę uwarunkowań genetycznych, bez negatywnego wpływu na poziom umięśnienia jagniąt obu ras. Wykazano zdecydowanie większy zakres oddziaływania roku doświadczenia, a więc czynnika środowiskowego, na poziom umięśnienia jagniąt obu ras w wieku 70 dni niż typu urodzenia, płci, pochodzenia po ojcu czy klasy oporności genetycznej na trzęsawkę.

*MS. received in November 2014*

**Author's address:**

Roman Niżnikowski  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Owiec i Kóz  
ul. Ciszewskiego 8, 02-786 Warszawa  
Poland  
e-mail: roman\_niznikowski@sggw.pl



## **Analysis of fattening results of Polish Holstein-Friesian bulls and PHF × Belgian Blue crossbreeds bulls**

TOMASZ PRZYSUCHA, MAGDALENA STEFANIUK, MARCIN GOŁEBIEWSKI, JAN SŁÓSZARZ, KAROLINA WNEK, MAŁGORZATA KUNOWSKA-SŁÓSZARZ

Department of Cattle Breeding, Warsaw University of Life Sciences – SGGW

**Abstract:** *Analysis of fattening results of Polish Holstein-Friesian bulls and PHF × Belgian Blue crossbreeds bulls.* The aim of study was the comparison of fattening results of Polish Holstein-Friesian (PHF) and PHF × Belgian Blue crossbreeds (PHF × BB) bulls fattened in intensive system up to age of 18 months. Fattening results of 50 bulls in each genotype group were analyzed. Bulls were kept in freestall system at seven private farms located in Central Poland and up to about 60 days of life fed with milk replacement, hay and concentrates and since that maize-silage, hay-silage and concentrates were introduced to their diet. Animals were weighted on digital scale every 3 months. After slaughter the dressing percentage and the carcasses evaluation by EUROP method were collected from processing plant. Standardized averages of bulls body weight at 120th, 210th and 540th day of life were calculated as well as the average daily body gains in particular fattening periods. Results revealed that crossbreeds PHF × BB were heavier by 94.2 kg (11.61%) than purebreds PHF at age of 18 months. The average daily body gains were high and accounted 1081 g for PHF and 1183 g for PHF × BB bulls, respectively. Daily body gains of crossbreeds were higher by 10.94%. Observed dressing percentage was high for the both genotype groups but by 3.4 percentage points higher for crossbreeds which also obtained the better notes in EUROP carcasses evaluation.

*Key words:* fattening, commercial crossing, Belgian Blue, Polish Holstein-Friesian

## INTRODUCTION

Belgian Blue is a double-muscle breed with the muscular hypertrophy caused by myostatin mutation (Grobet et al. 1997, Kambadur 1997, Grobet et al. 1998, Casas 1999, Albera 2006). The last decades, BB have met a worldwide development, wider than expected. The use of BB as cross breed all over the world is a major fact at the end of the last century (Hanset and Georges 1997). Belgian Blue crossbreeds are predisposed for the intensive fattening with the high daily body weight gain at the moderate feedstuffs use. Animals kept for slaughtering can be fattened to the high body weight without any risk of over fattening (Grodzki 1999, Grodzki and Przysucha 2004, Jasirowski and Przysucha 2004). The high performance of the modern BB as pure breed had caught attention of farmers to be used it as terminal sire (Albera et al. 2001). Similarly in Poland the role of BB in commercial crossing is growing up. There were 13,524 inseminations with BB semen in 2012, which was 4.4% of all commercial crossings (Przysucha et al. 2014).

The aim of the study was the comparison of fattening performance of Polish Holstein-Friesian and PHF × Belgian Blue crossbreeds bulls.

## MATERIAL AND METHODS

The material for the analysis were fattening results of Polish Holstein-Friesian (PHF) and PHF × Belgian Blue crossbreeds (PHF × BB) bulls fattened in an intensive system up to age of 18 months. Crossbreeds mothers (PHF) were multiparous cows without any problems with delivery course in the past.

Fattening results of 50 bulls in each genotype group were analyzed. Bulls were kept in freestall system at seven private farms located in Central Poland and up to about 60 days of life fed with milk replacement, hay and concentrates and since that maize-silage, hay-silage (*ad libitum*) and concentrates (from 1 to 4 kg depending on age and body weight) were introduced to their diet. Animals were weighted on digital scale every 3 months. After slaughter the dressing percentage and the carcasses evaluation by EUROP method were collected from processing plant. Standardized averages of bulls body weight at 120th, 210th and 540th day of life were calculated as well as the average daily body gains in particular fattening periods.

Calculation of standardized body weight of the animal for the particular day of its life (120, 210, 540) was executed according to the following formula:

$$MCS = [(MCB - MCU) / WW] \times WS + MCU$$

where:

MCS – standardized body weight of the animal (kg);

MCB – real body weight of the animal in the day of weighing (kg);

MCU – real body weight of the animal at 48 h after birth (kg);

WW – age of the animal in the day of weighing (days);

WS – standardized age of the animal, 210 or 420 (days).

Average daily body weight gains of the animals were calculated in the periods:

- from birth up to 120th day of life;
- from birth up to 210th day of life;
- from birth up to 540th day of life.

Average daily body weight gains of the animals were calculated according to the following formula:

$$PDMC = (MCK - MCP) \times 1000 / (WK - WP)$$

where:

PDMC – daily body weight gain of the animal (g);

MCK – final body weight of the animal in the day of weighing (kg);

MCP – initial body weight of the animal in the day of weighing (kg);

WK – final age of the animal in the day of weighing (days);

WP – initial age of the animal in the day of weighing (days).

Statistical analysis of the study data was carried out by GLM procedure on IBM SPSS 21.0 (Statistical Product and Service Solution 2013).

RESULTS AND DISCUSSION

Results presented in Table 1 revealed that PHF × BB crossbreeds were heavier by 94.2 kg (11.61%) than PHF purebreeds at age of 18 months. The average daily body gains were high and accounted 1081 g for PHF and 1183 g for PHF × BB bulls, respectively. Daily body gains from birth to the age of 540 days of the crossbreeds were higher by 10.94%. Observed dressing percentage was high for the both genotype groups but by 3.4 percentage points higher for crossbreeds which also obtained the better notes in EUROP carcasses evaluation. Hanset and Georges (1997) showed, that

average carcass yield of Holstein-Friesian bulls was 53.03% when BB × HF bulls was 59.19%. The same authors in the earlier studies obtained 55 and 60% of dressing percentage, respectively. The daily body weight gains of bulls were 1.3 kg for HF and 1.5 kg for BB × HF. Hanset and Georges (1997) gave according to Langholz and Gerardy studies, that 33% of HF carcasses were evaluated in EUROP scale as “R” grade and 67% as “O”. In case of BB × HF crossbred it was 38% as “U” and 62% as “R”.

CONCLUSIONS

Commercial crossbreeds bulls PHF × BB fattened in intensive system up to age of 18 months showed much higher daily body weight gains in every fattening period as well as higher dressing percentage and better EUROP grade than

TABLE 1. The results of experimental bulls fattening

Specification	Bulls PHF		Bulls PHF × BB		Difference (%)
	Average	SD	Average	SD	
Body weight at birth (kg)	38.4	1.15	39.2	1.73	2.08
Body weight at age of 120 days (kg)	142.4	2.56	152.1	2.98	1.07
Body weight at age of 210 days (kg)	220.5	4.32	277.3	4.12	12.58
Body weight at age of 540 days (kg)	583.8	5.43	678.0	4.77	11.61
Average daily body weight gain up to age of 120 days (g)	867	63	941	89	10.85
Average daily body weight gain up to age of 210 days (g)	987	84	1134	102	11.49
Average daily body weight gain up to age of 540 days (g)	1081	111	1183	132	10.94
Number of carcasses according to EUROP evaluation system	U	–		17	–
	R	17		31	
	O	33		2	
Dressing percentage (%)	57.20	1.34	60.60	1.12	

purebred PHF bulls. Therefore PHF × × BB bulls are significantly better fattening material than PHF one kept under intensive production systems.

## REFERENCES

- ALBERA A., 2006: Selection for beef traits and calving performance in Piemontese cattle. Ph.D. Dissertation. Wageningen University, Netherlands.
- ALBERA A., MANTOVANI R., BITTANTE G., GROEN A.F., CARNIER P., 2001: Genetic parameters for daily live-weight gain, live fleshiness and bone thinness in station-tested Piemontese young bulls. *Anim. Sci.* 72, 449–456.
- CASAS E., KEELE J.W., FAHRENKRUG S.C., SMITH T.P., CUNDIFF L.V., STONE T., 1999: Quantitative analysis of birth, weaning, and yearling weights and calving difficulty in Piedmontese crossbreds segregating an inactive myostatin allele. *J. Anim. Sci.* 77, 1886–1692.
- GROBET L., PONCELET D., ROYO L.J., BROUWERS B., PIROTTIN D., MICHAUX C., MENISSIER F., ZANOTTI M., DUNNER S., GEORGES M., 1998: Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. *Mamm. Genome* 9, 210–213.
- GROBET L., ROYO L.J., PONCELET D., PIROTTIN D., BROUWERS B., RIQNET J., SCHOEBERLEIN A., DUNNER S., MENISSIER F., MASSABANDA J., FRIES R., HANSET R., GEORGES M., 1997: A deletion in the bovine myostatin gene causes the double-muscling phenotype in cattle. *Nature Genetics* 17, 71–74.
- GRODZKI H., 1999: Chów bydła mięsnego. *Wieś Jutra* 7–8, 29–30.
- GRODZKI H., PRZYSUCHA T., 2004: Krzyżowanie towarowe: szansa na dobrą wołowinę. *Top Agrar Polska* 12, 18–21.
- HANSET R., GEORGES M., 1997: A deletion in the bovine myostatin gene causes the double-muscling phenotype in cattle. *Nature Genetics* 17, 71–74.
- JASIOROWSKI H., PRZYSUCHA T., 2004: Bydło mięsne: wybór rasy. *Top Agrar Polska* 1, 102–104.
- KAMBADUR R., SHARMA M., SMITH T.P.L., BASS J.J., 1997: Mutations in myostatin in double-muscling Belgian Blue Cattle. *Genome Res.* 7, 910–916.
- PRZYSUCHA T., GRODZKI H., 2004: Przydatność różnych ras bydła mięsnego do chowu w czystości rasy i krzyżowania towarowego. *Mat. Konf. „Hodowla i produkcja bydła mięsnego”*. Międzynarodowe Targi Poznańskie POLAGRA FARM, Poznań, 63–73.
- PRZYSUCHA T., GRODZKI H., GOŁĘBIEWSKI M., 2014: Krzyżowanie towarowe – dobry wybór, ale targuj się. *Top Agrar Polska* 3, 40–43.

**Streszczenie:** *Analiza wyników opasu buhajków phf i mieszańców phf × belgijska bialo-błękitna.* Celem pracy było porównanie wyników opasu buhajków phf i mieszańców phf × belgijska bialo-błękitna opasanych w systemie intensywnym do wieku 18 miesięcy. Analizowano wyniki opasu 50 buhajków w każdej grupie genotypowej. Buhajki obu grup były opasane w siedmiu gospodarstwach prywatnych w centralnej Polsce. Buhajki były utrzymywane luzem w kojcach grupowych i żywione do ok. 60. dnia życia preparatami mlekozastępczymi, sianem i paszami treściwymi, a po tym okresie kiszonką z kukurydzy, sianokiszonką i paszą treściwą. Opas prowadzono w systemie półintensywnym do ok. 18. miesiąca życia. Zwierzęta były ważone co trzy miesiące na wadze elektronicznej. Po odstawieniu do uboju z zakładów mięsnych uzyskano dane dotyczące wydajności rzeźnej i wydajności poubojowej oceny tusz metodą EUROP. Wyliczono średnie standaryzowane masy ciała buhajków na 120., 210. i 540. dzień życia oraz średnie dobowe przyrosty w poszczególnych okresach opasu. Buhajki mieszańce phf × bbb uzyskały w wieku 18 miesięcy większą o 94,2 kg masę ciała, czyli o 11,61% od czystorasowych buhajków rasy phf. Średnie dobowe przyrosty za cały okres opasu

były bardzo duże i wynosiły 1081 g dla buhajków phf i 1183 g dla buhajków phf × bbb. Przyrosty mieszańców były o 10,94% większe niż cieląt czystorasowych. Wydajność rzeźna była wysoka w obu grupach genetycznych, ale o 3,4% wyższa w grupie mieszańców, które uzyskały również wyższe oceny uformowania tuszy w systemie klasyfikacji poubojowej tusz EUROP.

*MS. received in November 2014*

**Author's address:**

Tomasz Przysucha  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
02-786 Warszawa, ul. Ciszewskiego 8  
Poland  
e-mail: tomasz\_przysucha@sggw.pl



## The effect of fibre level in the mixture on the state of intestinal epithelium of fatteners

ANNA REKIEL<sup>1</sup>, JUSTYNA WIĘCEK<sup>1</sup>, MARZENA CICHOWICZ<sup>2</sup>, WOJCIECH BIELECKI<sup>3</sup>, PAULINA WIESZCZY<sup>4</sup>

<sup>1</sup> Department of Pig Breeding, Warsaw University of Life Sciences – SGGW

<sup>2</sup> Department of Pathomorphology, Military Medical Institute in Warsaw

<sup>3</sup> Department of Pathology of Exotic, Warsaw University of Life Sciences – SGGW

<sup>4</sup> Department of Gastroenterology, Hepatology and Clinical Oncology Centre of Postgraduate Medical Education

**Abstract:** *The effect of fibre level in the mixture on the state of intestinal epithelium of fatteners.* The purpose of the work was to determine the effect of the fibre content in the mixture on morphometric traits of intestinal epithelium of fatteners. In one-phase fattening, 14 crossbred pigs were classified into two groups and *ad libitum* fed the mixtures, differing in the composition and nutritional value, including the level of crude fibre (control group – C – 3.4%; experimental group – E – 12%) but with the maintained energy-protein ratio (1 : 13). At the age of 180 days, the fattening was completed; the weight of the pigs from C group was equal to ca. 100 kg and that one of the pigs from group E was by ca. 26 kg lower. The rate of growth of pigs from group E as compared to the animals from group C was slower ( $P \leq 0.01$ ). After slaughter, morphometric evaluation of the scrapes from the following three segments of the small intestine was carried out: duodenum, jejunum and ileum. There was found a lower mitosis index (the number of divisions per one crypt) of the intestinal epithelium cells of the pigs from group E as compared to group C (duodenum,  $P \leq 0.01$ ). The height of the epithelial cells in crypts and depth of crypts in three examined segments of small intestine (D, J, I) was higher in group E vs. group C ( $P \leq 0.05$ ;  $P \leq 0.01$ ), whereas the villus height was lower in group E vs. group C. The fibre level in the mixture affected the direction and level of changes in the examined indicators, characterizing the morphometric traits of intestinal epithelium.

*Key words:* fatteners, nutrition, fibre level, intestinal epithelium

### INTRODUCTION

The application of the mixtures with the increased fibre content, containing e.g. dried substances and/or pectins in the nutrition of monogastric animals constitutes the basis for the studies on the physiology of nutrition and digestion and utilization of nutrients and also, on the morphology and morphometry of intestines in the pigs and model animals (Jin et al. 1994, Pluske et al. 1997, Wenk 2001, Fusch et al. 2003, Hedemann et al. 2006, Drzikova et al. 2007, Rekiel et al. 2007, Serena et al. 2008, Rekiel et al. 2010, Hanczakowska and Świątkiewicz 2012, Święch et al. 2012).

Feed materials, containing considerable quantities of fibre, dried grass and cotyledonous plants, wheat brans, oats grain are not employed in intensive production of slaughter pigs, or they are administered in small quantities. It results from limitation of digestibility and assimilability of nutrients (Fusch et al.

2003). For growing pigs, piglets, weaners and fatteners under intensive production, it is recommended to employ full-ration mixtures with a low content of fibre and high digestibility. In extensive management, *inter alia*, ecological one, feed materials with a higher fibre content are used. Nutritional preferences of the pigs differing in age and physiological condition, connected with the production systems (intensive, organic) are the basis for the studies on feed materials (Fabijańska et al. 2003). They concern also the effect of diet, containing nutritional fibre on development of gastrointestinal tract. Depending on the source and level of fibre in the mixtures, the growth of small intestine and internal organs, liver and pancreas was examined in the rats (Faraldo Corrêa et al. 2009); in the pigs, the length and volume of gastrointestinal tract was studied (Jin et al. 1994). As affected by the feed components and the present fibre, intestinal mucus is changed. Its optimal level stimulates growth of intestinal villi and increases the number of crypts. In the crypts, the epithelial cells are proliferating and the factors of antibacterial resistance are produced (Paneth cells produce peptides – defensins) as well as endocrine factors, e.g. chromogranine A. The changes within the intestinal crypts increase or lower proliferating activity of enterocytes, contributing to the change in secreting activity of bactericidal factors and secretion of mucus (Mc Culough et al. 1998, Ayabe et al. 2000).

The aim of the work was to determine the effect of fibre content in the mixture on the morphometric traits of intestinal epithelium of fatteners.

## MATERIAL AND METHODS

One-phase fattening of 14 crossbred pigs of 4 breeds (PL × PLW) × (DUR × PIETR) was carried out; the animals were *ad libitum* fed from the initial body weight of ca. 25 kg in the intensive system (control group – C), or in the extensive system (experimental group – E). The groups consisted of 7 animals in each group, including 4 gilts and 3 barrows, from 7 litters. The mixtures for animals from groups C and E differed in composition and nutritional value (AOAC 1990), including crude fibre content (C – 3.4%; E – 12%) but they had the same ratio of energy to protein (Table 1).

TABLE 1. Composition of raw materials of the mixtures and their nutritive values

Specification	Group	
	C	E
Content		
Barley meal	64.0	64.0
Wheat meal	15.0	–
Extraction soy meal	13.5	–
Lard	7.0	–
Premix	0.5	0.5
Dried papilionaceous plants	–	35.5
Nutritive value		
Metabolizable energy (MJ/kg)	13.53	9.81
Crude protein (%)	17.75	12.80
Crude fiber (%)	3.4	12.0
Ratio of energy to protein (–)	1 : 13.1	1 : 13.0

After completion of fattening (age of 180 days), the animals were subject to standard slaughter procedures. From all pigs, the following samples from three segments of small intestines were *post mortem* collected: duodenum (D), the first loop of jejunum (J) and ileum (I), with dimensions of  $5 \times 20$  mm each. The collected material was washed out with 0.9% solution of physiological salt and then, preserved in 10% buffered formalin solution (for 24 h). The samples were immersed in paraffine (paraplast-Sigma). To protect material, Vacuum Infiltration Processor (V.I.P.) by SAKURA company was used.

Paraffine blocs, containing specimens of intestines were cut into series of scrapes of  $4 \mu\text{m}$  thickness in microtome and placed on basic slides, covered with protein (for histological staining). Staining of the scrapes was carried out using a review method: hematoxyline – eosine (H-E), using colouring machine DIVERSIFIED STAINER DRS-601 by SAKURA company. Then, the preparations were closed with a band, using closing device Coverslipper by SAKURA company.

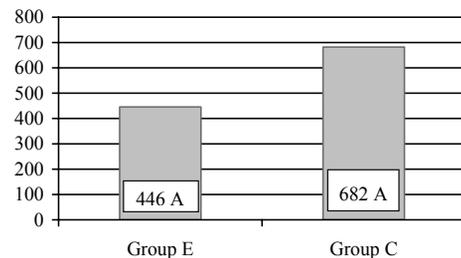
The evaluation of the preparations with magnitude of  $400\times$  was performed with the use of light microscope BX 50 Olympus. The number of partitions of enterocytes in crypt (mitotic index per 1 crypt), counting each time 100 cells in three repetitions was calculated. The height of villi and depth of crypts was also determined. The height of epithelial cells in crypts was evaluated, using

analysis of the image AnalySIS 3.0. The result was expressed as the mean for 10 measured cells.

The number of cells' division per 1 crypt – mitotic index, height of epithelium cells in crypts, villus height, depth of crypts in each intestine segment (duodenum, jejunum, ileum) among the groups were compared with the Wilcoxon test with the Bonferroni correction using R software.

## RESULTS AND DISCUSSION

The rate of growth of pigs from group E as compared to the animals from group C was slower (446 vs. 682;  $P \leq 0.01$ ) – Figure 1. The results of the own studies are confirmed by the results of another



A, A –  $P \leq 0.01$ .

FIGURE 1. Growth rate (g)

experiment (Fusch et al. 2003) in which various levels of fibre were employed in the mixtures for weaners. The increased participation of the fibre in feed for growing pigs caused abbreviation and decrease of the absorptive area of small intestine and prolongation of colon what slowed down the growth rate of the animals. Lower by 25.9 kg body weight of

the pigs from group E vs. group C (extensively fed in comparison to those ones fed intensively) as being found in own studies, confirmed a negative effect of the increased fibre level in the mixture on physiology of digestion and, in effect, on the growth rate (Wenk 2001).

The correct histological structure of intestinal wall was recorded. The detailed evaluation included image of intestinal crypts and border of mucus and intestinal villi. Any univocal directional changes in the research groups E and C, as being expressed by value of the examined indicators (number of partitions into 1 crypt, height of epithelial cells in crypts and height of villi and depth of crypts) in small intestine (Table 2) were recorded.

The number of partitions per 1 crypt was higher in group C vs. group E in one from three examined segments of small intestine – in duodenum ( $P \leq 0.01$ ). Values of the two examined indicators: height of enterocytes in crypts and depth

of crypts revealed domination in group E vs. group C in each from three studied segments of small intestine. In D, J, I segments of intestine, the height of epithelial cells in crypts was higher in group E vs. group C by 0.20 (0.94%), 2.84 (13.58%) at  $P \leq 0.05$ , and by 4.12 (22.34%) at  $P \leq 0.01$ , respectively; the depth of crypts was higher by 80.92 (22.01%), 51 (13.74%) and 48 (14.81%) at  $P \leq 0.05$ , respectively. In D, J, I segments of intestine, villi were shorter in group E vs. group C by 6.67 (1.48%), 6.04 (1.46%), 5.7 (1.31%).

Nutritional factors and bacteria, present in intestines, may modify their microstructure and change functioning of mucus (Babińska et al. 2005). It was also demonstrated that the level of fibre and feed components changed microflora of intestinal content of growing pigs (Pluske et al. 1997). The results, obtained in own studies, indicate that the increased level of fibre in the mixture (group E) changed

TABLE 2. Morphometry of intestines of fatteners mixtures with different fibre level (mediana and IQR)

Item	Intestine segment	Group	
		C	E
Number of cells' division per 1 crypt – mitotic index	Duodenum (D)	2 (2.00) A	1 (1.00) A
	Jejunum (J)	2 (1.00)	2 (0.50)
	Ileum (I)	2 (2.00)	2 (1.00)
Height of epithelium cells in crypts ( $\mu\text{m}$ ) – magnif. 20 $\times$	Duodenum (D)	21.35 (4.73)	21.55 (6.01)
	Jejunum (J)	20.91 (5.39) a	23.75 (8.55) a
	Ileum (I)	18.44 (4.33) A	22.56 (5.09) A
Villus height ( $\mu\text{m}$ ) – magnif. 4 $\times$	Duodenum (D)	450.49 (220.78)	443.82 (154.04)
	Jejunum (J)	413.12 (118.42)	407.08 (99.97)
	Ileum (I)	433.89 (101.53)	428.19 (82.40)
Depth of crypts ( $\mu\text{m}$ ) – magnif. 4 $\times$	Duodenum (D)	367.58 (129.76)	448.50 (119.50)
	Jejunum (J)	374.62 (97.75) a	426.10 (87.42) a
	Ileum (I)	326.60 (146.27)	374.96 (107.02)

A,  $A - P \leq 0.01$ ; a,  $a - P \leq 0.05$ .

the structure of intestinal epithelium of fatteners and its morphometric parameters. It was a “response” to different mixture which was administrated to experimental pigs. Slower rate of growth of pigs from group E vs. group C resulted from worse utilization (conversion) of nutrients in feed with the increased fibre level. The obtained results indicate and confirm the limited capacities of utilizing hardly digestible feed components by monogastric animals where enzymatic digestion is dominating. In case of pigs, the participation and activity of favourable intestinal microflora in digestion process is small (Pluske et al. 1997, Babińska et al. 2005).

## CONCLUSIONS

The pigs from the experimental group as compared to the animals from the control group were characterized by slower growth rate ( $P \leq 0.01$ ). It could be associated, *inter alia*, with morphological changes of intestinal epithelium. The number of partitions per 1 crypt was higher in group C vs. group E in case of duodenum ( $P \leq 0.01$ ). The height of the epithelial cells in crypts and depth of crypts in three examined segments of small intestine (D, J, I) was higher in group E vs. group C ( $P \leq 0.05$ ;  $P \leq 0.01$ ), whereas the villus height was lower in group E vs. group C. The results indicate the effect of differentiated content of fibre (derivate of diet components) on morphometric traits of intestinal epithelium. Preliminary results require confirmation on a larger sample.

## REFERENCES

- Association of Official Analytical Chemists (AOAC), 1990: Official Methods of Analysis of the Associated Official Analytical Chemists, Charter 32, Washington, DC.
- AYABE T., SATCHELL D., WILSON C., PARKS W., SELSTED M., OUELLETTE A., 2000: Secretion of microbial  $\alpha$ -defensin by Paneth cell in response to bacteria. *Nature Immunol.* 1, 113–118.
- BABIŃSKA J., ROTKIEWICZ T., OTROCKA-DOMAGAŁA., 2005: The effect of *Lactobacillus acidophilus* and *Bifidobacterium* spp. Administration on the morphology of the gastrointestinal tract, liver and pancreas in piglets. *Pol. J. Vet. Sci.* 8, 29–35.
- DRZIKOVA B., FLORIAN S., DONGOWSKI G., 2007: Histological and immunohistological evaluation of colon of rats fed dietary fibre-rich oat-based diets. *Nutr.* 31 (11), 445–454.
- FABIJAŃSKA M., KOSIERADZKA I., BEKTA M., 2003: Owies nagi w żywieniu trzody chlewnej i drobiu. Część I. Owies nagi w żywieniu tuczników i drobiu. Część II. Owies nagi w żywieniu tuczników. *Biul. Inst. Hod. Aklim. Roślin* 229, 317–328.
- FARALDO CORRÊA T.A., REIS S.M.P.M., DE OLIVEIRA A.C., 2009: Increase in digestive organs of rats due to the ingestion of dietary fiber with similar solubility to that of common bean. *Archiv. Latin. Nutr.* 59 (1), 47–53.
- FUSCH B., LEROCH R., SZUBA-TRZNADEL A., 2003: Wpływ różnego źródła i poziomu włókna surowego w mieszankach na długość i pojemność przewodu pokarmowego świń ubitych w wieku 90 i 180 dni. *Acta Scient. Polon. – Zootechn.* 2 (1), 35–46.
- HANCZAKOWSKA E., ŚWIĄTKIEWICZ M., 2012: Effect of herbal extracts on piglet performance and small intestinal epithelial villi. *Czech J. Anim. Sci.* 57 (9), 420–429.
- HEDEMANN M.S., ESKILDSEN M., LÓRKE H.N., PEDERSEN C., LINDBERG J.E., LAURINEN P., BACH KNUDSEN K.E., 2006: Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fibre concentrations and fiber properties. *J. Anim. Sci.* 84 (6), 1375–1386.

- JIN L., REYNOLDS L.P., REDMER D.A., CANTON J.S., CRENSHAW J.D., 1994: Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. *J. Anim. Sci.* 72 (9), 2270–2278.
- Mc CULLOUGH J.S., RATCLIFFE B., MANDIR N., CARR K.E., GOODLAD R.A., 1998: Dietary fibre and intestinal microflora: Effects on intestinal morphometry and crypt branching. *Gut* 42, 799–806.
- PLUSKE J.R., HAMPSON D.J., WILLIAMS I.H., 1997: Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest. Prod. Sci.* 51 (1–3), 215–236.
- REKIEL A., BIELECKI W., WIĘCEK J., 2010: The Effect of Probiotics on the Morphological Characteristics of the Small Intestinal Mucosa. *Acta Vet. Brno* 79, 519–524.
- REKIEL A., WIĘCEK J., BIELECKI W., GAJEWSKA J., KULISIEWICZ J., CICHOWICZ M., BATORSKA M., ROSZKOWSKI T., BEYGA K., 2007: Effect of addition of feed antibiotic flavomycin or prebiotic BIOS on production results of fatteners, blood biochemical parameters, morphometric indices of intestine and composition of microflora. *Archiv Tierz. Dummerstorf* 50, Special Issue, 172–180.
- SERENA A., HEDEMANN M.S., BACH KNUDSEN K.E., 2008: Influence of dietary fiber on luminal environment and morphology in the small and large intestine of sows. *J. Anim. Sci.* 86 (9), 2217–2227.
- ŚWIĘCH E., TUŚNIO A., TACIAK M., BORYCZKA M., BURACZEWSKA L., 2012: The effects of pectin and rye on amino acid ileal digestibility, threonine metabolism, nitrogen retention, and morphology of the small intestine in young pigs. *J. Anim. Feed Sci.* 21, 89–106.
- WENK C., 2001: The role of dietary fibre in the digestive physiology of the pig. *Anim. Feed Sci. Techn.* 90 (1–2), 21–33.

**Streszczenie:** *Wpływ poziomu włókna w mieszance na stan nabłonka jelit tuczników.* Celem pracy było określenie wpływu zawartości włókna w mieszance na cechy morfometryczne nabłonka jelit tuczników. W tuczu jednofazowym, 14 świń mieszańców podzielono na dwie grupy i żywiono do woli mieszankami różniącymi się składem i wartością pokarmową, w tym zawartością włókna surowego (grupa kontrolna – C – 3,4%, grupa doświadczalna – E – 12%), ale o zachowanym stosunku energetyczno-białkowym (1 : 13). W wieku 180 dni zakończono tucz; świnię z grupy C ważyły ok. 100 kg, a z grupy E o ok. 26 kg mniej. Tempo wzrostu świń z grupy E w porównaniu z grupą C było wolniejsze ( $P \leq 0,01$ ). Poubojowo dokonano oceny morfometrycznej skrawków trzech odcinków jelita cienkiego: dwunastnicy, jelita czczego i jelita biodrowego. Stwierdzono niższy indeks mitotyczny (liczba podziałów na 1 kryptę) komórek nabłonka jelit świń (dwunastnica,  $P \leq 0,01$ ) otrzymujących mieszankę o dużej zawartości włókna (grupa E) w porównaniu z grupą otrzymującą jego standardową zawartość w paszy (grupa C). Wysokość komórek nabłonka w kryptach i głębokość krypt w trzech badanych odcinkach jelita cienkiego (dwunastnica, jelito czcze i jelito biodrowe) miały większe wartości w grupie E w porównaniu z grupą C ( $P \leq 0,05$ ;  $P \leq 0,01$ ), a wysokość kosmków mniejszą. Poziom włókna w mieszance wpłynęła na kierunek i poziom zmian badanych wskaźników charakteryzujących cechy morfometryczne nabłonka jelit.

*MS. received in November 2014*

**Author's address:**

Anna Rekiel  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Trzody Chlewnej  
ul. Ciszewskiego 8, 02-786 Warszawa  
Poland  
e-mail: anna\_rekiel@sggw.pl

## Effect of sex on results of slaughter analysis of grey partridge *Perdix perdix*

AGNIESZKA WNUK<sup>1</sup>, MONIKA ŁUKASIEWICZ<sup>1</sup>, NATALIA MROCZEK-SOSNOWSKA<sup>1</sup>, JAN NIEMIEC<sup>1</sup>, BARTŁOMIEJ POPCZYK<sup>2</sup>, MAREK BALCERAK<sup>1</sup>

<sup>1</sup>Department of Animal Breeding, <sup>2</sup>Department of Animal Environment Biology  
Warsaw University of Life Sciences – SGGW

**Abstract:** *Effect of sex on results of slaughter analysis of grey partridge *Perdix perdix*.* The experimental material included grey partridges *Perdix perdix* planned for re-introduction into the natural habitat, reared at the Center of Game Breeding on the area of Mazovia Province. 10 hens and 10 roosters were selected for slaughter. After exsanguination, plucking and evisceration, the birds were cooled for 24 h to a temperature of 4°C. The carcasses were subjected to dissection to enable determination of dressing percentage and calculation in the carcass contents of: breast muscles, leg muscles, adipose fat and giblets. The study showed no effect of sex on results of slaughter analysis of grey partridge.

**Key words:** grey partridge, dressing percentage, game

### INTRODUCTION

It is estimated that ca. 94 species of partridges occur worldwide. They belong to the galliforms order (Galliformes), pheasant family (Phasianidae) and partridge subfamily (Perdicinae). Most of these birds inhabit open areas. Likewise pheasants, they are mainly represented by settled, non-migrating species. In Poland, grey partridge *Perdix perdix* occurs on the entire area of the country. This species inhabits open areas and prefers

habitats with high biological productivity (clumps of bushes, belts of brushwoods, midfield shrubs, foliage-fields) and with good access to water (Okarma and Tomek 2008). Partridges have been hunted for years but today this tradition is abandoned due to a significant decrease in the population number of these species on hunting grounds. Contemporarily, game birds appear on tables sporadically. However, in some homes and cultures, the hunter's cuisine is still meticulously cultivated and brings the plenitude of ideas, courses and flavors onto our tables. Unfortunately, numerous changes in the natural habitat of birds caused their lower number on hunting grounds (Panek 2012). The tradition of bird hunting is vanishing, however, hunting for pheasants and partridges is still popular in some regions of Poland, especially these where environmental conditions are unfavorable to the big game (Rancew-Sikora 2009).

Game meat has for years been playing the most significant role among meat courses. Partridge game is highly valued by consumers as the most delicate and

the most tasty meat of all game birds (Łebkowska and Łebkowski 1995). Only a few species of birds are still economically significant, therefore bird hunting has lost its commercial character. Nevertheless, in some countries, courses prepared from wild fowl are a local delicacy, are perceived as a luxury course, often claimed a tourist attraction (Wnuk et al. 2013). They are, simultaneously, a valuable dietary complement and variety (Wójcik et al. 2010).

The aim of this study was to determine the effect of sex on results of slaughter analysis of grey partridge.

## MATERIAL AND METHODS

The experimental material included grey partridges *Perdix perdix* planned for re-introduction into natural habits, reared at the Game Breeding Center on the area of Mazovia Province.

Complete feed mixtures were applied in the rearing period. In first 4 weeks of birds life, the feed mixtures contained: 29% of total protein, 11.5 MJ of metabolizable energy and 3.6% of crude fiber. Since 6<sup>th</sup> till 10<sup>th</sup> week of birds life the mixtures contained: 23% of total protein, 11.5 MJ of metabolizable energy and 4% of crude fiber. From 10<sup>th</sup> week of birds life till the end of rearing, the birds were fed diets, with a daily feed ration including up to 50 g of wheat and maize grain and *ad libitum* grass which included a mixture of maize, sunflower, alfalfa, grasses and marrows stem kale.

For first 4 weeks, partridges were kept indoors. Since then, they had free access to rearing aviaries that were partly roofed, with the roof covered with an electric cord to protect against predators. The aviaries with gravel-sand bottom were planted with vegetation and possessed natural hideaways in the form of rootstocks and large stones.

10 hens and 10 roosters aged of 14 weeks were selected for slaughter. After exsanguination, plucking and evisceration, the birds were cooled for 24 h to a temperature of 4°C. Cooled carcasses were weighed and subjected to a simplified carcass analysis according to methodology provided by Ziolecki and Doruchowski (1989). Dissection was performed at the laboratory of the Department of Poultry Breeding, Warsaw University of Life Sciences – SGGW. Once the carcasses had been cooled, dissection was performed to enable determining dressing percentage and calculating in the carcass contents of: breast muscles, leg muscles, abdominal fat and giblets.

Results achieved were elaborated statistically with the use of Student's t-test in SPSS 19.0 PL software (SPSS Inc., Chicago, IL, USA). Differences were found significant at  $P \leq 0.05$  and  $P < 0.01$ .

## RESULTS AND DISCUSSION

The conducted experiment did not show any differences in results of the slaughter analysis between sexes of grey partridge (Table 1). Body weight of roosters

TABLE 1. Results of slaughter analysis [g] of grey partridge *Perdix perdix*

Group	Body weight		Breast muscles		Leg muscles		Fat	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
♂♂	387.33	12.50	73.68	4.16	40.67	2.08	2.00	0.00
♀♀	380.67	12.22	83.67	4.16	45.33	11.24	1.33	0.57

was higher (387.33 g) than that of hens (380.67 g). A similar tendency was demonstrated by Kokoszyński et al. (2013), however, in their study the final body weight of birds was definitely lower and reached 301.8 g in roosters and 299.5 g in hens. Body weight of grey partridge changes in the annual cycle. Since November till January, they reach the maximum body weight, whereas since June till August, namely in the breeding period, they reach the lowest body weight.

significantly higher body weight reaching ca. 460 g is reported for rock partridges *Alectoris graeca*. When reared in closed runs, they reach a significantly ( $P \leq 0.01$ ) higher body weight of ca. 480 g (Ozdemir and Esen 2006).

In the conducted experiment, dressing percentage was similar in both sexes and reached ca. 68% (Table 2). According to Adamski (2012), carcasses of partridges are characterized by high dressing percentage of ca. 71.1%, which is similar to

TABLE 2. Results of slaughter analysis (%) of grey partridge *Perdix perdix*

Group	Dressing percentage		Breast muscles		Leg muscles		Fat	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
♂♂	68.55	3.58	26.78	2.49	15.37	2.08	0.65	0.05
♀♀	68.34	2.05	32.30	2.71	17.47	11.24	0.53	0.23

Males weigh from 340 to 410 g, and females from 350 to 395 g (Krupka 1986). Putaala and Hissa (1995) demonstrated that partridges reared in closed runs had higher ( $P < 0.05$ ) body weight compared to wild birds. A similar tendency may be observed in case of pheasants. Many authors (Hofbauer et al. 2010, Brudnicki et al. 2012) point to differences between birds reared in the hunting ground and birds reared in aviaries. Among partridges being of dietary importance, a

dressing percentage of broiler chickens that accounts for 70–71% on average in the EU Member States (Łukaszewicz 2008). Kokoszyński et al. (2013) also showed no effect of sex on dressing percentage, demonstrating a slightly higher value of this parameter at 72.1–72.4%. In turn, Łukasiewicz et al. (2011) demonstrated the effect of sex in pheasants, where dressing percentage was lower in roosters (71.90%) compared to hens (73.50%). The study of Ozdemir and

Esen (2006) shows that dressing percentage of rock partridge ranges from 71.88 to 72.85%. In the group of game birds, a similar dressing percentage as in our study was demonstrated for game pheasants: 70.4–70.8% (Kokoszyński et al.

No differences were demonstrated in the edible giblets between sexes of grey partridge (Table 3). Roosters were characterized by a higher mass of liver (7.67 g) and gizzard weight (6.67 g) compared to hens (5.33 and 6.00 g, re-

TABLE 3. Content of edible giblets in carcass of grey partridge *Perdix perdix*

Group	Liver				Heart				Gizzard			
	g		%		g		%		g		%	
	$\bar{x}$	SE										
♂♂	7.67	1.52	3.09	0.48	3.00	0.00	1.11	0.08	6.67	0.57	2.57	0.17
♀♀	5.33	0.57	2.04	0.55	3.00	0.00	1.16	0.07	6.00	0.00	2.32	0.14

2011), and slightly lower one for Mallard duck: 64.9–65.9% (Murawska et al. 2013), and for hazel grouses: 65.5% (Dzierżyńska-Cybulko and Fruziński 1997).

In our study, roosters were characterized by a higher percentage content of breast muscles and leg muscles, and a lower content of fat compared to hens. As reported by Adamski (2012), the content of breast muscle in partridge carcass reaches ca. 24.4%, that of leg muscle – 14.8%, and that of fat – 3–4% (skin + fat). In turn, Kokoszyński et al. (2013) demonstrated higher percentage contents of breast muscles at 30.7–31.1%, legs at 17.0–17.4%, and fat with skin at 5.3–5.5%. Similar contents of breast and leg muscles are found in game pheasants (27.5 and 19.1%). All cited works and our study showed no effect of sex on the percentage content of muscles.

The heart weight was equal in both sexes (3 g). In terms of percentage content in the carcass, a higher content was determined for liver and gizzard in roosters and for heart in hens. According to Adamski (2012), the content of edible giblets (heart, liver and gizzard) in carcass of grey partridge reaches 4.6%, whereas in game pheasant 4%. In the conducted experiment, the content of edible giblets was higher in roosters than in hens (4.46 vs. 3.76%). The study by Ozdemir and Esen (2006) demonstrated that in rock partridge it was significantly higher and reached 7.42%. In case of red-legged partridge, it was comparable to that noted in grey partridge, i.e. 4.07% (Millan et al. 2002). According to Putaala and Hissa (1995), wild partridges are characterized by higher heart, liver and gizzard weight. Similar conclusions were also reached by Liukkonen-Anttila et al. (1999).

## CONCLUSIONS

The conducted study showed no effect of sex on results of slaughter analysis of grey partridge. Noteworthy is the high dressing percentage of these birds.

## REFERENCES

- ADAMSKI M., 2012: Kuropatwy. [In:] Hodowla i użytkowanie drobiu. Red. J. Jankowski. Powszechnie Wydawnictwo Rolnicze i Leśne, Warszawa. 469–476.
- BRUDNICKA A., KUŁAKOWSKA A., PIETRUSZYŃSKA D., ŁOŻYCKA-KAPŁON M., WACH J., 2012: Differences in the amino acid composition of the breast muscle of wild and farmer pheasants. Czech J. Anim. Sci. 30(4), 309–313.
- DZIERŻYŃSKA-CYBULKO B., FRUZIŃSKI B., 1997: Dziczyzna jako źródło żywności. Wartość żywieniowa i przetwórcza. Państwowe Wydawnictwo Rolnicze i Leśne, Poznań.
- HOFBAUER P., SMULDERS F.J.M., VODNANSKY M., PAULSEN P., EL-GHAREEB W.R., 2010: A note on meat quality traits of pheasants (*Phasianus colchicus*). Eur. J. Wildl. Res. 56, 809–813.
- KOKOSZYŃSKI D., BERNACKI Z., CISOWSKA A., 2011: Growth and development of young game pheasants (*Phasianus colchicus*). Archiv Tierzucht. 54, 83–92.
- KOKOSZYŃSKI D., BERNACKI Z., KORYTKOWSKA H., WILKANOWSKA A., FRIESKE A., 2013: Carcass composition and meat quality of Grey Partridge (*Perdix perdix* L.). JCEA. 14(1), 378–387.
- KRUPKA J., 1986: Łowiectwo. Wyd. Rolnicze i Leśne, Warszawa.
- LIUKKONEN-ANTTILA T., PUTAALA A., HISSA R., 1999: Does shifting from a commercial to a natural diet affect the nutritional status of hand-reared grey partridges *Perdix perdix*? Wildlife Biol. 5(3), 147–156.
- ŁEBKOWSKA D., ŁEBKOWSKI D., 1995: Dziczyzna. Tenten, Warszawa, 1–93.
- ŁUKASIEWICZ M., MICHALCZUK M., GŁOGOWSKI R., BALCERAK M., POPCZYK B., 2011: Carcass efficiency and fatty side content of farmer pheasants (*Phasianus colchicus*) meat. Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 49, 199–203.
- ŁUKASZEWICZ E., 2008: Choroby drobiu oraz ptaków ozdobnych, materiały szkoleniowe. Ed. M. Mazurkiewicz. Wrocław.
- MILLAN J., GORTAZAR C., BUENESTRADO F.J., RODRIGUEZ P., TORTOSA F.S., VILLAFUERTE R., 2002: Effect of a fiber-rich diet on physiology and survival of farm-reared red-legged partridges (*Alectoris rufa*). Comp. Biochem. Phys. A. 134, 85–91.
- MURAWSKA D., JANISZEWSKI P., MICHALIK D., HANZAL V., ZAWADZKA M., 2013: Carcass and meat characteristics of mallards (*Anas platyrhynchos* L.) hunter-harvested in North-Eastern Poland. XXV International Poultry Symposium PB WPSA. 02–04 September. Zegrze, Poland, 143.
- OKARMA H., TOMEK A., 2008: Łowiectwo. Wyd. Edukacyjno-Naukowe H2O, Kraków, 73–77.
- OZDEMIR G., ESEN F., 2006: Feed performance and carcass characteristics of Rock Partridges (*A. graeca*) in intensive conditions. Journal of Animal and Veterinary Advances 5(5), 356–360.
- PANEK M., 2012: Demografia kuropatwy w zależności od struktury krajobrazu rolniczego. Wyd. SGGW. Warszawa (rozprawa habilitacyjna).
- PUTAALA A., HISSA R., 1995: Effects of hand-rearing on physiology and anatomy in the grey partridge. Wildlife Biol. 1, 27–31.
- RANCEW-SIKORA D., 2009: Sens polowania. Wyd. Naukowe Scholar, Warszawa.
- WNUK A., MROCZEK-SOSNOWSKA N., ŁUKASIEWICZ M., 2013: Dzikie ptactwo ze staropolskiego stołu. Polskie Drob. 10, 40–43.
- WÓJCIK K., SOBCZAK M., ŻOCHOWSKA-KUJAWSKA J., ZIELIŃSKI K., 2010: Porównanie tekstury i struktury oraz podatności na proces masowania mięśni danieli (*Dama dama*). Żywność. Nauka. Technologia. Jakość 1(68), 93–104.
- ZIOŁECKI J., DORUCHOWSKI W., 1989: Metoda oceny wartości rzeźnej drobiu. Wyd. COBRD, Poznań.

**Streszczenie:** *Wpływ płci na wyniki analizy rzeźnej kuropatwy polnej *Perdix perdix*. Materiał badawczy stanowiły kuropatwy polne *Perdix perdix* przeznaczone do wsiedlenia do środowiska naturalnego, odchowywane w Ośrodku Hodowli Zwierzyny na terenie województwa mazowieckiego. Do uboju wybrano po 10 kur i 10 kogutów. Ptaki po skrwawieniu, oskubaniu i wypatroszeniu schłodzone w ciągu 24 h do temperatury 4°C, przeprowadzono dysekcję, na podstawie której określono wydajność rzeźną oraz obliczono udział w tuszce: mięśni piersiowych, mięśni nóg, tłuszczu sadełkowego i podrobów. Nie wykazano wpływu płci na wyniki analizy rzeźnej kuropatwy polnej.*

*MS. received in November 2014*

**Authors' address:**

Agnieszka Wnuk, Monika Łukasiewicz,  
Natalia Mroczek-Sosnowska, Jan Niemiec,  
Marek Balcerak  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Drobiu  
Ciszewskiego 8, 02-786 Warszawa  
Poland

Bartłomiej Popczyk  
Wydział Nauk o Zwierzętach SGGW  
Katedra Biologii Środowiska Zwierząt  
Ciszewskiego 8, 02-786 Warszawa  
Poland

## The role of vestibulum in the nests of the red mason bee *Osmia bicornis* L.

BARBARA ZAJDEL<sup>1</sup>, JAKUB GĄBKA<sup>1</sup>, KORNELIA KUCHARSKA<sup>2</sup>,  
DARIUSZ KUCHARSKI<sup>3</sup>

<sup>1</sup>Bee Division, Warsaw University of Life Sciences – SGGW

<sup>2</sup>Department of Zoology, Warsaw University of Life Sciences – SGGW

<sup>3</sup>Department of Ecology, Warsaw University

**Abstract:** *The role of vestibulum in the nests of the red mason bee Osmia bicornis* L. The function of vestibulum (the outermost empty brood cell, without provisioning mass and larvae) has not been precisely determined so far. It is most probably a behavioral relic, which currently has no adaptive value. However, red mason bees often build vestibulum. In this study, we examined the nest tubes of red mason bees in three sites (Kłoda, Kanie and Warsaw) in Mazovian Province and in one site (Sapłaty) in Warmian-Masurian Province. About 70% of the nest tubes in Kłoda, Kanie and Warsaw had vestibulum, whereas only 29% of the tubes in Sapłaty had vestibulum. It can be assumed that the vestibulum protects the brood against unstable atmospheric conditions, and the proximity of the lake Sapłaty reduced the temperature amplitude. It was found, however, that the vestibulum did not protect the brood cell against nest parasitoides and cleptoparasites. More cells were infested by parasites and kleptoparasites in tubes with vestibulum.

*Key words:* *Osmia bicornis* L., red mason bees, vestibulum, parasitoides, cleptoparasites

### INTRODUCTION

Nests of many Aculeata have empty cells in variable numbers and positions (Linsley 1958, Krombein 1967, Tepedino et al. 1979). Red mason bees

*Osmia bicornis* L. build linear nests in horizontal holes in wood or reed tubes *Phragmites australis*. The bees leave the outermost cell of the nest (so-called vestibulum) empty, without provisioning mass and egg/larvae. Many authors have tried to explain the purpose of the vestibulum built by bees of the genus *Osmia*. In nests which are exposed to unstable weather conditions, the vestibulum protects the brood against temperature fluctuations (Seidelmann 1999). Another purpose of vestibular cell is to discourage parasites and predators from entering the nest (Krombein 1967). According to the probability models by Tepedino et al. (1979), empty, but closed cells can reduce the success of parasitoides of mud-nesting Hymenoptera. According to Krombein (1967), the vestibulum is a behavioral relic, which currently has no adaptive value. Accompanying fauna and parasitoides are major problems in mason bee keeping and may restrict the mason bee population, particularly in large bee aggregations (Wójtowski and Wilkaniec 1969, Wójtowski and Szymaś

1973, Szymaś 1991). Parasitoides and cleptoparasites usually enter open nest cells at the time the female is collecting pollen. The longer the female leaves the nest unattended, the more likely are parasites and cleptoparasites to lay their eggs inside. The risk of parasitism in open cells is correlated with the provisioning time (Seidelmann 2006). This is why in mason bee keeping it is important to provide bees with food base rich in flower pollen, which shortens the time of collecting pollen by the female and, additionally, results in obtaining larger and more effective pollinators (Giejdasz et al. 2005). The aim of this research was to determine the frequency at which bees build vestibulum and whether its presence limits the number of cells occupied by parasitoides and cleptoparasites.

## MATERIAL AND METHODS

The material consisted of reed tubes nested by red mason bees *Osmia bicornis*, taken from four nesting sites:

M1 – Kłoda, M2 – Sąplaty, M3 – Kanie, M4 – SGGW. Artificial nest and cocoons placed first time in M1, M2 and M3, while in M4 red mason beehad been nesting every year for 10 years. All cocoons became from one populations breded of Apicultural Division Warsaw Uvniversity of Life Science – SGGW. All the nesting sites had similar foraging conditions for the development of the solitary bee populations (mixed orchard apple and plum, home gardens, mixed forest). The M1, M3 and M4 sites were located in the Mazovian Province, while the M2 site was located in the Warmian-Masurian Province, in the vicinity of the lake Sąplaty. Table 1 presents the mean daily temperatures in the period of bee flights. Four hundreds nest tubes were chosen randomly from each nesting site and analyzed. The nest contents were examined between October 2012 and February 2013 in the Bee Division of the Warsaw University of Life Sciences – SGGW. All the tubes were

TABLE 1. Daily temperature and nest exposure at annual and perennial nesting sites

Specification	Mean daily temperature* (°C)			
	M1 – Kłoda	M2 – Sąplaty	M3 – Kanie	M4 – SGGW
April	9.4	5.6	10.0	9.6
May	15.1	14.2	14.0	15.5
June	17.6	16.3	17.6	17.1
July	22.3	19.7	20.6	20.8
August	19.2	17.9	19.1	19.4
Overall mean temperature	16.7	14.7	16.3	16.5
Nest exposure	south-east	south-east	south-east	south

\*The weather data from the nearest meteorological stations were provided from <http://pogoda.ekologia.pl/>.

opened with a scalpel and the cells with well developed cocoons, dead larvae and nest parasitoides and cleptoparasites were counted. The frequency of building a vestibulum by bees was determined. Whether the presence of vestibulum in a reed tube affected the numbers of well-developed cocoons, dead larvae and the number of brood cells infested by three species of the most commonly found parasitoides and cleptoparasites (*Cacoxenus indagator*, *Monodontomerus obscurus*, *Chaetodactylus osmiae*) was examined by analyzing the data on the nest contents of red mason bees. The statistical analyses were performed by the SPSS 17 software. The normality of data distributions was verified using Kolmogorov-Smirnov test and Shapiro-Wilk test. Non-parametric Chi-square, Kruskal-Wallis and Mann-Whitney U tests were used for comparing the groups.

## RESULTS

### Distributions of the analyzed data

The distributions of all analyzed features differed significantly from the normal distribution (Kolmogorov-Smirnov test and Shapiro-Wilk test,  $P \leq 0.001$ ). The distribution of the number of vestibular cells was slightly to moderately skewed – between  $-1$ ;  $1$ , the number of fully formed cocoons, dead larvae, parasitoides and cleptoparasites was slightly to highly skewed – between  $-1$ ;  $9$ .

### Frequency of constructing vestibulum by the red mason bee

Almost 70% of tubes in the M1, M3 and M4 sites had a vestibulum. Bees nesting in the M2 site left the outermost brood cell empty significantly less (in every third tube) than in sites M1 ( $\chi^2 = 44.63$ ,  $df = 1$ ,  $P < 0.001$ ), M3 ( $\chi^2 = 42.98$ ,  $df = 1$ ,  $P < 0.001$ ) and M4 ( $\chi^2 = 48.38$ ,  $df = 1$ ,  $P < 0.001$ ) – Table 2.

TABLE 2. Frequency of a vestibular cell in nest traps for solitary bees *O. bicornis* L. in new and perennial nesting sites

Nesting site	Number of tubes	Tubes with vestibulum			
		No	%	Median	Skewness
M1 Kłoda	400	276	69.0	1 a*	0.97
M2 Sapłaty	400	116	29.0	0 b	0.93
M3 Kanie	400	272	68.5	1 a	-0.77
M4 SGGW	400	287	71.8	1 a	0.12

\*Different letters in columns indicate significant differences in mean ranks of the groups (Kruskal-Wallis test:  $\chi^2 = 799.00$ ,  $df = 3$ ,  $P < 0.001$ ; pairwise comparisons: Chi-square test,  $P < 0.01$ ).

### Impact of vestibular cells on the number of fully formed cocoons, dead larvae and parasitism

The results demonstrated that significantly more healthy cocoons were obtained from nest tubes lacking vestibulum than from tubes without vestibulum in M1, M3 and M4 sites (Mann-Whitney U test,  $P < 0.00$ ). The numbers of parasitized cells and cells with dead larvae were similar in tubes with and without vestibulum (Tables 3 and 4).

### DISCUSSION

In all the nests (M1, M2, M3 and M4) bees built vestibulum. The vestibulum protects the brood from unstable en-

vironmental conditions (Seidelmann 1999). In nests exposed to sun and high temperature bees built vestibulum more frequently (Seidelmann 1999). The M2 site, Sapłaty, is located next to the lake Sapłaty in the Warmian-Masurian Province, which has a cooler climate (Table 1), but also smaller temperature amplitudes. This is probably why bees from the M2 site built fewer vestibulum – only in ca. 30% of the tubes, while at the other sites (M1, M3 and M4) vestibuli were found in ca. 70% of the tubes (Table 2). It can be concluded that the frequency of building vestibulum by mason bees is mainly influenced by environmental conditions (e.g. temperature amplitudes, lower diurnal temperatures).

TABLE 3. Fully formed cocoons and dead larvae in nest tubes with and without vestibulum in M1 (Kłoda), M2 (Sapłaty), M3 (Kanie) and M4 (SGGW)

Specification	Mean	Median	Skewness	Mean	Median	Skewness
M1 – Kłoda	With vestibulum (71.3%*)			Without vestibulum (28.7 %)		
No of cocoons	6.77 a**	7	0.2	7.23 b	7	-0.15
No of dead larvae	0.74 a	0	2.13	0.71 a	0	2.54
M2 – Sapłaty	With vestibulum (29%)			Without vestibulum (71%)		
No of cocoons	7.08 a	7	0.06	6.91 a	7	0.01
No of dead larvae	0.85 a	0	1.28	0.84 a	0	1.71
M3 – Kanie	With vestibulum ( 69%)			Without vestibulum (31%)		
No of cocoons	6.94 a	7	-0.04	6.01 b	6	-0.15
No of dead larvae	0.28 a	0	2.25	0.33 a	0	1.14
M4 – SGGW	With vestibulum (68%)			Without vestibulum (32%)		
No of cocoons	5.93 a	6	-0.11	5.37 b	6	-0.7
No of dead larvae	0.66 a	0	2.57	1.13 a	1	1.74

\*Percentage of tubes with/without vestibulum in all analyzed tubes.

\*\*Different letters in the same lines indicate significant differences in mean ranks of the groups (Kruskal-Wallis test,  $P < 0.01$  and Mann-Whitney U test,  $P < 0.01$ ).

TABLE 4. Parasitoids and cleptoparasites in nest tubes with and without vestibulum in M1 (Kłoda), M2 (Sapłaty), M3 (Kanie) and M4 (SGGW)

Specification	Mean	Median	Skewness	Mean	Median	Skewness
M1 – Kłoda	With vestibulum (71.3%*)			Without vestibulum (28.7%)		
<i>C. indagator</i>	0.27 a**	0	2.18	0.29 a	0	4.05
<i>M. obscurus</i>	0.05 a	0	5.07	0.02 a	0	7.77
<i>Ch. osmiae</i>	0.11 a	0	5.04	0.16 a	0	4.48
M2 – Sapłaty	With vestibulum (29%)			Without vestibulum (71%)		
<i>C. indagator</i>	0.36 a	0	3.79	0.24 a	0	2.91
<i>M. obscurus</i>	0.13 a	0	3.60	0.02 a	0	8.82
<i>Ch. osmiae</i>	0.11 a	0	3.30	0.09 a	0	4.81
M3 – Kanie	With vestibulum (69%)			Without vestibulum (31%)		
<i>C. indagator</i>	0.47 a	0	3.29	0.54 a	0	3.06
<i>M. obscurus</i>	0.13 a	0	2.77	0.18 a	0	2.60
<i>Ch. osmiae</i>	0.03 a	0	7.23	0.05 a	0	4.34
M4 – SGGW	With vestibulum (68%)			Without vestibulum (32%)		
<i>C. indagator</i>	0.91 a	0	1.56	1.2 a	1	1.65
<i>M. obscurus</i>	0.13 a	0	3.60	0.20 a	0	2.95
<i>Ch. osmiae</i>	0.32 a	0	3.30	0.35 a	0	2.01

\*Percentage of tubes with/without vestibulum in all analyzed tubes.

\*\*Different letters in the same lines indicate significant differences in mean ranks of the groups (Kruskal-Wallis test:  $P < 0.01$  and Mann-Whitney U test:  $P < 0.01$ ).

It has been hypothesized that these vestibular cells function to discourage penetration of parasitoides and predators to the stored cells (Krombein 1967). Nevertheless, our observations during the present study demonstrated that the occurrence of vestibulum did not decrease the number of cells in the tubes occupied by parasitoides/cleptoparasites (Table 4). Unexpectedly, more cocoons were obtained from the nesting tubes which had no vestibular cell in M1, M3 and M4 sites (Table 3).

It may be concluded, therefore, that the main function of vestibular cells built by mason bees is most probably to protect the nest against harmful at-

mospheric conditions (e.g. overheating) rather than against parasites and accompanying fauna.

## CONCLUSIONS

1. The frequency of building vestibular cells by red mason bees is probably influenced by unstable environmental conditions. In Sapłaty where the temperatures during bee flights were lower and the temperature amplitudes were smaller, only 29% of the nest tubes had a vestibulum.
2. The vestibulum does not protect mason bee nests against nest parasitoides and cleptoparasites.

## REFERENCES

- GIEJDASZ K., WILKANIEC Z., PIECH K., 2005: Effects of seed onion pollination by red mason bee females *Osmiarufa* L. (Apoidea: Megachilidae) with different body weights. *J. Apic. Sc.* 49 (2), 21–27.
- KROMBEIN K.V., 1967: Trap-nesting wasps and bees: Life Histories, Nests, and Associates. Smithsonian Press, Washington.
- LINSLEY E.G., 1958: The ecology of solitary bees. *Hilgardia* (Berkeley) 27(19) 543–599.
- SEIDELMANN K., 1999: The function of the vestibulum in nests of a solitary stem-nesting bee, *Osmiarufa* (L.). *Apidologie* 30 (1), 19–29.
- SEIDELMANN K., 2006: Open-cell parasitism shapes maternal investment patterns in the red mason bee *Osmiarufa*. *Behav. Ecol.* 17(5): 839–848.
- SZYMAŚ B., 1991. Entomofauna pasożytnicza ograniczająca populację pszczół samotnie żyjących (Apidaesolitariae). 35 (3-5), 307–313.
- TEPEDINO V.J., MCDONALD L.L., ROTHWELL R., 1979: Defence against parasitization in mud-nesting Hymenoptera: Can empty cells increase net reproductive output? *Behav. Ecol. Sociobiol.* 6, 99–104.
- WÓJTOWSKI F., SZYMAŚ B., 1973: Entomofauna pasożytnicza i towarzysząca pszczołom samotniczym (Apoideasolitariae) w pułapkach gniazdowych. *Rocz. AR. w Poznaniu* 66, 171–179.
- WÓJTOWSKI F., WILKANIEC Z., 1969: Próby hodowli pszczół miesiarek i murarek (Hymenoptera, Apoidea, Megachilidae) w pułapkach gniazdowych. *Rocz. AR. w Poznaniu* 42, 153–165.
- Streszczenie:** Funkcja vestibulum w gnieździe murarki ogrodowej *Osmia bicornis* L. Funkcja vestibulum (najbardziej zewnętrznej komory gniazdowej) nie została dotychczas dokładnie określona. Prawdopodobnie jest to behawioralny relikwyt obecnie bez żadnej wartości adaptacyjnej. Jednak pszczoły chętnie budują vestibulum. W trzech miejscach badań: Kłoda, Kanie, Warszawa (woj. mazowieckie), w 70% rurek gniazdowych znajdował się przedsionek. Stwierdzono, że murarki gniazdujące w Sapłatach (woj. warmińsko-mazurskie) vestibulum wybudowały jedynie w 29% rurek. Można przypuszczać, że przedsionek chroni czerw przed zmiennymi warunkami atmosferycznymi, a bliskość jeziora łagodziła amplitudy temperatury. Stwierdzono natomiast, że vestibulum nie zabezpieczało komórki z czerwiem przed pasożytami i kleptopasożytami gniazdowymi. W rurekach, które miały vestibulum, stwierdzono więcej komórek porażonych przez pasożyty i kleptopasożyty.

*MS. received in November 2014*

### Authors' addresses:

Barbara Zajdel, Jakub Gąbka  
Wydział Nauk o Zwierzętach SGGW  
Pracownia Pszczelnictwa  
02-787 Warszawa, ul. Nowoursynowska 166  
Poland

e-mail: bzajdel@o2.pl

Kornelia Kucharska  
Wydział Nauk o Zwierzętach SGGW  
Katedra Biologii Środowiska Zwierząt  
Zakład Zoologii  
02-787 Warszawa, ul. Ciszewskiego 8  
Poland

Dariusz Kucharski  
Uniwersytet Warszawski  
Wydział Biologii  
Instytut Zoologii, Zakład Ekologii  
02-097 Warszawa, ul. Żwirki i Wigury 101  
Poland

## Behaviors of the corvids towards common buzzard *Buteo buteo* on urban and extra-urban areas of the Mazowieckie Province

IWONA BIELA<sup>2</sup>, MARLENA KOŹLIŃSKA<sup>2</sup>, PAULINA SOSNOWSKA<sup>2</sup>,  
DOROTA JĘDRUCHÓW<sup>2</sup>, MONIKA ŁUKASIEWICZ<sup>1</sup>

<sup>1</sup> Department of Animal Breeding, <sup>2</sup> „Aves” Scientific Circle  
Warsaw University of Sciences – SGGW

**Abstract:** *Behaviors of the corvids towards common buzzard Buteo buteo on urban and extra-urban areas of the Mazowieckie Province.* The study was based on observations of birds of the crow family (Corvidae) since May till November, during intensive training of a raptor common buzzard *Buteo buteo* on various areas – urban and extra-urban, of the Mazowieckie Province at various times of the day. A distinct difference was noted in the activity of the corvids depending on land development. In the study period, 305 birds were recorded, the majority of which (n = 276) were noted on the urban area, which constituted 91% of all birds observed in the entire study period. The evaluation was also significantly affected by day time. In the afternoon hours, the activity of birds towards common buzzard was significantly higher and reached 65% on average. A lower activity of the birds was observed on extra-urban areas where representatives of the crow family – Eurasian jay *Garrulus glandarius* and Eurasian magpie *Pica pica* – showed minimal interest in the bird of prey. They were undertaking individual attempts of scaring the raptor off, without intensive alerting signals and calling other individuals.

**Key words:** corvids, raven, common buzzard, behaviors

## INTRODUCTION

Behaviors of birds are currently addressed in many studies, while our knowledge on this exquisitely fascinating subject is

still fragmentary. The problem relies, in part, in the fact that these behaviors often attain many forms that also result from various environments.

In Europe, the corvids are represented by 12 species. In Poland, regular hatches are observed for 7 species: rook *Corvus frugilegus*, hooded crow *Corvus cornix*, raven *Corvus corax*, magpie *Pica pica*, jackdaw *Corvus monedula*, jay *Garrulus glandarius*, and spotted nutcracker *Nucifraga caryocatactes*. The raven *Corvus corax* (Linnaeus, 1758) is the least “singing” and at the same time the largest representative of the order of passerines *Passeriformes*. The syntaxonomic position of raven, determined based on DNA analysis, is as follows: order Passeriformes, sub-order Oscines, superfamily Corvoidea, family Corvidae, subfamily Corvinae, and species *Corvus* (Glangt 2003, after Sibeley and Ahlquist 1990). The raven is a large bird, in size resembling common buzzard, with completely black (raven-black) plumage having a metallic sheen. Also feathers of raven are in size similar to those of common buzzard and, additionally, have a characteristic scent absent in other Accipitriformes (Zawadzka 2006).

Great environmental plasticity and capability of utilizing food residues left by man made that for hundreds of years the corvids have been accompanying man. In recent years, the population of corvid family birds (Corvidae) has rapidly increased on urban areas (Mazgajski and Szczepanowski 2005). It was ascribed to the easiness of acquiring feed and a reduced number of raptors. The corvids have been shown to exert a strong impact on the nesting success of many species of birds (Tomiałojć and Stawarczyk 2003, Zduniak 2005). In addition, their role in ecosystems stirs up many emotions amongst biologists, hunters, officials administering the bird-protecting areas and institutions responsible for natural environment preservation in Poland, by making this group a conflicting one (Mazgajski and Szczepanowski 2005). For a few decades, a very large (over 100,000 birds) population of the corvids is observed to appear each year in Warsaw at the beginning of November and to leave the city at the turn of February and March. In Poland, the corvids occur both in forests, on open areas as well as on fringes of arable lands and forests. The majority of this population is nesting in forests. If they do nest in large agglomerations, it is always on their outskirts (Luniak et al. 2001, Tomiałojć and Stawarczyk 2003).

Common buzzard *Buteo buteo* is a slender bird of prey with elongated tail and long sharp-ended wings that during winging are kept in the form of a shallow letter "V". Its population is averagely numerous. It inhabits the entire area of the country and its biotopes include forests (usually their fringes) or fringes of forest enclaves (Dudziński 1988). Common buzzard feeds with small rodents of the

rural and field landscape (Perrins 1998), small passerines and other birds. Birds constitute the second in line component of its diet (Goszczyński and Piłatowski 1986, Jędrzejewska and Jędrzejewski 2001, Reif et al. 2001, Goszczyński et al. 2005, Skierczyński 2006).

The biology of birds, their behaviorism, migrations and geography of routes are very interesting and increasingly better recognized. But still little is known on the dynamics of the flock – including species and spatial dynamics, causes of sudden "plays" during flight, behaviors towards other bird species, or alerting signals. Therefore, any action that allows extending knowledge on birds is valuable.

In view of the above, this study was aimed at evaluating behavioral differences of the corvids on the urban and extra-urban areas of the Mazowieckie Province toward birds of prey.

## MATERIAL AND METHODS

The study was based on observations of the corvid family birds (Corvidae) since May till the end of November, during intensive trainings of a raptor common buzzard *Buteo buteo* in various areas – urban and extra-urban, of the Mazowieckie Province at different times of the day without rain. Observations were registered with photograms and short videos, and meticulously described.

## RESULTS AND DISCUSSION

The analysis of results of observations demonstrated a significant difference in the activity of the corvids depending on land development. A similar tendency

was noted by Mazgajski and Szczepanowski (2005).

In the course of the study, a total of 305 birds were noted, the majority of which were observed on the rural areas (i.e. 276 birds, 91% of all birds observed in the study period). The evaluation of birds behavior was also significantly affected by the time of the day. The activity of the corvids towards common buzzard was remarkably higher in the hours before the afternoon and reached 65% on average (Table 1). Since the results of the observations were often similar in the amount of observed birds in the diagram shows the chosen dates.

The corvids (Fig. 1) are fiercely territorial birds (Mazgajski and Szczepanowski 2005, Zduniak 2005). Our study showed that most of the birds colonizing the area, where the raptor common buzzard *Buteo buteo* was appearing, were raising a significant verbal alert. It needs

to emphasized that the birds are recognizing one another inside the flock. After alerting signals, the corvids were suddenly flying off and were hovering in smaller and smaller circles around the raptor or were sitting on nearby trees, brickworks, roof of buildings, and were calling their fellows. We also observed attempts of attacks on the claw bird with, e.g. twigs, acorns and other materials available to the corvids, that were aimed at scaring the intruder off. The main aggressors were birds with predominating positions in the flock. According to Henrich (1989, 1999), the group actions of the corvids are aimed, most of all, at satisfying the needs of an individual, and not the community. These actions include the need to break the defense line of protecting the resources by owners of the territory (hatching mates of ravens) and scaring off the species competing for feed – carrion.

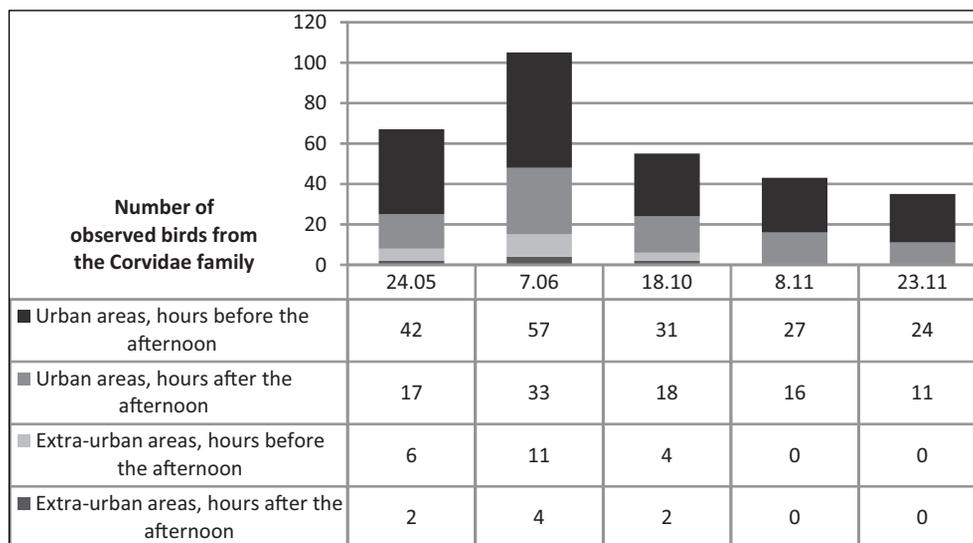


TABLE 1. Activity of the corvids depending on the site and time of observations



FIGURE 1. The corvids – from left raven, hooded crow and jackdaw (authoress: Paulina Sosnowska)

Lower activity was observed on the extra-urban areas, where representatives of the corvids – jay and magpie – expressed minimal interest in the raptor. They were undertaking individual attempts of scaring the raptor off, without intensive alerting signals and calling other individuals. Such a different behavior was, probably, due to conditions occurring in these two extremely different environments. Excellent living conditions, lack of natural enemies, easiness in acquiring food, and living in large flocks – that are characteristic for the developed area – contributed to the elimination of the instinct of natural escape from the raptor in the group of the corvids. Most of relations with large birds of prey are examples of commensalism, dietary parasitism, competitiveness or predation.

It is likely that the enhanced activity of the corvids in the hours before afternoon could be due to disturbance of

uneventful feeding of their community by the predator. Slightly different observations were made by Selva (2004) in the Białowiecki Primeval Forest. This author stated that although ravens were attacking all birds except for jay, most of their aggressive behaviors were targeted at individuals of their own species, i.e. as many as 94% of cases, and that only 4% of cases referred to attacks on common buzzard and 2% to attacks on white-tailed eagles. The white-tailed eagle, definitely predominating in this community, was attacking most of all ravens (90%). Ravens were also targets of attacks by common buzzard (89%), whereas other common buzzards were attacked significantly less frequently.

Because attacks hardly ever ended in a definite victory of one of the sides, it is difficult to determine explicitly the hierarchy between common buzzard and raven. It may be hypothesized that, in

this case, the common buzzard was the “dominant” owing to the presence of man during bird training.

## SUMMARY

The corvids are characterized by specific intelligence and interesting behaviors in the flock; what is more, they are capable of acting in a group. Raven being a numerous and controversial species in Poland, ought to be definitely more often a subject of complex investigations.

## REFERENCES

- DUDZIŃSKI W., 1988: Ptaki łowne, PWRiL Warszawa.
- GOSZCZYŃSKI J., PIŁATOWSKI T., 1986: Diet of common buzzards (*Buteo buteo* L.) and goshawks (*Acipiter gentilis* L.) in the nesting period. *Ekologia Polska* 34: 655–667.
- GOSZCZYŃSKI J., GRYZ J., KRAUZE D., 2005: Fluctuations of a Common Buzzard *Buteo buteo* population in Central Poland. *Acta Ornithologica* 40: 75–78.
- HENRICH B., 1989: Ravens in Winter. Vintage Books, New York.
- HENRICH B., 1999: Mind of the Raven. Harper Collins Publisher Inc., New York.
- JĘDRZEJEWSKA B., JĘDRZEJEWSKI W., 2001: Ekologia Zwierząt Drapieżnych Puszczy Białowieskiej. Wydawnictwo Naukowe PWN, Warszawa.
- LUNIAK M., KOZŁOWSKI P., NOWICKI W., PLIT J., 2001: Ptaki Warszawy 1962–2000 – Atlas Warszawy 8. IGPiP PAN, Warszawa.
- MAZGAJSKI T.D., SZCZEPANOWSKI R., 2005: Liczebność zgrupowania ptaków krukowatych zimujących w Warszawie. [In:] Ptaki krukowate Polski. Bogucki. Wyd. Nauk., Poznań: 427–434.
- PERRINS C., 1998: The Complete Birds of the Western Palearctic [CD-ROM]. Oxford University Press, Oxford.
- REIF V., TORNBORG R., JUNGELL S., KORPIMÄKI E., 2001: Diet variation of common buzzards in Finland supports the alternative prey hypothesis. *Ecography* 24: 267–274.
- SELVA N., 2004: The role of scavenging in the predator community of Białowieża Primeval Forest. PhD Thesis. Mammal Research Institute Academy of Sciences.
- SIBLEY C.G., AHLQUIST J.E., 1990: Phylogeny and classification of birds: A study in molecular evolution. Yale Univ. Press, New Haven, CT.
- SKIERCZYŃSKI M., 2006: Food niche overlap of three sympatric raptors breeding in agricultural landscape in Western Pomerania region of Poland. *Buteo* 15: 17–22.
- TOMIAŁOJĆ L., STAWARCZYK S., 2003: Awifauna Polski. Rozmieszczenie, liczebność i zmiany. PTPP “pro Natura”, Wrocław.
- ZAWADZKA D., 2006: Kruk. Wydawnictwo Klubu Przyrodników, Świebodzin: 1–196.
- ZDUNIAK P., 2005: Wrona siwa *Corvus cornix* w Polsce – stan wiedzy i perspektywy badań. [In:] Ptaki krukowate Polski. Bogucki. Wyd. Nauk., Poznań: 113–125.

**Streszczenie:** Zachowania ptaków krukowatych w stosunku do myszołowa zwyczajnego *Buteo buteo* na obszarach miejskich i pozamiejskich województwa mazowieckiego. Badania oparto na obserwacjach ptaków z rodziny krukowatych (Corvidae) od maja do końca listopada, podczas intensywnego treningu ptaka drapieżnego myszołowa zwyczajnego *Buteo buteo* w zróżnicowanych obszarach – miejskim i pozamiejskim, województwa mazowieckiego o różnych porach dnia. Stwierdzono wyraźną różnicę w aktywności krukowatych w zależności od zabudowania terenu. W trakcie prowadzonych badań odnotowano 305 sztuk ptaków, z czego zdecydowaną większość, tzn. 276 sztuk, na terenie miejskim, co stanowiło 91% obserwowanych osobników w ciągu

całego okresu badań. Istotny wpływ na ocenę miała również pora dnia. W godzinach przedpołudniowych aktywność ptaków w stosunku do myszolewa zwyczajnego była znacznie większa i wynosiła średnio 65%. Mniejszą aktywność obserwowano na terenach pozamiejskich, gdzie przedstawiciele krukowatych – sójka zwyczajna *Garrulus glandarius* oraz sroka *Pica pica* – wykazywały minimalne zainteresowanie ptakiem drapieżnym. Podejmowały one indywidualne próby odstraszenia drapieżnika, bez intensywnych sy-

gnałów ostrzegawczych i nawoływania innych osobników.

*MS. received November 2014*

**Authors' address:**

Monika Łukasiewicz  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Drobiu  
ul. Ciszewskiego 8, 02-786 Warszawa, Poland

## Factors affecting prolificacy and lambs rearing results in Olkuska sheep population

WIOLETA DROBIK, ELŻBIETA MARTYNIUK

Department of Genetics and Animal Breeding, Warsaw University of Life Sciences – SGGW

**Abstract:** *Factors affecting prolificacy and lambs rearing results in Olkuska sheep population.* The aim of this study was to evaluate reproductive performance of the growing Olkuska sheep population and to analyse impact of a number of factors on lamb rearing results as well as the length of ewes' utilisation in the flock. Although reproductive performance of Olkuska sheep is still at a relatively high level, recently a decrease of average prolificacy and the continuously low percentage of lambs reared have been observed. This might be related to the rapid growth of the population in the recent years and rather short flock-life of ewes, on average 3.17 years. The results of this study confirm that the size of the litter had the highest impact on lamb rearing results. Substantial differences in reproductive performance depending on the age of the dam were also observed. Despite of poor rearing of lambs born in large litters, no significant correlation between the average prolificacy of ewes in the flock and the percentage of lambs being reared was observed. The results indicate that a major influence on lamb rearing performance depends upon individual characteristics of a dam, as well as husbandry conditions in the flock and skills of the breeder.

*Key words:* Olkuska sheep, litter size, lamb rearing

### INTRODUCTION

Olkuska sheep, developed in the early 20<sup>th</sup> century in the Olkusz region, is a native Polish longwool sheep breed.

The breakdown of the sheep sector in the period of 1980s and 1990s resulted in the rapid decrease of the Olkuska sheep population size, down to 100 breeding ewes only. Since 2000, as result of the coordinated efforts undertaken within the genetic resources conservation programme, and especially enhanced financial support to maintain endangered local sheep breeds provided through the Rural Development Programme 2004–2006, the Olkuska sheep population systematically increased and the number of flocks is growing (Sikora 2010).

At present, the population of Olkuska sheep is developing steadily. In 2013, 1550 purebred ewes were registered in the flock books (PZO 2013). Of these, 952 breeding ewes (kept in 50 flocks) have been included in the genetic resources conservation programme (IZ-PIB 2005).

Olkuska sheep are characterised by exceptionally high prolificacy and good maternal abilities. With single lambing per year, the mean litter size of two and over two years old ewes is at least two (IZ-PIB 2005). Such high prolificacy is genetically determined by a single gene of a major effect increasing ovulation rate in carriers (Martyniuk 2009). Recent research has shown that in the

Olkuska sheep population, a novel mutation *FecX<sup>O</sup>* in *BMP15* gene on X chromosome, is segregating (Kaczor 2011; Demars et al. 2013). Contrary to six other known mutations in *BMP15* gene, in Olkuska sheep, the homozygous carriers are fertile and hyperprolific. The litter size in Olkuska sheep ranges from one to seven.

Rearing lambs from large litters puts substantial demands on breeders' time and investment. As a result of the rapid development of Olkuska sheep population and high number of newly established flocks, more farmers, without adequate experience are becoming involved in Olkuska sheep breeding. Given this trend, better understanding of the specific characteristics of reproductive performance of Olkuska sheep is needed to enhance management and further develop the breed.

The aim of this paper was to analyse selected traits of reproductive performance of the breed in the last several years. Due to substantial lamb mortality observed within some flocks, it was also important to evaluate the impacts of various factors on lamb rearing results, and the length of ewes' utilisation within the flock to be able to conduct informed discussion with Olkuska sheep breeders participating in the genetic resources conservation programme.

## MATERIAL AND METHODS

The material used in this study included data on lambings of 1718 Olkuska ewes participating in the reproduction in years 1996–2012. Based on data availability and quality, the lamb rearing results were

analysed only in the years 2003–2012. Two reproductive traits were considered in this study at the flock level:

- Prolificacy, defined as the percentage of all lambs born (alive and dead) of all ewes lambing in the flock;
- Lamb rearing, defined as the percentage of lambs reared out of all lambs being born (alive and dead).

Similarly, reproductive traits analyzed for individual ewes included litter size and percentage of lambs reared.

Data for this study were obtained from various sources: lambing reports since 2007 provided by regional branches of the Polish Union of Sheep-Farmers of Nowy Targ, Opole, Lublin, Warszawa, Malbork, and Poznań; lambing databases of the National Research Institute of Animal Production (since 2002) and breeding documentation obtained directly from Olkuska flocks (Imbramowice and Żelazna since 1996).

The study on relationship between the length of utilisation and the reproductive performance of ewes was conducted on 399 ewes that completed their flock-life and had at least three lambings. In total, 1937 lambing results of 399 ewes were analysed. Out of 399 ewes, 124 ewes had three litters only, 99 four litters, 56 five and 120 six or more litters. A separate analysis was conducted for sheep kept at Warsaw University of Life Sciences – SGGW flock in Żelazna (77 out of 399 ewes).

In order to identify factors having a significant impact on lamb rearing results, data on 10 676 lambs born by 1718 dams were analyzed. Due to the gaps in data, 25 dams from the initial group were eliminated, leaving 1693 ewes for the final analysis. For each

lamb the following data were recorded: year of birth, type of birth (range from 1 to 6 and more), age of dam (range from 1 to 8 and older), herd ID, dam ID, and survival up to 56 days (values 0 or 1).

The reproductive performance of Olkuska ewes belonging to different age classes was analyzed for 1693 ewes. Age categories were determined according to similar age-related reproductive potential of ewes, what reduced data loss to minimum. Litters of ewes older than 7 years accounted for only 5.3% of the data and therefore older ewes were grouped in one category (7–12). The age structure of ewes participating in the lambings was analyzed for 1058 ewes participating in year 2012 reproductive season. Age distribution in 2012 was representative for the last few years.

The relationship between lamb rearing results and mean prolificacy of the dam in individual flocks was estimated

using the sample of flocks (44) with available relevant data for the years 2008–2012.

The Spearman rank correlation test (cor.test, method="Spearman"), linear Pearson correlation test (cor.test, method="Pearson") and the test for equality of proportions based on chi-square statistics (prop.test and pairwise.prop.test with Bonfferoni correction) in R environment (R Development Core Team 2008) were used in statistical analysis.

## RESULTS

The average prolificacy of Olkuska sheep in 2012, was 207% (N = 1058) with lamb rearing at the level of 74.01%. In the period studied, the lamb rearing was in the range of 72.3% (in 2004) to 84.7% (in 2007), while the mean prolificacy was in the range of 208% (2012) to 221% (in 2004) as shown on Figure 1.

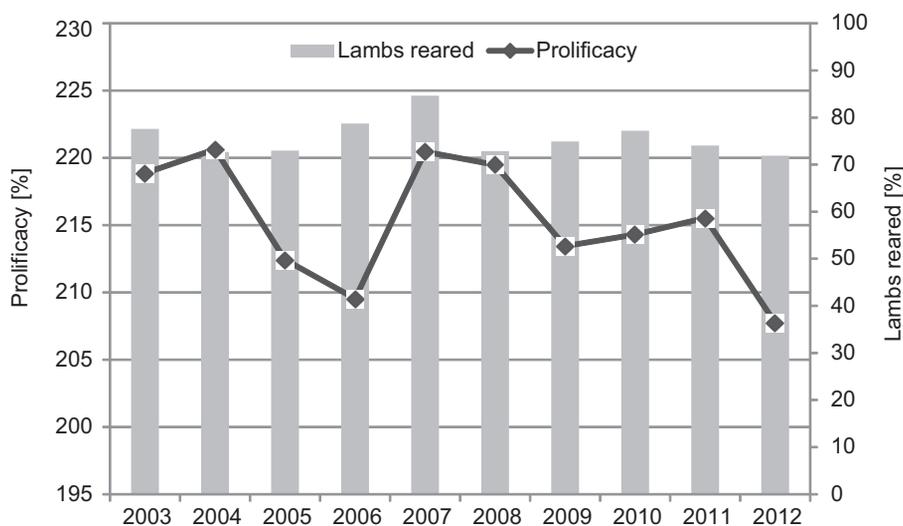


FIGURE 1. Average prolificacy and lambs' rearing percentage in Olkuska sheep flocks in years 2003–2012

The mean reproductive performance in Olkuska ewes increased with age, having the highest values in 4–6 years old ewes (Table 1). The substantially higher prolificacy was observed in 2 or 3 years old ewes (217%) in comparison with 1 year old ones (185%). The increase of average prolificacy in older ewes was mainly due to a higher share of large lit-

ters in this age group. For instance, triplets constituted over 20% of litters in 2 or 3 years old ewes, while in 1 year old ewes, their share was 8.3% only.

Lamb rearing percentage was the highest in ewes between 2 and 6 years old, on average 76% (Table 1). A substantial decline was observed in 7 or more years old ewes, down to 70.5%.

TABLE 1. Mean prolificacy, litter size distribution and lamb rearing results in years 2003–2012 in ewes of different age (N = 1693)

Age of dam [years]	Average prolificacy [%]	Litter size	Litters			Average share of lambs reared [%]
			Number	Share in the flock [%]	Share of lambs reared [%]	
1	185	1	175	25.9	86.9	73.6
		2	438	64.8	74.1	
		3	56	8.3	59.5	
		4	6	0.9	66.7	
		5	1	0.1	40.0	
2–3	217	1	401	16.0	86.3	76.0
		2	1447	57.9	80.4	
		3	532	21.3	71.6	
		4	93	3.7	62.9	
		5	20	0.8	54.0	
		6	5	0.2	16.7	
		7	3	0.1	38.1	
4–6	227	1	209	13.9	90.0	76.0
		2	836	55.4	80.5	
		3	361	23.9	70.4	
		4	70	4.6	70.4	
		5	22	1.5	78.2	
		6	6	0.4	25.0	
		7	4	0.3	35.7	
7–12	228	1	47	18.0	70.2	70.5
		2	128	49.0	77.7	
		3	61	23.4	63.9	
		4	17	6.5	57.4	
		5	7	2.7	82.9	
		6	0	0	0	
		7	1	0.4	42.9	

The age of the dam had significant effect on lamb rearing results, both in case of large litters and in twins and singles. Significant differences were observed:

- in twins and singles ( $p < 0.05$ )
  - between 1, 2–3 and 4–6 years old ewes;
- in litters of three lambs and more ( $p < 0.05$ ) – between 1 and 4–6 years old ewes.

A highly significant relationship (propability test;  $p < 0.01$ ) was observed between the litter size and the number of lambs reared (Table 2). The lamb rearing was rather low in triplets and litters consisting of four or five lambs (69%). The most difficult to rear were lambs born in litters of six or seven, only 28% of them were weaned.

The mean prolificacy and percentage of lambs reared in 44 selected flocks in years 2008–2012 are presented at Figure 2. The average prolificacy in examined flocks ranged between 170 and 312%, while the average percentage of lambs reared was between 33.3 and 92.1%. No significant phenotypic correlation was observed between these two traits ( $+0.039$ ,  $p = 0.703$ ). Among the 44 flocks included in the analysis, there were flocks of relatively low prolificacy for Olkuska breed (192%) and poor rear-

ing results (50.7% only), as well as flocks with outstanding reproduction results (225 and 91.8% respectively) – Figure 2.

The average length of ewe utilisation in the flock estimated on the basis of a sample of 399 ewes was rather short, with a high standard deviation, indicating substantial differences between individual dams ( $3.17 \pm 1.64$ ). It has to be underlined that a high share of young ewes was observed in the examined flocks.

The age structure of ewes participating in the lambing in 2012 ( $N = 1058$ ) is presented at Figure 3. Due to the early maturity of Olkuska breed and good husbandry conditions, some flocks used to practice an early lambing, therefore a small share (10.9%) of 1 year old ewes was observed in lambing in 2012. The highest contribution to 2012 lambing had ewes 2, 3 and 4 years old (20.0, 18.5 and 20.6% respectively), while only 28.1% of all litters were born by older ewes.

A significant relationship between mean litter size and a maximum litter size of ewes and the length of their utilisation in a flock was observed for the whole population of the Olkuska sheep (Table 3). However, such relationship was not significant for the Żelazna flock, probably due to the small sample size. In opposition to the rest of the population,

TABLE 2. Lamb rearing results in litters of 1–7 lambs

Specification	Litter size							Total
	1	2	3	4	5	6	7	
Number of lambs born	832	5 698	3 030	744	250	66	56	10 676
Number of lambs reared	719	4 520	2 121	486	171	14	21	8 052
Share of lambs reared [%]	86.4 <sup>A</sup>	79.3 <sup>B</sup>	70.0 <sup>C</sup>	65.3 <sup>C</sup>	68.4 <sup>C</sup>	21.2 <sup>D</sup>	37.5 <sup>D</sup>	75.4

Groups with different letters (A, B, C, D) have highly significant differences in the percentage of lambs reared.

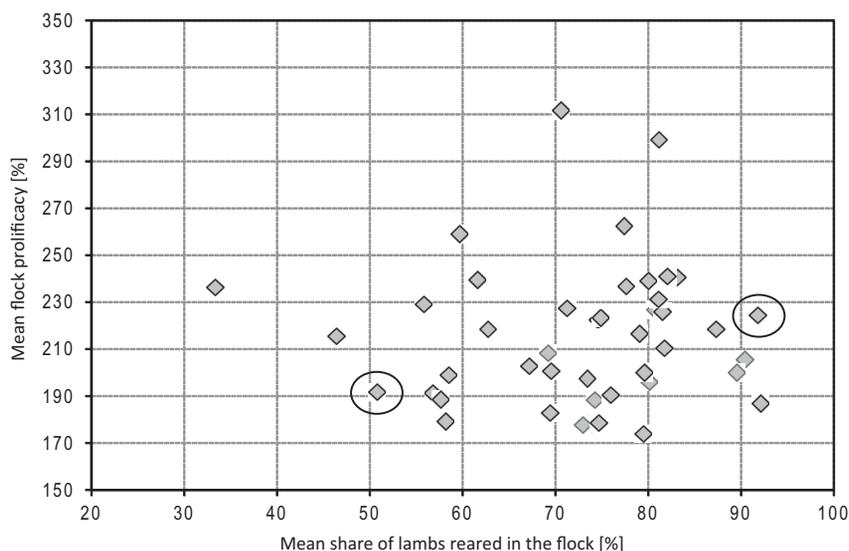


FIGURE 2. Mean prolificacy and % of lambs reared in selected 44 flocks of Olkuska sheep. Circles represent flocks, in which rearing of lambs is very high or very low despite their similar prolificacy level

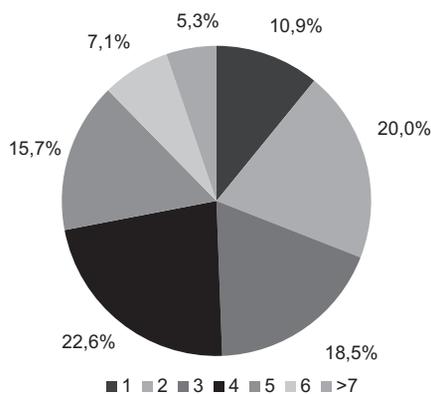


FIGURE 3. The age structure of ewes participating in the lambing in 2012 (N = 1058)

TABLE 3. The Spearman rank correlation coefficient between length of ewe utilisation on the flock and mean and maximum litters size and mean lamb rearing

Specification	Correlation coefficient		
	Population (N = 322)	RZD Żelazna (N = 77)	Total (N = 399)
A length of ewe utilisation in the flock and mean litter size	+0.134 <sup>a</sup>	+0.218	+0.149 <sup>b</sup>
maximum litter size	+0.347 <sup>b</sup>	+0.193	+0.317 <sup>b</sup>
mean share of lambs reared [%]	+0.006	+0.315 <sup>b</sup>	+0.068

<sup>a</sup> Significant (<0.05), <sup>b</sup> highly significant correlation (<0.01).

positive correlation between lamb rearing results and the length of ewes' utilisation was observed in Żelazna flock (+0.315;  $p < 0.01$ ).

## DISCUSSION

Olkuska sheep is a breed of high reproductive potential and good maternal abilities, which combined with breeders' experience and knowledge, enable rearing lambs from numerous litters. The average prolificacy of Olkuska ewes in the analyzed period of 2003–2012 was significantly higher than the minimum level, set in the breed standard. However, in recent years, a slight decrease in the average litter size was observed. High variation in prolificacy levels between years and the clear downward trend was also reported by Piwczyński et al. (2013). In 2012, the average prolificacy of Olkuska ewes was the lowest of all analyzed years, amounting to 208%; also a percentage of lambs reared remained at a relatively low level. These results may be associated with a high proportion of primiparous ewes in the population (30.9% in 2012; Fig. 3), as well as a higher share in the recent years of breeding rams that were not carriers of the *FecX<sup>O</sup>* gene. A frequency of the *FecX<sup>O</sup>* gene in the population, regional distribution of the gene carriers, as well as its effect on litter size and other reproductive traits, requires further research.

The significant correlation between the average prolificacy and maximum litter size of ewes and the length of their utilization in the flock could indicate that breeders prefer to keep more prolific ewes longer in the flock. However, it should be also noted that the age related physiological increase of the litter size

could have contributed to this relationship. In Żelazna flock, in the opposition to the rest of the population the length of ewes' flock-life was most affected by their performance in lambs' rearing. This trend is in line with flock management strategy, to cull dams that have poor lamb rearing results.

The relationships between litter size and survival of lambs were studied in a number of prolific sheep breeds (Fogarty et al. 2000; Kleemann and Walker 2005; Gootwine et al. 2008). In studies conducted by Gootwine et al. (2008) lamb survival at birth in Awassi and Asaf breeds was 98, 92, 86, 78 and 65% for litters consisting of respectively of a single lamb, twins, triplets, quadruplets and quintuplets. Also, in previous studies on Olkuska sheep, a high mortality at birth and poor lamb rearing were observed in litters consisting of many lambs. In Drozdy flock (Klewiec and Baranowski 1999), 7% of still born lambs were observed in twins, 14% in litters of triplets and 25% in quadruplets. High lamb losses were also reported during the rearing period. On average, in this flock, 1.4 lambs and were weaned from twin litters and 1.1 lambs from triplet litters at the age of 56 days.

Rearing results in Olkuska sheep is also influenced by the age of dams, however, the impact of this factor is much smaller than the litter size. It should be underlined, that poor rearing results observed in this study can be attributed to a large share of primiparous in the current population of Olkuska sheep. The average length of utilisation of ewes in the flock, only a little over 3 years, is shorter than recommended. The average flock-life, about 5 years, would be most beneficial to farmers. The present low

flock-life may be associated with the dynamic development of the Olkuska sheep population, a large number of newly established flocks and resulting exchange of adult females between farmers. The number of flocks participating in the sheep genetic resources conservation programme has increased from 10 to 50 in the period 2005–2013.

Analysis conducted in the selected 44 flocks indicated high differences both in the level of prolificacy, as well as the percentage of lambs reared among flocks. Despite the high effect of litter size on the success of lambs' rearing, and thus the strong relationship between dam prolificacy and lamb rearing results, no significant correlation was observed between the average prolificacy of ewes in the flock, and the percentage of lambs being reared. This result indicates the importance of individual maternal abilities of the ewes and quality of husbandry conditions provided individual flocks. The flock effect is a combination of a number of factors, such as the overall husbandry conditions, nutrition and animal welfare; and most of all engagement, experience and the skills of a breeder. Management of a flock of such a high prolificacy, as observed in the case of Olkuska breed, requires greater efforts than in the case of the majority of Polish sheep breeds which have an average litter size not exceeding 1.50. It is recommended that beginner breeders, start from a lower level of flock prolificacy, and with increased knowledge and experience, select their flocks towards reaching the full potential of fecundity offered by Olkuska sheep breed (Smętek and Korczyński 2010).

In light of the establishment of a large number of new flocks and the high differences in performance among individual flocks, it becomes particularly important to ensure that breeders have access to the most complete information about the breeding material. Genotyping of all rams available for sale to make sure if they are carrying the *FecX<sup>O</sup>* gene would greatly facilitate a choice of appropriate rams and enable better management of flock prolificacy. The genotype information would assist in the selection of breeding pairs for mating to control reproduction level as well as inbreeding in the flock. At present, the increasing genetic relationship between ewes and rams impose a significant management problem in the Olkuska sheep population (Martyniuk 2011; Drobik 2014).

## CONCLUSIONS

Olkuska sheep is a highly prolific breed therefore maintaining its overall reproductive performances is of a major importance. Although, as expected, the impact of litter size on lamb mortality is substantial, also other factors, such as age of dam and flock effect, play an important role. At the current rate of population growth there is a high risk of further decrease in reproductive performance. The flock effect and the length of ewe utilisation in the flock are factors which improvement is possible through a better husbandry and could lead to a higher reproductive performance of Olkuska sheep in Poland. It is very important to discuss this issue with breeders and facilitate sharing experience of lamb rearing techniques and overall flock manage-

ment to improve flocks' outputs and the breed performance. Monitoring and analysing performance results of the Olkuska population, especially flocks that are participating in sheep genetic resources conservation programme, and communicate it to breeders is essential for the future of the breed, as many farmers experiencing difficulties in lamb rearing are getting disappointed with the breed.

### Acknowledgments

The authors wish to thank Dr Jacek Sikora and Dr Andrzej Szewczyk, the National Research Institute of Animal Production (IZ-PIB), as well as the Directors and Employees of the Regional Trade Breeders Sheep and Goats for providing data.

### REFERENCES

- DEMARS J., FABRE S., SARRY J., ROSETTI R., GILBERT H., PERSANI L., TOSSER-KLOPP G., MULSANT P., NOWAK Z., DROBIK W., MARTYNIUK E., BODIN L. 2013. Genome-Wide Association Studies Identify Two Novel *BMP15* Mutations Responsible for an Atypical Hyperprolificacy Phenotype in Sheep. *PLoS Genet* 9 (4), e1003482.
- DROBIK W., 2014: Analiza genetyczna stad owiec rasy olkuskiej uczestniczących w krajowym programie ochrony zasobów genetycznych. Praca doktorska. Szkoła Główna Gospodarstwa Wiejskiego w Warszawie, Warszawa.
- FOGARTY N., HOPKINS D., Van Der VAN R., 2000: Lamb production from diverse genotypes. 1. Lamb growth and survival and ewe performances. *Anim. Sci.* 70: 135–145.
- GOOTWINE E., REICHER S., ROZOV A., 2008: Prolificacy and lamb survival at birth in Awassi and Assaf sheep carrying the *FecB* (Booroola) mutation. *Anim. Reprod. Sci.* 108: 402–411.
- IZ-PIB, 2005: Program Ochrony Zasobów Genetycznych owiec rasy olkuskiej. Kraków.
- IZ-PIB. Retrived from location <http://owce.bioroznorodnosc.izoo.krakow.pl/> [Accessed 14.07.2014].
- KACZOR U., 2011: Identyfikacja markerów plenności owiec rasy olkuskiej na podstawie polimorfizmu genów kodujących białka z nadrodziny TGF- $\beta$ . Rozprawy Naukowe i Monografie. Wydawnictwo Uniwersytetu Rolniczego w Krakowie, Kraków.
- KLEEMANN D.O., WALKER S.K., 2005: Fertility in South Australian commercial merino flocks: source of reproductive wastage. *Theriogenology* 63: 2075–2088.
- KLEWIEC J., BARANOWSKI A., 1999: Analiza śmiertelności jagniąt matek różnych genotypów. *Pr. Mat. Zoot.* 55: 97–105.
- MARTYNIUK E., 2009: Genetyczne uwarunkowanie wysokiej plenności owcy rasy olkuskiej. Rozprawy Naukowe i Monografie. Wydawnictwo SGGW, Warszawa.
- MARTYNIUK E., 2011: Problemy hodowlane populacji o małej liczebności na przykładzie owcy rasy olkuskiej. *Wiad. Zoot.* XLIX, 1: 21–31.
- PIWCZYŃSKI D., CZAJKOWSKA A., ZALEWSKA A., 2013: Zmiany cech reprodukcyjnych wybranych ras plennych owiec w Polsce w latach 1997-2010. *Przegl. Hod.* 2: 20–22.
- PZO, 1987–2013: Hodowla owiec i kóz w Polsce, roczniki 1987-2013. Polski Związek Owczarski, Warszawa.
- SIKORA J., 2010: Charakterystyka populacji owiec rasy olkuskiej w latach 1989-2010. Owce w krajobrazie Wyżyny Krakowsko-Częstochowskiej. Towarzystwo Miłośników Ziemi Zawierciańskiej, Zawiercie: 32–39.
- SMĘTEK J., KORCZYŃSKI T., 2010: Specyfika hodowli owiec olkuskich. Owce w krajobrazie Wyżyny Krakowsko-Częstochowskiej. Towarzystwo

Miłośników Ziemi Zawierciańskiej, Zawiercie: 40–50.

R Development Core Team, 2008: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

**Streszczenie:** *Analiza czynników wpływających na plenność oraz odchów jagniąt w populacji owiec rasy olkuskiej.* Celem niniejszej pracy było scharakteryzowanie populacji owiec olkuskich pod względem użytkowości reprodukcyjnej na przestrzeni ostatnich lat oraz analiza wpływu wybranych czynników na wyniki odchowu jagniąt oraz długość użytkowania matek w stadzie. Użytkowość reprodukcyjna owiec olkuskich kształtuje się na stosunkowo wysokim poziomie, w ostatnich latach obserwuje się jednak obniżenie poziomu plenności oraz utrzymujący się dość niski poziom odchowu jagniąt. Może to być związane z dynamicznym rozwojem populacji oraz krótkim

okresem użytkowania matek w stadach wynoszącym średnio jedynie 3,17 lat. Czynnikiem, który ma największy wpływ na odchów jagniąt u owiec rasy olkuskiej, jest wielkość miotu. Obserwuje się również wyraźne zmiany w użytkowości reprodukcyjnej w zależności od wieku matki. Pomimo słabszych wyników odchowu w przypadku licznych miotów nie obserwuje się istotnej korelacji między średnią plennością matek w stadzie a poziomem odchowu jagniąt. Wskazuje to na fakt, że decydujący wpływ na odchów mają indywidualne predyspozycje matki, warunki utrzymania zwierząt oraz umiejętności hodowcy.

**Authors' address:**

Wioleta Drobik  
Wydział Nauk o Zwierzętach SGGW  
Katedra Genetyki i Ogólnej Hodowli Zwierząt  
ul. Ciszewskiego 8, 02-786 Warszawa, Poland  
e-mail: wioleta\_drobik@sggw.pl

## Young chinchillas weight gain, depending on their body mass at birth

DANUTA DZIERŻANOWSKA-GÓRYŃ<sup>1</sup>, MARIAN BRZozowski<sup>1</sup>,  
KATARZYNA GÓRAL-RADZISZEWSKA<sup>2</sup>

<sup>1</sup> Department of Animal Breeding and Production, <sup>2</sup> Department of Genetics and Animal Breeding  
Warsaw University of Life Sciences – SGGW

**Abstract:** *Young chinchillas weight gain, depending on their body mass at birth.* The aim of the study was to determine if the results of raising and growth for up to 4th months of age in chinchilla depends on body weight and the litter size at birth. There were also attempts to answer the question, when sexual dimorphism begins to be visible in young chinchillas. Males are heavier females from the first month of life in chinchilla, however up to the 4th months of age the differences are not statistically significant. During the study it was found, that young chinchillas mortality depends on their body weight at birth. When the body weight at birth is higher, the greater chances of survival of the young are. Number of puppies in the litter affects their body weight: the more young per litter are, the less average body weight is.

*Key words:* chinchillas, body gain, litter size

## INTRODUCTION

One of the parameters to assess the reproductive performance of female mammals is the number of young born and weaned per year. The research carried out on pigs (Quiniou et al. 2002, Foxcroft et al. 2009;) shows that a large litter size reduces the average birth weight of young and increases the variability of birth weight in the litter. Body weight of piglets at birth is the primary factor for their survival, growth rate and body weight at weaning and significantly affects their subse-

quent fattening efficiency (Gondret et al. 2005; Rehfeldt and Kuhn 2006). Reaching high daily gains in fattening is only possible when achieved good growth already during the rearing period of piglets (Wećkiewicz and Haraśny 1992). Low body weight of piglets at birth is associated with an increasing number of piglets born dead and the increasing number of falls during the rearing period (Milligan et al. 2002a, b).

In breeding chinchillas also it comes to obtaining the largest possible number litters, and the largest possible number of young from the female during the year. A positive result is already two litters a year, although some authors point to a higher reproductive potential chinchillas. On the other hand, the excessive exploitation of females weakens their bodies and as a result may cause a reduction in fertility and shorten the length of the period of use (Barabasz 2001; Socha et al. 2001a; Socha et al. 2001b, Socha and Kasjaniuk 2003).

In fur animals especially important is the size of the skin, which is a determinant of its value. In studies carried out by different authors at finn racoons, foxes and mink shown that there is a positive correlation between the body weight and the size of the skin of animals (Gugolek et al. 2002).

Many studies have been carried out to take into account the indicators of fertility chinchillas depending on their age, color variety or a group of genetic origin (Socha and Wrona 2000a, b; Socha et al. 2003).

The aim of the study was to determine if the results of raising and growth for up to 4th months of age in chinchilla depends on body weight and the litter size at birth. There were also attempts to answer the question, when sexual dimorphism begins to be visible in young chinchillas.

## MATERIAL AND METHODS

The studies were performed on a farm chinchillas in WULS-SGGW in 4 consecutive years. 98 litters (173 individuals) were rated together. The pups body weight measurements were performed once a week, starting from birth to 16th weeks of age (4 months). Chinchillas were kept in standard netting cages. The animals were fed pelleted feed ration (18.4% protein, 2.8% fat and 12% fiber) and hay *ad libitum*. The animals had continuous access to water.

The following parameters were calculated:

- the number of litters per female attributable to the year;
- the number of young born per litter;
- the average birth weight of pups;
- an overall mortality of pups.

Data analysis was performed by using one-way ANOVA and post hoc test and test NIR.

## RESULTS AND DISCUSSION

Fertility rates and prolificacy of females in the experiment are shown in Table 1.

Fertility and prolificacy are the parameters defining the reproductive performance of females. Both genetic and environmental factors have an impact on the reproductive performance of females. Since many factors, particularly environmental ones may affect the farms breeding results. The average litter size in conducted throughout the period of 4-year studies were obtained at the level of 1.93 young. Literature sources indicate that chinchillas female in one litter give birth from 1 to 5 young, with an average litter size ranged from 1.79 to 2.15 (Socha et al. 2001a; Seremak 2007). Obtained in the study results are consistent with the literature cited.

Ratio of the average number of litters from female per year in the analyzed herd ranged from 1.31 to 1.63. The results are also similar to the literature data. Sulik and Barabasz (1995) received 1.21, Barabasz et al. (2000) reported 1.54–1.90 litters per female per year.

Percentage distribution of litter size is shown in Table 2.

TABLE 1. Reproductive parameters of chinchillas female

Parameters	1st observation year	2nd observation year	3th observation year	4th observation year	Average
Average number of pups in litter	1.88	1.87	2.13	1.81	1.93
Average number of litters per year	1.53	1.63	1.36	1.31	1.46

TABLE 2. Litter size, the percentage distribution and average body weight of born pups

Parameters	Litter size (number of pups)			
	1	2	3	4
Number of born pups	22	100	39	12
Share of in litter (%)	12.72	57.80	22.54	6.94
Average body weight at birth (g) ±SD	57.25 ±4.42	50.60 ±6.61	44.72 ±7.34	41.92 ±9.22

Most in terms of numbers were obtained from litters of young chinchillas double – 57.8%. Next were litters of three young constituting 22.54% and single – 12.72%. Received 12 chinchillas born in litters of 4 pups, which accounted for only 6.94%. By Neira et al. (1989), most chinchillas give birth to 1 young – 47.2%; 2 in the litter born 29.7%, 3 – 7.6% and 4 puppies – 0.6% of females.

The mean body weight for all litters of puppies at birth was 49.5 g. The highest average body weight at birth had a chinchilla from individual litters, and the lowest mass characterized by quadruplets.

The survival rate of young according to their birth weight are shown in Table 3.

Best results are obtained with birth weight above 60 g. Good results were obtained from rearing puppies from the group with birth weight 50–59 g and 40–49 g.

TABLE 3. Percentage of falls chinchillas pups depending on birth weight

Body weight at birth (g)	Share of in all falls (%)
30–39	47.40
40–49	23.20
50–59	19.30
Over 60	6.00

The overall mortality pups from birth to weaning was 19.52%, while in the first 2 weeks 17.9% of puppies have died. This is consistent with literature data. According to Jarosz and Rzewska (1996) and Seremak (2007), the first 2 weeks after birth are considered crucial to the survival of young chinchillas. This period is characterized by the highest mortality rate (around 20%), which may be due to low resistance and the absence of maternal milk.

Lower body weight of puppies at birth is correlated with high mortality: more susceptible to falls are lighter chinchillas.

Figure 1 shows the development of the body weight of young chinchillas from birth to 16<sup>th</sup> week of life, taking into account their body weight at birth. Puppies belonging to the lightest group (30–39 g) at the age of 4 months weighed an average of 362.8 g. Animals of the heaviest group (60–69 g at birth) in the same time weighed average until 480.2 g. Difference in body weight between individuals of the extreme groups was approximately 117.4 g. Pearson correlation coefficient calculated for birth weight and weight at week 16 was 0.496. It indicates, that the relationship exists: the heavier the newborns are, the heavier 4-month youth will be. The strength of this relationship, however, is average.

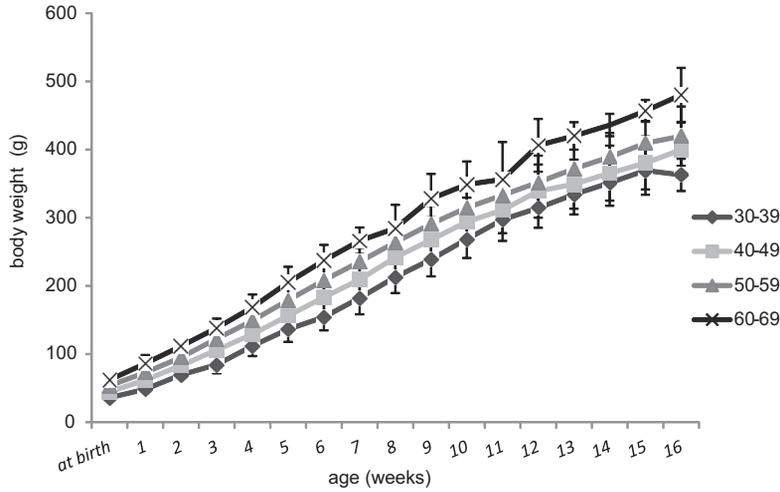


FIGURE 1. Changes in average body weight of chinchilla pups depending on body weight at birth

The analysis of young chinchillas changes in body weight with respect to gender are shown in Table 4.

TABLE 4. Changes in chinchillas body weight from birth to 16th weeks of age disaggregated by gender: x – mean, V – variability (%)

Age (weeks)	Average body weight (g)			
	male		female	
	x	V	x	V
At birth	50.30	16.20	48.58	15.52
1st	68.72	21.57	66.75	17.74
2nd	91.73	18.16	89.33	18.46
3th	114.75	18.48	111.40	20.25
4th	141.23	17.28	136.84	18.37
5th	170.21	15.96	166.26	18.05
6th	200.72	15.07	195.09	17.56
7th	228.18	13.82	219.70	15.07
8th	253.56	13.64	247.45	14.59
9th	282.67	12.47	276.44	14.02
10th	308.14	13.01	291.28	18.52
11th	328.87	13.40	316.54	11.90
12th	348.59	12.99	343.15	11.89
13th	362.37	12.67	353.18	11.19
14th	382.09	11.76	376.15	11.41
15th	401.57	9.88	392.84	10.05
16th	418.54	11.74	410.52	10.14

There was no observed gender impact on body weight (in any of the analyzed weeks, animals of different sexes did not differ statistically from each other).

In the literature there are reports (Jarosz and Rzewaska 1996), that the male chinchilla are smaller than female. In this study the observed period to 4th months of age, females were characterized by lower body weight. Age of 4 months is the beginning of puberty chinchillas and this may have an impact on the subsequent differences in body weight between females and males. However, this requires further observations on a larger population of animals, but also in a longer period of time.

The relationship between litter size and the body weight of the animals are shown in Table 5.

Litters with one puppy characterized the highest body weight throughout the rearing period and the difference was highly significant compared with the other groups. Group of triplets characterized by a highly significant lower aver-

TABLE 5. Effect of chinchilla litter size on body weight from birth to 16th weeks of age

Age (weeks)	Litter size (number of pups)												P
	1			2			3			4			
	N	X	V	N	X	V	N	X	V	N	X	V	
At birth	22	57.25	7.73	100	50.60	13.06	39	44.72	16.42	12	41.92	21.99	<0.001
1st	21	79.00	15.92	85	69.25	17.36	36	58.81	21.64	8	64.75	16.38	<0.001
2nd	20	102.20	14.78	82	93.21	16.43	32	78.31	17.17	8	84.88	16.93	<0.001
3th	21	128.81	14.67	83	116.53	17.05	33	97.82	18.44	6	96.67	22.13	<0.001
4th	20	156.65	14.12	78	143.36	16.28	35	122.60	15.66	6	125.50	17.72	<0.001
5th	18	189.89	14.21	82	172.41	15.91	37	152.30	14.40	6	148.67	15.99	<0.001
6th	20	220.40	11.16	72	203.24	14.98	35	178.54	15.27	6	180.83	18.71	<0.001
7th	20	247.40	8.86	70	230.74	12.73	36	202.10	14.36	8	209.38	16.71	<0.001
8th	16	270.88	9.32	69	258.59	12.10	36	227.78	14.86	5	247.20	19.50	<0.001
9th	15	310.93	8.58	68	284.60	11.70	35	256.31	14.16	8	284.13	16.92	<0.001
10th	16	302.71	24.97	64	309.71	12.22	32	279.53	14.47	7	311.71	16.40	0.025
11th	14	343.07	9.96	53	330.09	11.92	23	300.43	11.66	5	305.80	10.69	0.003
12th	13	359.54	9.44	56	352.29	12.14	23	322.87	11.24	7	349.71	17.74	0.026
13th	11	392.09	7.13	52	360.67	12.01	23	344.09	10.59	5	325.20	17.19	0.005
14th	13	407.69	6.65	51	382.88	11.79	25	361.52	10.92	5	361.60	14.36	0.012
15th	12	421.75	6.71	44	402.84	9.52	17	377.18	9.87	3	347.33	3.17	<0.001
16th	10	434.70	6.11	41	418.83	10.78	17	391.06	10.00	4	429.75	20.06	0.064

N – number of litters; X – average body weight of one puppy (g); V – variability (%); P – statistical significance of the difference.

age body weight than the other groups of litters, also of quadruplets. It may result because of a small sample of quadruplets litters. Such results may also be affected by different numbers of individual in observed litters.

Barabasz and Łapiński (2008) in their study observed significant differences in body weight of pups from a single litters and “triplets” throughout the period of lactation (up to 5th weeks of age). Significant differences also occurred between puppies from litters of single and double in the first period of lactation. Differences decreased from the moment when pups have started to eat a pellets. The average body weight of pups from the “twins” were significantly higher than those of

the triple in 14th, 21st and 28th days of age. The author suspects that it could be related to competition for nipple access.

### CONCLUSIONS

1. Young chinchillas mortality depends on their body weight at birth. When the body weight at birth is higher, the greater chances of survival of the young are.
2. Birth weight determines the body weight of puppies chinchilla at the age of 4 months in the average degree.
3. Number of puppies in the litter affects their body weight. The more young per litter are, the less average body weight is.

4. Males are heavier females from the first month of life, but to the age 4 months the differences are not statistically significant.

## REFERENCES

- BARABASZ B., 2001: Szynszyle, hodowla i użytkowanie (Chinchillas – breeding and use). PWRiL, Warszawa (in Polish).
- BARABASZ B., FORTUŃSKA D., BIE-NIEK J., 2000: Ocena intensywności użytkowania rozplodowego samic szynszyli. Zesz. Nauk. AR Kraków 369(35): 121–133.
- BARABASZ B., ŁAPIŃSKIS., 2008: Growth rate of sucking chinchilla pups and lactating performance of their dams. Anim. Sci. Pap. Rep. 26, 3: 227–234.
- FOXCROFT G.R., DIXON W.T., DYCK M.K., NOVAK S., HARDIN G.J.C.S., ALMEIDA F.C.R.L., 2009: Prenatal programming of postnatal development in the pig. In: Control of pig reproduction, VIII: 213–233.
- GUGOLEK A., LOREK M.O., ZABŁOCKA D., 2002: Studies on the relationship between the body weight, trunk length and pelt size in arctic foxes. Czech J. Anim. Sci. 47(8): 328–332.
- GONDRET F., LEFAUCHEUR L., LOU-VEAU I., LEBRET B., PICHOD O X., Le COZLER Y., 2005: Influence of piglet birth weight on postnatal growth performance, tissue lipogenic capacity and muscle histological traits at market weight. Livest. Prod. Sci. 93: 137–146.
- JAROSZ S., RZEWSKA E., 1996: Szynszyle, chów i hodowla. PWRiL, Warszawa.
- MILLIGAN B.N., DEWEY C.E., De GRAU A.F., 2002a: Neonatal-piglet weight variation and its cycles and season of the year and its influence on fertility and litter weight. Ann. Anim. Sci., Suppl. 2/1: 107–111.
- MILLIGAN B.N., FRASER D., KRAMER D.L., 2002b: Within-litter birth weight variation in the domestic pig and its relation to pre-weaning survival, weight gain, and variation in weaning weights. Livest. Prod. Sci. 76: 181–191.
- NEIRA R., GARCIA X., SCHEN R., 1989: Reproduction and growth in confined chinchillas (*Chinchilla lanigera*). Avances en Production Animal, Chile 14 (1–2): 109–110.
- QUINIOU N., DAGORN J., GAUDRE D. 2002: Variation of piglets' birth weight and consequences on subsequent performance. Livest. Prod. Sci. 78: 63–70.
- REHFELDT C., KUHN G., 2006: Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J. Anim. Sci. 84 (E. Suppl.): E113–E123.
- SEREMAK B., 2007: Wybrane aspekty rozrodu szynszyli w chowie fermowym (Selected aspects of chinchilla (*Chinchilla laniger* M.) reproduction under farm management). Zeszyty Naukowe AR w Szczecinie, Rozprawy 240: 1–60 (Eng. Summary).
- SOCHA S., JEŻEWSKA G., GONTARZ A., 2001a: Quantitative characterisation of chinchillas (*Chinchilla velligera* M.) litters. Deutsche Veterinärmedizinische Gesellschaft e.V. DVG, Arbeitstagung über Haltung und Krankheiten der Kaninchen Pelztiere und Heimtiere 9–10: 231–235.
- SOCHA S., KASJANIUK M., 2003: Analiza czynników wpływających na plenność wybranych odmian barwnych szynszyli. Acta Sci. Pol. Pol., Zootechnica 2(2): 113–124.
- SOCHA S., MAĆKOWIAK I., JEŻEWSKA G., GONTARZ A., DĄBROWSKA D., 2001b: Analiza plenności szynszyli (*Chinchilla velligera* M.) odmiany standardowej i beżowej polskiej w wybranych fermach. Zeszyty Naukowe Prz. Hod. 58: 39–46.
- SOCHA S., WRONA A., 2000a: The analysis of the seasonal character of the chinchilla (*Chinchilla velligera* M.) reproduction. Proceedings of the VIIth International Scientific Congress in Fur animal Production, Kastoria. SCIENTIFUR 24, 4. Reproduction, III-A: 49–52.

- SOCHA S., WRONA A., 2000b: Plenność samic szynszyli (*Chinchilla velligera* M.) należących do różnych grup genetycznych. Zeszyty Naukowe Prz. Hod. 53: 87–95.
- SULIK M., BARABASZ B., 1995: Porównanie systemów utrzymania rozplodowego na przykładzie wybranych ferm. Zesz. Nauk. AR w Krakowie 297: 159–165.
- WĘCKOWICZ E., HARAŚNY Z., 1992: Badania rozwoju prosiąt. Prz. Hod. 8: 16–17.
- ju młodych zwierząt uwidacznia się dymorfizm płciowy. Od początku samce są cięższe od samic, lecz aż do 4. miesiąca życia różnica ta nie jest istotna statystycznie. Podczas badania stwierdzono zależność między urodzeniową masą ciała a śmiertelnością młodych. Większa masa ciała wpływa na większe szanse na przetrwanie. Liczba szczeniąt w miocie wpływa na ich wagę ciała: im więcej młodych, tym przeciętnie mają mniejszą masę ciała.

*MS. received November 2014*

**Authors' address:**

Danuta Dzierżanowska-Góryń  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
ul. Ciszewskiego 8, 02-786 Warszawa, Poland  
e-mail: danuta\_dzierżanowska\_goryn@sggw.pl

**Streszczenie:** *Wpływ urodzeniowej masy ciała na wyniki odchovu szynszyli.* Celem badań było określenie, czy wyniki odchovu szynszyli do 4. miesiąca życia zależą od masy ciała i wielkości miotu przy urodzeniu. Spróbowano odpowiedzieć również na pytanie, w którym momencie rozwo-



## **Effect of ensiling pumpkin *Cucurbita maxima* with the addition of inoculant or without it on chemical composition and quality of silages**

GABRIELA DOROTA HALIK, ANDRZEJ ŁOZICKI,  
AGATA KOZIORZĘBSKA, MARIA DYMNIĆKA, EWA ARKUSZEWSKA  
Department of Animal Nutrition and Biotechnology, Warsaw University of Life Science – SGGW

**Abstract:** *Effect of ensiling pumpkin Cucurbita maxima with the addition of inoculant or without it on chemical composition and quality of silages.* The aim of the studies was to determine the effect of ensiling the pumpkin on chemical composition and quality of silages. The silages were produced from pumpkin of Justynka variety. Before ensiling, the disintegrated pumpkin fruits were mixed with the dried beet pulp in ratio equals 80 : 20. Two variants of silages were prepared: with the inoculant and without it. In the silages, the following basic chemical composition was determined: the content of dry matter, crude ash, crude protein, crude fat, crude fibre, NDF, ADF and ADL. The indicators, being an evidence of the run of ensiling process and the quality of the obtained silages, were also determined, i.e. pH, lactic, acetic and butyric acids, ammonia nitrogen, ethanol and aerobic stability. In the obtained silages, as compared to the material before ensiling, the lower content of crude fibre and ADF was found whereas in the silage with inoculant, NDF level was also lowered. The silages with the inoculant were characterized by higher content of lactic and acetic acids and lower level of nitrogen, ammonia and ethanol. The silages with the inoculant had also higher aerobic stability. The conducted studies indicate that the application of inoculant has affected the improvement of the quality of the obtained silages.

*Key words:* silage, pumpkin, dried beet pulp, inoculants, chemical composition

## **INTRODUCTION**

Pumpkin *Cucurbita maxima* and especially its new varieties with a higher content of dry matter and carotenoids may become a valuable feedstuff for farm animals. The fruits of pumpkin, containing many carbohydrates, are easily digested by animals and they are rich in mineral, carotenoids and vitamins (Danielenko 2000; Korzeniewska et al. 2004; Niewczas et al. 2005). The pumpkin may be utilized in nutrition of animals in a form of fresh fruits, dry solids and silage. The seasonal availability of fresh fruits causes that their ensiling may become a good solution for their preservation. Owing to the increased content of dry matter, fruits of new varieties of pumpkin reveal better suitability for ensiling and the obtained feed may possess a high nutritional value. It may become especially valuable feedstuff for the dairy cows, having a positive influence on palatability of the ration and improvement of dietetic values of the obtained milk (Kuczyńska 2011). The discussed feed may constitute an interesting proposal for organic farms. The increase of dry

matter content from few to several percent (Korzeniewska et al. 2004) increases the suitability of the pumpkin for ensiling but it does not give the possibility of ensiling it alone. If we want to ensure the correct run of fermentation and obtain the feed with a high nutritional value, we must ensile the pumpkin together with the dry substance, e.g. with straw (Hashemi and Razzaghzaden 2007). The ensiling of the pumpkin with dry sugar beet root pulp seems to be an interesting solution. The high level of easily-fermenting carbohydrates in the pumpkin and the beet pulp, as mixed altogether, should ensure the correct fermentation run. The improvement of the ensiling process and the silage quality may be also obtained *via* application of the additives which facilitate the ensiling, e.g. inoculants (Raczkowska-Werwińska et al. 2008; Kilic and Saricicek, 2010; Alves et al. 2011; Selwet 2011).

The aim of the studies was to evaluate the nutritional value and quality of the silage, obtained from the pumpkin, ensiled with the dried beetroot pulp in variants with inoculant and without it.

## MATERIAL AND METHODS

The study used the Justynka variety of pumpkin (*Cucurbita maxima* D.), which has an increased content of dry matter (14–20%) and carotenoids (8–12 mg per 100 g fresh matter). Pumpkin was grown in an experimental field at the Department of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences – SGGW. Fruits were harvested from the field in late September and used to prepare silages at the Depart-

ment of Animal Nutrition and Biotechnology, Warsaw University of Life Sciences – SGGW in October.

Silages were prepared in mini-silos, made of 20-L plastic bags. To increase the dry matter content of the ensiled material, ground pumpkin fruits were mixed with dried sugar beet pulp at 80 : 20. The material was compressed in an automated press and packed into mini-silos. The ensilage treatments were: with a bacterial-enzyme inoculant, and no inoculant. The bacterial-enzyme inoculant contained *Lactobacillus plantarum* bacteria, endo-1,4-beta-glucanase, xylanase, and glucoamylase. The preparation was applied at 0.2% of the ensiled material. Ten mini-silos were prepared for each ensiling treatment.

Mini-silos were stored in the laboratory for 10 weeks in shaded conditions (19–23°C). After 10 weeks mini-silos were opened and their contents were sampled for analysis.

Samples for analyses were collected from fresh pre-ensiled material and from silages. Fresh material samples were collected from 10 mini-silos (5 from inoculant treatment and 5 from control treatment) before sealing them up. After collection, the samples were frozen at –30°C until chemical analyses. Silage samples were collected from all the mini-silos.

The chemical composition of the fresh material and silage was determined according to AOAC (2005): DM by drying at 104°C for 24 h, ash by incineration at 550°C for 6 h, crude protein (N × 6.25) by using the micro-Kjeldahl technique (Kjeltec System 1026 Distilling Unit, Foss Tecator, Sweden) and crude fat after extraction with petroleum ether by the Soxhlet method. NDF was

determined according to Van Soest et al. (1991) and expressed as the ash free residue after extraction with boiling neutral solutions of sodium lauryl sulfate and EDTA in a Tecator apparatus. The silage pH was determined using a CP-315 ELMETRON pH-meter equipped with a replaceable electrode. The concentrations of L-form lactic acid, L-form acetic acid and D-3-hydroxybutyric acid were determined spectrophotometrically using a commercial enzymatic test combination (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany).

The content of ammonia nitrogen (N-NH<sub>3</sub>) in the silage was determined by Conway method. Ethanol was determined by oscillometric method (PB-ZF/GS-11). The aerobic stability of the silages was determined by measurement of temperature, using thermometer (automatic, electronic, multi-channel thermometer LB-711), performing the measurements of temperature each hour during the suc-

cessive 7 days. The measurements were conducted under the standardized conditions – in air-conditioning room, at constant temperature of 21°C.

The effects of ensiling and silage additive on chemical composition of the silages were evaluated by using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Statistical analyses were carried out, using Statgraphics 6.0 Plus.

## RESULTS

In Table 1, the basic chemical composition of the studied silages has been given.

When we compared the basic composition of the silages and the material before the ensiling, we found a significant decrease of crude fibre content in the silages (for the silage without the inoculant  $p \geq 0.05$ ; for the silage with the inoculant

TABLE 1. Effect of ensiling on chemical composition of the silages

Component	Fresh material	Silages		p-value	SE
		without inoculant	with inoculant		
Dry matter (g · kg <sup>-1</sup> )	289.56	303.31	298.01	0.083	4.140
Crude ash (g · kg <sup>-1</sup> DM)	71.13	70.50	68.59	0.194	0.998
Crude protein (g · kg <sup>-1</sup> DM)	114.69	109.72	108.97	0.131	1.873
Crude fat (g · kg <sup>-1</sup> DM)	46.58	41.43	42.33	0.109	1.754
Crude fibre (g · kg <sup>-1</sup> DM)	189.24 <sup>Bb</sup>	168.80 <sup>a</sup>	162.01 <sup>A</sup>	0.003	3.994
WSC g · kg <sup>-1</sup> DM)	57.83 <sup>B</sup>	60.95 <sup>A</sup>	61.71 <sup>A</sup>	0.005	0.616
NDF (g · kg <sup>-1</sup> DM)	340.85 <sup>B</sup>	314.39 <sup>A</sup>	307.32 <sup>A</sup>	0.001	6.502
ADF (g · kg <sup>-1</sup> DM)	257.74 <sup>B</sup>	213.34 <sup>A</sup>	215.42 <sup>A</sup>	0.001	8.426
ADL (g · kg <sup>-1</sup> DM)	51.35	45.51	45.32	0.147	2.366
UFL (kg <sup>-1</sup> DM)	1.07	1.05	1.08	0.321	0.034
PDIN (g kg <sup>-1</sup> DM)	83.72	81.87	82.26	0.725	2.925
PDIE (g kg <sup>-1</sup> DM)	123.87	127.12	128.32	0.436	2.391

AB – differences between the selected rows ( $P \leq 0.01$ ); ab – differences between the selected rows ( $P \leq 0.05$ ); WSC – water-soluble carbohydrates; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid neutral lignin; UFL – unit of energy for milk production; PDIN – protein digested in the small intestine depending on rumen degraded protein; PDIE – protein digested in the small intestine depending on rumen-fermented organic matter.

$p \geq 0.01$ ), NDF ( $p \geq 0.01$ ) and ADF ( $p \geq 0.01$ ). The content of WSC in silages was significantly higher compare to fresh material ( $p \geq 0.01$ ). On the other hand, the comparison of the basic composition of two studied variants of the silages did not reveal any statistically significant differences (Table 1). Any significant differences between the variants of ensiling in respect of pH of the silages were not found (Table 2). The silages produced with the addition of inoculant were characterized by significantly higher acetic acid ( $p \geq 0.01$ ) and of lactic acid ( $p \geq 0.05$ ) content (Fig. 1). The content of butyric acid in both variants of ensiling was recorded on the similar, very low level.

Statistically significant differences between the silages were found in the

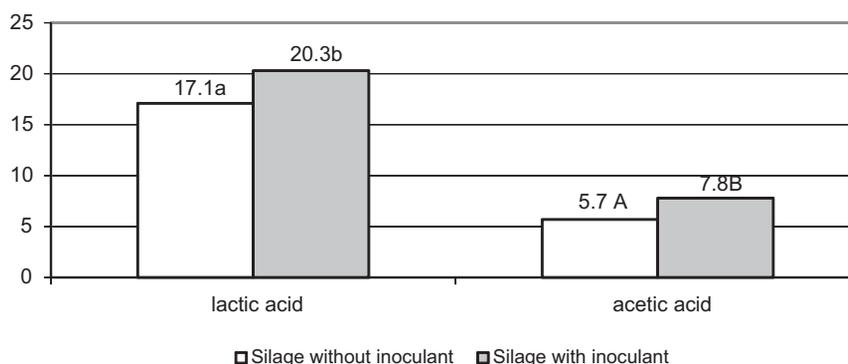
content of ammonia nitrogen (N-NH<sub>3</sub>), the higher concentration of which occurred in the silage without the participation of the additive, facilitating the ensiling process ( $p \geq 0.01$ ). The discussed silage was also characterized by the higher content of ethanol ( $p \geq 0.01$ ), resulting from the alcohol fermentation (Table 2).

Aerobic stability of the silage is the important parameter, indicating the susceptibility of the silage to the secondary fermentation. There was found a positive effect of the employed inoculant on aerobic stability of the examined silages, i.e. significantly higher stability of the silages produced with the addition of inoculant as compared to the silages without the mentioned additive ( $p \geq 0.01$ ).

TABLE 2. The effect of the ensiling method on the parameters, determining quality of the silages

Quality parameters of silage	Silage without inoculant	Silage with inoculant	SE	p-value
pH	4.5	4.4	0.123	0.548
Ammonia nitrogen ( $\text{g} \cdot \text{kg}^{-1}$ DM)	11.06 <sup>A</sup>	8.23 <sup>B</sup>	0.421	<0.001
Ethanol ( $\text{g} \cdot \text{kg}^{-1}$ DM)	9.72 <sup>A</sup>	7.13 <sup>B</sup>	0.512	0.003
Aerobic stability (h)	21.63 <sup>A</sup>	44.25 <sup>B</sup>	5.201	0.002

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



AB – different letters indicate significant differences ( $P \leq 0.01$ );

ab – different letters indicate significant differences ( $P \leq 0.05$ ).

FIGURE 1. The mean content of volatile fatty acids in the silages and the statistical analysis ( $\text{g} \cdot \text{kg}^{-1}$  DM)

## DISCUSSION

Freshly disintegrated fruits of pumpkin are characterized by a low content of dry matter; they are the material which is difficult to be ensiled. Due to this fact, it is recommended to enrich the ensiled biomass with the additional components, increasing the content of dry matter in the ensiled material. In the conducted experiment, dried beet pulp was the additional source of dry matter and organic substance, containing simple sugars. The application of the dried beet root pulp increased the dry matter content in the ensiled material and additionally, it increased the level of easily fermenting carbohydrates. The mentioned additives were the factors favourable for ensiling process and the obtained silage was characterized by high dry matter content and a high nutritional value. The addition of the beet root pulp could constitute a specific stimulator of fermentation in the initial stage of lactic fermentation (Bodarski et al. 2004). Hashemi and Razzaghzadeh (2007) ensiled pumpkin with the wheat straw (in the quantity of 28.6%) and also, added molasses to the ensiled material with the aim to improve the conditions of lactic fermentation.

The ensiling process, irrespectively of the examined variant, did not have any effect on the dry matter content in the silages as compared to the fresh material. Kilic and Saricicek (2010) when ensiling beet root pulp with different additives, including the bacterial ones, did not find any effect of ensiling on the content of dry matter in the silages. In the studies of Kilic and Saricicek (2010), the effect of the application of the inoculant on the crude protein content in the silages was

also not found. In our studies, the content of crude protein in the silages and also, of the crude fat was similar as the content of the mentioned components in the fresh material. Any effect of the inoculant on the content of the discussed components in the silage was also not recorded. The application of the inoculant did not affect the content of crude protein in the grass silages in the studies of Jalč et al. (2009). On the other hand, the cited authors stated the decline of fiber content when the bacterial preparations were employed. In the studies of Kilic and Saricicek (2010) on the ensiling of beet root pulp, any effect of inoculant on fiber content in the obtained silages was not found. In our studies, the decrease of the crude fiber content and ADF in the silages vs. the fresh material was recorded and in the silages with inoculant, also NDF decline was stated. On the other hand, any differences in the fiber content (crude fiber, NDF, ADF and ADL) between the examined silages were not stated. In the pumpkin as well as in the beet pulp, there is a high content of sugars and pectins, which are greatly decomposed by fermentation bacteria. Perhaps in the case of the mentioned raw materials, the intensive fermentation, even without the participation of bacteria from inoculant, affects the decomposition of a considerable part of structural carbohydrates, being present in the discussed raw materials. Hence, there is a lack of distinct effect of the inoculant application whereas the effect of ensiling process is visible.

Any significant differences in respect of pH value between the examined variants of silages were not found. On the basis of the above-mentioned fact, we

may state that the process of ensiling in the both cases was equally efficient and it was favourable for lowering of pH of the ensiled biomass. In the both silages, the pH value was found within the limits of 4.0–4.5. Such value of pH inhibits development of putrefying bacteria and fungi and activity of proteolytic vegetal enzymes, so it constitutes a preserving factor (Bodarski et al. 2004).

The bacterial-enzymatic preparation, as being employed in the experiment contained as follows: *Lactobacillus plantarum* bacteria, endo-1,4-beta-glucanase, xylanase and glucoamylase. *Lactobacillus plantarum* bacteria belong to the group of lactic acid bacteria and their addition should support fermentation towards lactic acid. The enzymes which are present in the preparation, i.e. endo-1,4-beta-glucanase, xylanase and glucoamylase, increase the decomposition of crude fiber in the ensiled material what increases the production of simple sugars, being a substrate for growth of lactic acid bacteria (Kowalik and Michalski 2006).

The content of ammonia nitrogen (N-NH<sub>3</sub>) in the silage is an indicator, i.a. of putrefaction processes which run in the silage usually due to the activity of butyric acid bacteria. Therefore, we may usually observe a positive correlation between the butyric acid and ammonia nitrogen content in the silage (Kowalik and Michalski 2006). In the examined silages, significantly lower N-NH<sub>3</sub> content was recorded in the silage with inoculant. The discussed silages were characterized by a higher content of lactic and acetic acid. The higher level of the mentioned acids could limit the development of proteolytic bacteria, decomposing protein to ammonia.

The examined silages differed significantly in respect of aerobic stability – the variants of the silages without inoculant were characterized by its lower value. Certain studies indicate that the silages with a higher content of lactic acid are more sensitive to the secondary fermentation as compared to the silages with the higher level of acetic acid (Szyszkowska et al. 2010). We may, therefore, assume that the silage produced from pumpkin with the higher content of lactic acid will be characterized by lower aerobic stability. Any such relationship was not, however, stated and the silage produced with the inoculant with higher lactic acid content had also higher aerobic stability. In the case of the examined silages in variant with the inoculant, apart from the higher level of lactic acid, there was also stated higher content of acetic acid which limits the development of secondary fermentation-inducing bacteria. It was stressed in the studies of Pyś et al. (2008), who analyzed the effect of *Lactobacillus buchneri* bacteria on the process of ensiling the maize.

We should pay the attention to the fact that the water-soluble simple sugars as contained in the ensiled material are the main substrate for development of bacteria, yeasts and moulds. Aerobic stability of the silage is decreasing proportionally to the increase of their content in the silage after completion of fermentation (Szyszkowska et al. 2010). The addition of beet root pulp to the ensiled pumpkin had a favourable effect on the ensiling process and nutritional value of the obtained silage. It constituted, however, a certain threat to its quality. The higher content of water-soluble carbohydrates in the silage increased significantly its sen-

sitivity to aerobic decomposition. Raczkowska-Werwińska et al. (2008) inform that a limited development of undesirable bacteria (*Clostridium* sp., *Coli* sp.) and fungi in the silages is obtained by the application of chemical and microbiological additives. It was confirmed in our studies, i.e. in lower content of ethanol and ammonia nitrogen in the silage with inoculant, and its higher aerobic stability.

## CONCLUSIONS

The obtained results of the studies indicate that mixing the disintegrated pumpkin fruits and dried pulp before ensiling allows obtaining the feed with a high nutritional value which may be employed, first of all, in nutrition of ruminants but also of monogastric animals. The application of inoculant during ensiling process increases the quality of the silage and improves its aerobic stability.

## Acknowledgements

This study was supported by the Polish National Research Council grant N CN NN311 521740.

## REFERENCES

- ALVES S.P., CABRITA A.R.J., JEROMINO E., BESSAR.J.B., 2011: Effect of ensiling and silage additives on fatty acid composition of ryegrass and corn experimental silages. *J. Anim. Sci.* 89: 2537–2545.
- AOAC, 2005: Official Methods of Analysis of AOAC International 16th Edition. Association of Analytical Chem., Arlington, VA, USA.
- BODARSKI R., KRZYWIECKI S., PASTERNAK A., 2004: Wpływ nawożenia azotem oraz dodatków kiszonkarskich na skład chemiczny i jakość oraz stabilność tlenową kiszzonek z pszenżyta. *Zesz. Nauk. AR we Wrocławiu Zootech.* LII, 505: 47–54.
- DANIELENKO H., 2000: The research on biochemical composition, culinary values and usability for processing of pumpkin family vegetables. *Rocz. AR w Poznaniu* 31(2): 245–252.
- HASHEMI A., RAZZAGHZADEH S., 2007: Investigation on the Possibility of Ensiling Cucurbit (*Cucurbita pepo*) Residues and Determination of Best Silage Formula. *J. Anim. Vet. Adv.* 6 (12): 1450–1452.
- JALČ D., LAUKOVAL A., SIMONOVA M., VARADYOVALZ., HOMOLKAP., 2009: The use of bacterial inoculants for grass silage: their effects on nutrient composition and fermentation parameters in grass silages. *Czech J. Anim. Sci.* 54: 84–91.
- KILIC U., SARICICEK B.Z., 2010: The Effects of Different Silage Additives on *in vitro* Gas Production, Digestibility and Energy Values of Sugar Beet Pulp Silage. *Asian J. Anim. Vet. Adv.* 5: 566–574.
- KORZENIEWSKA A., SZTANGRET J., SEROCZYŃSKA A., NIEMIROWICZ-SZCZYK K., 2004: The carotenoid content in the fruits of winter squash (*Cucurbita maxima* Duch). *Adv. Agri. Sci. Probl. Issues* 497: 339–345.
- KOWALIK I., MICHALSKI T., 2006: Wpływ niektórych czynników agrotechnicznych na parametry jakościowe kiszonki z kukurydzy odmiany „stay green”. *Pam. Puł.* 142: 201–224.
- KUCZYŃSKA B., 2011: Składniki bioaktywne i parametry technologiczne mleka produkowanego w gospodarstwach ekologicznych i konwencjonalnych. *Rozprawy Naukowe i Monografie. Wydawnictwo SGGW, Warszawa.*
- NIWCZAS J., SZWEDA D., MITEK M., 2005: Zawartość wybranych składników prozdrowotnych w owocach dyni olbrzymiej (*Cucurbita maxima*). *Żyw. Nauk. Tech. Jakość* 2 (43): 147–155.
- PYŚ J.B., KARPOWICZA., KANIA K., 2008: Wpływ dodatków – białkowego, bakteryj-

- nego lub chemicznego na skład chemiczny, mikrobiologiczny oraz stabilność tlenową kiszzonek z całych roślin kukurydzy. Zesz. Nauk. WSA Łomża. 37: 240–250.
- RASZKOWSKA-WERMIŃSKA K., POKAŃSKI A., GRAJEWSKI J., TWA-  
RUŻEK M., MIKLASZEWSKA B., ŁU-  
KOMSKA W., GUBAŁA A., SELWET  
M., 2008: Wpływ wybranych dodatków na  
wartość pokarmową i mikroflorę kiszzonek  
z kukurydzy inokulowanej *Penicillium ver-  
rucosum* 410. Med. Wet. 64 (2): 240–244.
- SELWET M., 2011: The effect of bacterial  
silage inoculants on the fermentation,  
cell wall contents and aerobic stability of  
maize silage. Acta Sci. Pol., Zootech. 10  
(3): 83–92.
- SZYSZKOWSKA A., KRZYWIECKI S.,  
SOBCZYK I., 2010: Czynniki wpływają-  
ce na intensywność procesu wtórnej fer-  
mentacji oraz wpływ skarmiania niesta-  
bilnych tlenowo kiszzonek na ryzyko wy-  
stąpienia jednostek chorobowych u krów  
mlecznych. Zesz. Nauk. UP Wroc., Biol.  
Hod. Zwierz. LX, 577: 205–2015.
- Van SOEST P.J., ROBERTSON J.B., LEWIS  
B.A., 1991: Method for dietary fibre, neu-  
tral detergent fibre, and non starch poly-  
saccharides in relation to animal nutrition.  
J. Dairy. Sci. 74: 3583–3597.
- Streszczenie:** Wpływ zakiszania dyni olbrzymiej z dodatkiem lub bez dodatku inokulanta na skład chemiczny i jakość kiszzonek. Celem badań było określenie wpływu zakiszania dyni na skład che-  
miczny i jakość kiszzonek. Kiszzonek przygotowano z dyni odmiany Justynka. Przed zakiszeniem rozdrobnione owoce dyni wymieszano z suszonymi wysłodkami w proporcji wynoszącej 80 : 20. Przygotowano dwa warianty kiszzonek z preparatem bakteryjno-enzymatycznym i bez preparatu. W kiszzonek oznaczono podstawowy skład chemiczny: zawartości suchej masy, popiołu surowego, białka ogólnego, tłuszczu surowego, włókna surowego, NDF, ADF, ADL. Oznaczono również wskaźniki świadczące o przebiegu procesu zakiszania i jakości uzyskanych kiszzonek: pH, zawartość kwasów mlekowego, octowego i masłowego, azotu amonowego, etanolu oraz stabilność tlenową. W uzyskanych kiszzonek w porównaniu do materiału przed zakiszeniem stwierdzono mniejszą zawartość włókna surowego, ADF, a w kiszzonek z inokulantem również NDF. Podstawowy skład chemiczny oraz pH w obu wariantach zakiszania były podobne. Kiszzonek z inokulantem charakteryzowały się większą zawartością kwasów mlekowego oraz octowego, natomiast mniejszą azotu amoniaku i etanolu. Kiszzonek z inokulantem miała również wyższą stabilność tlenową. Badania wskazują, że zastosowanie inokulanta wpłynęło na poprawę jakości uzyskanych kiszzonek.

MS. received November 2014

**Authors' addresses:**

Gabriela Halik  
Wydział Nauk o Zwierzętach SGGW  
Katedra Żywienia i Biotechnologii Zwierząt  
ul. Ciszewskiego 8  
02-786 Warszawa, Poland  
e-mail: gabrysiahalik@wp.pl

## **A survey of Deoxynivalenol and Zearalenone content in commercial dry foods for growing dogs**

KAROLINA HOŁDA, ROBERT GŁOGOWSKI

Department of Animal Breeding and Production, Warsaw University of Life Sciences – SGGW

**Abstract:** *A survey of Deoxynivalenol and Zearalenone content in commercial dry foods for growing dogs.* The Polish market of commercial dry dog food for growing dogs of small breeds was surveyed for the presence of DON and ZEN with its metabolites  $\alpha$ - and  $\beta$ -zearalenol ( $\alpha$ -,  $\beta$ -ZOL). LC/MS method was applied for 6 randomly selected foods. The low levels of toxins found, gives overall picture of the safe segment of the market. The content of masked  $\alpha$ -zearalenol, however, from the perspective of long term consumption of small amounts may pose a health risk for the reproductive system of bitches.

*Key words:* mycotoxin, growing dog, reproduction

### **INTRODUCTION**

In recent years a growing concern can be observed, regarding the potential health risks for pets via grain compounds of petfood, commonly contaminated with mycotoxins (Böhm et al. 2010).

From the perspective of animal nutrition, the most significant mycotoxins or mycotoxin groups are aflatoxins, zearalenone – ZEN, deoxynivalenol – DON, fumonisins and ochratoxin A – OTA (Schatzmayer and Streit 2013).

Among the *Fusarium* mycotoxins, DON and ZEN are of special importance because their occurrence cannot be completely excluded during plant production. Special attention has to be paid to the co-occurrence of those toxins. The intensity

of the effects observed may depend both on the concentration and as well as on the DON/ZEN. The proliferation-depressing potential of DON and ZEN on the same target cells serves as an example of their metabolic interactions (Döll and Dänicke 2011).

Numerous studies confirm, that the severity of mycotoxicoses, occurring after ingestion of feed contaminated with mycotoxins depends on dose, exposure duration, type of toxin, animal species and the age of the animal (Zachiarasova et al. 2014).

The most apparent clinical effect of DON is the voluntary intake depression. Its acute ability to induce vomiting has assigned it the trivial name “vomitoxin” (Döll and Dänicke 2011). Hughes et al. (1999) revealed, that the toxicity of DON in dogs is similar to that in swine, while dogs can be more prone to vomiting, because of their well-known tendency to rapidly consume their food.

Administered orally, ZEN is entirely absorbed in the gastrointestinal tract. It can be metabolized in monogastric animals and humans with the formation of  $\alpha$ - and  $\beta$ -ZOL, subsequently conjugated with glucuronic acid. The anatomopathological investigations have revealed, that ZEN administration of 200  $\mu\text{g}/\text{kg}$  of body weight per os for 7 days, caused apparent

structural changes in the ovarian follicle of the young, growing bitch (Gajęcka et al. 2004). It should be stressed, however, that both male and female dogs are affected by ZEN toxicity (Boermans and Leung 2007).

The capacity for ZEN metabolism in plants, leading to  $\alpha$ - and  $\beta$ -ZOL synthesis was described for rice and corn by Goliński et al. (1988) and further confirmed in a model study (Berthiller et al. 2007). The presence of ZEN and its metabolites in animal feed triggers a competition with the body estrogens and leads to a deregulation of estrogenic effects via impaired RNA and protein synthesis, clinically resulting in hyperestrogenism and reproductive disorders (Döll and Dänicke 2011).

Numerous scientific reports have been published on the prevalence of various mycotoxins in foods for companion animals, in particular dogs but, to our best knowledge there was no previous reports addressing the mycotoxin contamination within particular segment of the market, i.e. dry food for growing dogs.

The aim of the present study was to survey the Polish market of commercial dry foods intended for puppies and growing dogs of small breeds, for the presence of DON, ZEN,  $\alpha$ - and  $\beta$ -ZOL.

## MATERIAL AND METHODS

### Sample collection

A product database was created containing 47 dry extruded foods for growing dogs of small and mini/toy breeds that are commercially available on the Polish market (including retail sale and internet distribution). Six foods were randomly selected and purchased in medium weight packages (from 0.4 to 3 kg), with the special care for the remote “best before” date. All products were stored factory-sealed until analysis.

Table 1 presents the nutritional characteristics of the evaluated products, as pictured on the labels, complemented with the calculated content of nitrogen free extract (NFE) and metabolizable energy (ME) levels.

TABLE 1. Analytical constituents listed on the labels with the calculated values of ME

Product number	Protein (%)	Fat (%)	Crude ash (%)	Crude fibre (%)	NFE (%)	ME (kJ/100g)
1	28.5	14.5	7.8	2.9	36.3	1 541.6
2	30.0	20.9	11.9	4.0	25.2	1 602.7
3	25.0	12.0	7.5	2.5	43.0	1 504.7
4	29.0	16.0	6.5	2.5	36.0	1 602.7
5	32.0	22.0	7.0	2.5	26.5	1 716.2
6	31.0	21.0	7.5	1.9	29.6	1 723.7

NFE – nitrogen free extract; ME – metabolizable energy (calculated accordingly to NRC 2006).

### LC/MS analysis

DON and ZEN were assessed in the samples according to norms PN-EN 15791 and PN-EN 15792, respectively.

In brief, the high performance liquid chromatographic method with fluorescence detection and immunoaffinity column clean-up was used.

Statistical calculations were performed using SPSS ver. 21 software.

## RESULTS AND DISCUSSION

The results of the mycotoxin content analysis were summarized in Table 2. All assayed substances were present in all products, however in small amounts. It can be noticed, that there was relatively little variation in ZEN and  $\beta$ -ZOL content, unlike with DON and  $\alpha$ -ZOL.

In the recently published paper, Błajet-Kosicka et al. (in press) collected data of 5 foods for puppies. Among other mycotoxins assayed, the average levels of DON and ZEN were substantially higher than those presented in this study.

The authors named the primary components of all 49 products studied: wheat, maize, soybean, rice, meat and animal-origin (chicken and fish) and dairy products (Błajet-Kosicka et al. 2014).

Main compounds of foods evaluated in this study were listed in Table 3. Products 1 and 2 were not produced with the extrusion technology. The label of the Product 2, probably intentionally did not contain the full list of ingredients, and there was no reply for the inquiry send by an e-mail to the producer.

Indeed, it is a general rule of thumb that the first 5 ingredients in the list provide 80% nutrients of the product (Case 2014). As it is helpful when comparing foods, it does not provide a definite knowledge on the possible source of toxins, since the potential contamination was shown not only for plant but also for animal commodities (Duca et al. 2009). In animals continuously fed on diets containing significant mycotoxin levels there is a risk of the varying extent of toxin deposition and accumulation beyond compliance even if the feed used

TABLE 2. The content of deoxynivalenol (DON) zearalenone (ZEN) and  $\alpha$ -,  $\beta$ -zearalenol ( $\alpha$ -,  $\beta$ -ZEL) in dry foods for growing dogs

Product number	DON ( $\mu\text{g}/\text{kg}$ )	ZEN ( $\mu\text{g}/\text{kg}$ )	$\alpha$ -ZEL ( $\mu\text{g}/\text{kg}$ )	$\beta$ -ZEL ( $\mu\text{g}/\text{kg}$ )
1	5.86	3.11	6.51	5.49
2	23.25	2.13	7.49	3.16
3	5.19	2.74	2.92	3.74
4	9.07	2.65	6.57	3.13
5	6.16	3.01	4.15	2.81
6	17.57	2.52	4.23	5.59
Average	11.18 $\pm$ 7.49	2.69 $\pm$ 0.35	5.31 $\pm$ 1.79	3.98 $\pm$ 1.24

TABLE 3. First 5 ingredients listed on the labels of assayed products

Product number	Ingredients				
	1	2	3	4	5
1	dried beef	brown rice	dried duck meat	maize	menhaden meat
2	beef	proprietary			
3	cereals (min. 4% maize)	meat and animal-origin products	plant protein extracts	oils and fats	plant derived products
4	maize flour	poultry meal	pork scratchings	poultry fat	bovine fat
5	poultry meat	rice	wheat meal	animal fats	wheat
6	dried chicken	rice	maize	chicken fat	dried fish

does not exceed the actual recommendation (Völkel et al. 2011).

Therefore, the final level of mycotoxins in the foods may be a cumulative effect of the contamination of various components, on the contrary to the previously published reports (Pestka 2007).

One plausible explanation of the noticeable differences between the results published by Błajet-Kosicka et al. (in press) and this study may be the quality of the ingredients used in particular production plants. It was previously claimed, that among the major challenges in the pet food industry are grain processing and sampling for the quality control (Leung et al. 2006).

Nevertheless, the content of  $\alpha$ -ZOL in all assessed foods may be slightly disturbing, thus demanding further studies of toxicological safety in pet foods. This metabolite is often described as more biologically dangerous than its parent chemical (ZEN). Early study (Fitzpatrick et al. 1989) has shown, that  $\alpha$ -ZOL has greater binding affinity for estrogen receptors, than ZEN or  $\beta$ -ZOL.

Consequently, we speculate that an applied, feasible for a dog owner outcome of the present study, could be the assumption of the theoretical risk, linked with the consuming of evaluated foods by a “model” animal. Table 4 presents the calculated dose of assayed toxins,

TABLE 4. Hypothetic approximate total exposure on mycotoxins ( $\mu\text{g}$ ) of a model dog

Product number	DON	ZEN	$\alpha$ -ZOL	$\beta$ -ZOL
1	82.6	43.8	91.8	77.4
2 <sup>a</sup>	390.6–558.0	35.8–51.1	125.8–179.7	53.1–75.8
3	156.5	82.6	88.0	112.8
4	206.8	60.4	149.8	71.3
5	165.2	80.7	111.3	75.3
6	500.7	71.8	120.5	159.3

<sup>a</sup> Due to the given range of dosage, suggested by manufacturer.

that would be ingested by the dog during continuous offering of the particular food from 2 to 10 months of age (i.e. for 240 days – 8 months).

The model dog was set as a female of a small breed, with expected adult body weight of 5 kg, fed solely the particular product (1–6), consuming completely her daily ration, calculated accordingly to feeding suggestions, taken from the tables on the package. For the purpose of calculation, we assumed the constant level of mycotoxins in foods throughout the feeding period (batch to batch).

It seems reasonable to assume, that concentrations of DON and ZEN in dry pet foods, reported here, were below the no-observed-adverse-effect-level (NOAEL) proposed for dogs (EC 2002; Gajęcka et al. 2013b).

Boermans and Leung (2007) have described the main elements of the assessment of toxicity risk for pets, with its two main components being toxicity and exposure. Numerous reports emphasized chronic effects of consuming moderate to low amounts of mycotoxins as well as their co-occurrence or synergism (Leung et al. 2006, Döll and Dänicke 2011). Moreover, the special importance of fusariotoxins DON and ZEN interactions was discussed separately (Döll and Dänicke 2011).

Low doses of DON and ZEN (around NOAEL) administered in feed were shown to decrease the mRNA expression of genes controlling nitric oxide synthase (NOS), particularly in distal sections of the digestive tract of immature gilts (Gajęcka et al. 2013b). The prolonged effects of an impaired nitric oxide (NO) production can modify gastrointestinal functions, accelerating peri-

stalsis increasing tension of sphincters thus contributing to the inhibition of gastric emptying and digesta transfer in the intestines (Waśkiewicz et al. 2014).

Moreover, it should not be overlooked, that monotonic consumption of foods, containing small amounts (far below NOAEL) of mycotoxins, likely due to its cumulative effect in tissues, may result in female reproductive system dysfunctions, that were recently revealed (Gajęcka et al. 2013a).

Another physiologically important consequence of long term low dose oral exposure to ZEN was reported by Gajęcka et al. (2011). It was documented, that at 150% NOAEL dose of ZEN may disturb the fragile enzymatic equilibrium in pre-pubertal bitches by slowing down the steroidogenesis essential for instance in carbohydrate metabolism.

One interesting observation was increased body weight of pre-pubertal bitches intoxicated per os with NOAEL dose of ZEN: 50 µg/kg BW (Gajęcka et al. 2013a). Authors concluded, that very low doses of ZEN in commercial dog food do not result in clinical symptoms of intoxication, but they may enhance the somatic cells proliferation, stimulating/adaptive effect, consistent with the principle of hormesis (Calabrese 2005).

## CONCLUSIONS

The present study revealed low levels of DON, ZEN,  $\alpha$ - and  $\beta$ -ZOL in the dry food for growing dogs. The optimistic picture of obviously effective quality control in the manufacturing phase should not be overestimated, due to imminent adverse effects of the masked

mycotoxins of plant and animal origin, present in pet foods.

## REFERENCES

- BERTHILLER F., LEMMENS M., WERNER U., KRŠKA R., HAUSER M.-T., ADAM G., SCHUHMACHER R., 2007: Short review: metabolism of the *Fusarium* mycotoxins deoxynivalenol and zearalenone in plants. *Mycotoxin Res.* 23: 68–72.
- BŁAJET-KOSICKA A., KOSICKI R., TWARUŻEK M., GRAJEWSKI J., in press: Determination of moulds and mycotoxins in dry dog and cat food using liquid chromatography with mass spectrometry and fluorescence detection. *Food Addit. Contam. B*, DOI 10.1080/19393210.2014.933269.
- BOERMANS H.J., LEUNG M.C.K., 2007: Mycotoxins and the pet food industry: toxicological evidence and risk assessment. *Int. J. Food Microbiol.* 119: 95–102.
- BÖHM J., KOINING L., RAZZAZI-FAZELI E., BŁAJET-KOSICKA A., TWARUŻEK M., GRAJEWSKI J., LANG C., 2010: Survey and risk assessment of the mycotoxins deoxynivalenol, zearalenone, fumonisins, ochratoxin A, and aflatoxins in commercial dry dog food. *Mycotoxin Res.* 26: 147–153.
- CALABRESE E.J., 2005: Paradigm lost, paradigm found: The re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. *Environ. Poll.* 138: 378–411.
- CASE L.P., 2014: Who owns your dog food? And what is in it? In *dog food logic*. Dog-wise Publishing, Wenatchee: 131–149.
- DÖLL S., DÄNICKE S., 2011: The *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZON) in animal feeding. *Prev. Vet. Med.* 102: 132–145.
- DUCA R.C., BRAVIN F., DELAFORGE M., VLADESCU L., BADEA I.A., CRISTE R.D., 2009: Development of a new HPLC method used for determination of zearalenone and its metabolites in broiler samples. Influence of zearalenone on the nutritional properties of broiler meat. *J. Agric. Food Chem.* 57: 10497–10504.
- European Commission 2002: Opinion of the scientific committee on food on *Fusarium* toxins. Part 6: group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol. Available online: [http://ec.europa.eu/food/fs/sc/scf/out123\\_en.pdf](http://ec.europa.eu/food/fs/sc/scf/out123_en.pdf).
- FITZPATRICK D.W., PICKEN C.A., MURPHY L.C., BUHR M.M., 1989: Measurement of the relative binding affinity of zearalenone,  $\alpha$ -zearalenol and  $\beta$ -zearalenol for uterine and oviduct estrogen receptors in swine, rats and chickens: an indicator of estrogenic potencies. *Comp. Biochem. Physiol. C: Comp. Pharmacol.* 94: 691–694.
- GAJEĆKA M., JAKIMIUK E., POLAK M., OTROCKA-DOMAGAŁA I., JANOWSKIT., ZWIERZCHOWSKI W., OBREM-SKI K., ZIELONKA Ł., APOZNAŃSKI J., GAJEŃKI M., 2004: Zearalenone applied *per os* provides adverse effects in structure of chosen parts of bitch reproductive system. *Pol. J. Vet. Sci.* 7: 59–66.
- GAJEĆKA M., STOPA E., TARASIUK M., ZIELONKA Ł., GAJEŃKI M., 2013b: The expression of type-1 and type-2 nitric oxide synthase in selected tissues of the gastrointestinal tract during mixed mycotoxicosis. *Toxins* 5: 2281–2292.
- GAJEĆKA M., WOŹNY M., BRZUZAN P., ZIELONKA Ł., GAJEŃKI M., 2011: Expression of CYPsc and 3B-HSD mRNA in bitch ovary after long-term exposure to zearalenone. *Bull. Vet. Inst. Pulawy* 55: 777–780.
- GAJEŃKA M., ZIELONKA Ł., DĄBROWSKI M., MRÓZ M., GAJEŃKI M., 2013a: The effect of low doses of zearalenone and its metabolites on progesterone and 17 $\beta$ -estradiol concentrations in blood of pre-pubertal female Beagle dogs. *Toxicol.* 76: 260–269.
- GOLIŃSKI P., VESONDER R.F., LATUSZKIĘTKIEWICZ D., PERKOWSKI J., 1988: Formation of fusarenone X, niva-

- lenol, zearalenone,  $\alpha$ -trans-zearalenol,  $\beta$ -trans-zearalenol, and fusarin C by *Fusarium crookwellense*. Appl. Environ. Microbiol. 54: 2147–2148.
- HUGHES D.M., GAHL M.J., GRAHAM C.H., GRIEB S.L., 1999: Overt signs of toxicity to dogs and cats of dietary deoxynivalenol. J. Anim. Sci. 77: 693–700.
- LEUNG M.C.K., DÍAZ-LLANO G., SMITH T.K., 2006: Mycotoxins in pet food: a review on worldwide prevalence and preventative strategies. J. Agric. Food Chem. 54: 9623–9635.
- National Research Council, 2006: Nutrient requirements of dogs and cats. National Academies Press, Washington.
- PESTKA J.J., 2007: Deoxynivalenol: toxicity, mechanisms and animal health risks. Anim. Feed Sci. Technol. 137: 283–298.
- PN-EN 15791, 2012: Pasze. Oznaczanie deoksyniwalenolu w paszy. Metoda HPLC z oczyszczaniem na kolumnie powinowactwa immunologicznego. PKN, Warszawa.
- PN-EN 15792, 2012: Pasze. Oznaczanie zearalenonu w paszach. Metoda wysokosprawnej chromatografii cieczowej z detekcją fluorescencyjną i oczyszczaniem na kolumnie powinowactwa immunologicznego. PKN, Warszawa.
- SCHATZMAYR G., STREIT E., 2013: Global occurrence of mycotoxins in the food and feed chains: facts and figures. World Mycotoxin J. 6: 213–222.
- VÖLKEL I., SCHRÖER-MERKER E., CZERNY C.-P., 2011: The carry-over of mycotoxins in products of animal origin with special regard to its implications for the European food safety legislation. Food Nutr. Sci. 2: 852–867.
- WAŚKIEWICZ A., BESZTERDA M., KOSTECKI M., ZIELONKA Ł., GOLIŃSKI P., GAJĘCKI M., 2014: Deoxynivalenol in the gastrointestinal tract of immature gilts under per os toxin application. Toxins 6: 973–987.
- ZACHARIASOVA M., DZUMAN Z., VEPRIKOVA Z., HAJKOVA K., JIRU M., VACLAVIKOVA M., ZACHARIASOVA A., POSPICALOVA M., FLORIAN M., HAJSLOVA J., 2014: Occurrence of multiple mycotoxins in European feedingstuffs, assessment of dietary intake by farm animals. Anim. Feed Sci. Technol. 193: 124–140.

**Streszczenie:** *Charakterystyka rynkowego segmentu suchych karm pełnoporcjowych dla psów pod względem poziomu deoksyniwalenolu i zearalenonu. W reprezentatywnej próbie 6 losowo wybranych produktów, należących do rynkowego segmentu suchych karm pełnoporcjowych dla psów rosnących, oznaczono poziom deoksyniwalenolu i zearalenonu wraz z jego metabolitami ( $\alpha$ ,  $\beta$ -zearalenolem), z wykorzystaniem metody LC/MS. Uzyskane wyniki wskazują na stosunkowo niski poziom oznaczanych mykotoksyn w ocenianych karmach. Istotne wydaje się zwrócenie uwagi na poziom  $\alpha$ -zearalenolu (zaliczanego do tzw. toksyn ukrytych, po ang. masked toxins), który w kontekście długotrwałego przyjmowania z karmą może stwarzać potencjalne zagrożenie dla rozwoju układu rozrodczego młodych suk.*

*MS. received November 2014*

**Authors' address:**

Robert Głogowski  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
ul. Ciszewskiego 8, 02-786 Warszawa, Poland  
e-mail: robert\_glogowski@sggw.pl



## The effect of different ripening stages of durian *Durio zibethinus* fruit on zinc content in liver of rats loaded with cholesterol

IWONA JESION<sup>1</sup>, MARIA LEONTOWICZ<sup>2</sup>, HANNA LEONTOWICZ<sup>2</sup>,  
MIKOŁAJ A. GRALAK<sup>2</sup>, HUBERT KMIĘĆ<sup>1</sup>, SHELA GORINSTEIN<sup>3</sup>,  
RATIPORN HARUENKIT<sup>4</sup>

<sup>1</sup> Department of Biology of Animal Environment, Warsaw University of Life Sciences – SGGW

<sup>2</sup> Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW

<sup>3</sup> Department of Medicinal Chemistry and Natural Products, The Hebrew University – Hadassah Medical School

<sup>4</sup> Faculty of Agricultural Industry, King Mondkut's Institute of Technology Ladkrabang

**Abstract:** *The effect of different ripening stages of durian Durio zibethinus fruit on zinc content in liver of rats loaded with cholesterol.* Durian *Durio zibethinus* is an exotic, climacteric fruit, rich in dietary fiber, polyphenols, saccharides, vitamins and minerals. Postharvest changes in durian fruit influence its physical and chemical quality, which determined its health protective effect – antioxidant and hypocholesterolemic. Durian is a good source of zinc. The aim of this study was to investigate the zinc content in lyophilisate of durian fruit and in the diets supplemented with durian fruits at different stages of maturity. The *in vivo* experiment was designed to determine the influence of these diets on zinc content in the liver of rats loaded with cholesterol. The zinc content in durian fruit, in the diets, and in rats livers was examined using a flame atomic absorption spectrophotometry (Perkin-Elmer 1100B). The zinc content in durian fruits ranged from 8.1 to 12.3 mg/kg. The zinc content amounted 45.0, 44.2, 47.1, 47.3 and 48.0 mg/kg in C, ch, chDM, ch DR and chDOR diet, respectively. The content of zinc in the liver in all animal groups was within the normal limits and amounted from 25.0 to 33.0 mg/kg fresh weight. Diet supplemented with durian fruits at different stages of ripening increases accumulation of zinc in the liver of rats loaded with cholesterol. Durian fruits, especially ripe ones, could be used as a natural supplementation of zinc in the diet and might be helpful in prevention of diseases or disorders related to zinc deficiency.

**Key words:** durian fruit, climacteric, zinc, rat, liver

## INTRODUCTION

The exotic durian fruit *Durio zibethinus* is a precious source of bioactive compounds like dietary fiber, polyphenols, oligosaccharides, vitamins and minerals (Arancibia-Avila et al. 2008). It is one of the most important tropical fruit crops in Thailand, however the lack of an established technology for handling, transport and storage makes durian available abroad only frozen or lyophilised. Durian is a climacteric fruit what means that respiratory activity and ethylene production is rising also after the harvest and many enzymatic processes occur, which cause changes in content of saccharides and bioactive compounds (Ketsa and Daengkanit 1998). Ketsa and Pangkool (1994, 1995) and Ketsa and Daengkanit (1998) reported that temperature and humidity, and also ethylene and carbon dioxide production have particular importance in ripening process. They also

indicated that these parameters determined physical and chemical properties of durian, its consumption quality. Precise knowledge of the chemical composition of durian fruits during ripening is very important because durian is consumed in different stages of maturity. An unripe durian may be cooked as a vegetable, a ripe one is consumed mainly fresh, of course, where it is available. Overripe durian has a strong odour that attracts carnivorous animals and could be used as a dietary supplement which improves palatability and feed intake. Postharvest changes in durian fruit influence its physical and chemical quality, which determined its health protective effect – antioxidant and hypocholesterolemic. One of the most popular cultivar of durian is Mon Thong. The flesh, botanically called aril, is creamy, mild sweet with relatively moderate smell and have a higher content of polyphenols and antioxidant potential than in Chanee and Kan Yao cultivars (Leontowicz et al. 2008), which are also widely available at the Thai markets. Leontowicz et al. (2008) suggested that durian fruits cultivar Mon Thong, especially ripe ones, can be used like a dietary supplement for humans with metabolic disorders and in consequence can help people who suffer from cardio-vascular diseases.

Durian is a good source of zinc, however its concentration may differ depending on cultivar, stage of maturity, and ripening conditions (Haruenkit et al. 2007, 2010, Leontowicz et al. 2008, 2011, Poovarodom et al. 2010). Zinc is an essential mineral for multiple aspects of metabolism required for the catalytic activity of more than 200 enzymes (Osrredkar and Sustar 2011). Zinc is also

critical to tissue growth, wound healing, immune system function, prostaglandin production, bone mineralization, proper thyroid function, blood clotting, and normal functioning of the brain and central nervous system (Bhowmik et al. 2010). So far, studies on the ripening of durian fruits have been mainly related to physicochemical changes in its respiration, solids, starch, firmness, activities of polygalacturonase and pectinesterase, content of polyphenols, and antioxidant potential (Imsabai et al. 2002, Toledo et al. 2008). To the best of our knowledge no results of comparative studies describing the influence of diets supplemented with durian fruits at different stages of ripening (mature, ripe and overripe) on zinc content in liver of rats have been published.

The aim of this study was to investigate the zinc content in lyophilisate of durian fruit and in the diets supplemented with durian fruits at different stages of maturity. The *in vivo* experiment was designed to determine the influence of these diets on zinc content in the liver of rats loaded with cholesterol. The results of the investigation of durian at different stages of maturity *in vivo* on rats loaded with cholesterol would advance the use of this fruit in human nutrition.

## MATERIAL AND METHODS

### Samples preparation

In this investigation samples of Mon Thong cultivar of durian fruits at different stages of ripening were studied. All durian samples were harvested in May 2008, from a 25-year old commercial durian orchard, in Chantaburi province,

eastern Thailand. Harvesting and determination of maturity were carried out by Thai workers using the following techniques: day count, character of fruit spines, tapping the fruit, colour and shape of the fruit (Yaacob and Subhadrabandhu 1995). To get mature durian flesh with firm texture and no odour samples were left for 1 day at room temperature. Other fruits were left for another 4 days to ripen until their flesh became soft and overripe samples – having strong odour were obtained when fruits were left for another 3 days. All fruits were cleaned, weighed, chopped and homogenised in a high-speed blender (Hamilton Beach Silex professional model) for 1 min and lyophilised for 48 h (Virtis model 10-324).

### Rats, diets and management

The study was conducted in the Department of Physiological Sciences, Faculty of Veterinary Medicine Warsaw University of Life Sciences – SGGW. The results of plasma lipids, antioxidant activity, liver enzymes and histopathology of the aorta and liver of rats were presented in the previous publication (Leontowicz et al. 2011). The experimental model comprised the male Wistar rats ( $n = 30$ ) loaded with dietary cholesterol (1%) for 6 weeks. The body weight of rats at the beginning of the experiment was  $95.4 \pm 3.0$  g. The animals were randomly divided into 5 groups: control (C), control with cholesterol (ch) and with mature (chDM), ripe (chDR) and overripe (chDOR) durian fruits. The rats of all 5 groups were fed a basal diet (BD), which included wheat starch, casein, soybean oil, vitamin and mineral mixtures (Leontowicz et al. 2011). Control

group (C) was fed the BD only, 1% of cholesterol was added to the BD of the ch group, and BD of three other groups: chDM, chDR, chDOR was supplemented with 1% of cholesterol and 5% durian fruits (as freeze-dried powder) at the mentioned above different stages of ripening. All rats were fed *ad libitum* and had free access to water. The diets were offered once a day. The feed intake was monitored daily.

Before the section the rats were not fed for 24 h. At the end of the experiment, the rats were anaesthetized using Narcotan® (Zentiva) for inhalation, and from the liver of each experimental rats left lateral lobe were dissected. The Animal Care Committee of the Warsaw University of Life Sciences – SGGW, Poland approved this study (No. 10/2007).

### Zinc analysis

Approximately 0.5 g of lyophilized durian fruits in different stages of ripening, 0.5 g of five diets (C, ch, chDM, chDR and chDOR) and 1.0 g of liver were mineralized in microwave oven Milestone 900 with 5 ml 65% HNO<sub>3</sub> (Merck 1.00441) and 1 ml 30% H<sub>2</sub>O<sub>2</sub> (Merck 1.07298) and the content of zinc was determined using a flame atomic absorption spectrophotometer (Perkin-Elmer 1100B), at 210 nm. The method provides a linearity in the concentration range of 0–1 mg/l with a detection limit of 1 µg/l. The standards of 0.5 and 1.0 mg/l were prepared using 9953 Titrisol Zinc standard (Merck).

### Statistical analysis

One-way ANOVA analysis of variance (Duncan's test) was performed using Statistica 9 software. Differences were considered significant at  $P \leq 0.05$ . Data are

presented in figure as means  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

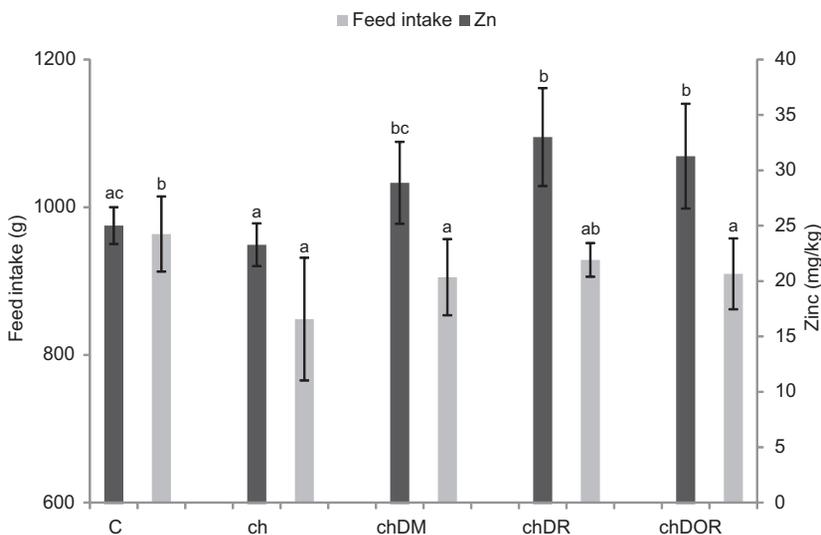
Fruits species vary widely in zinc concentration. The factors affecting the nutritional quality of crops can be assigned to two groups: pre-harvest like soil type, climate, disease, insects, harvest date, and post-harvest: storage conditions, post-harvest processing. It has been shown that out of four investigated exotic fruits: dragon fruit, star fruit, Longgong and durian, the last fruit cultivar Chanee has the highest (0.27 mg/100 g) content of zinc in fresh flesh (Charoensiri and Kongkachuichai 2006). Leontowicz et al. (2008) have reported, that the content of zinc in all three studied cultivars of durian: Mon Thong, Kan Yao and Chanee was comparable ( $P > 0.05$ ) and ranged from 49.5 to 78.4  $\mu\text{g}/100$  g fresh weight. Our study indicates that the content of zinc ranged from 8.1 to 12.3 mg/kg in durian fruit Mon Thong harvested in 2008. It must be noted that analytical determination was made in lyophilisate of durian. Our previous studies, conducted since 1994, have confirmed that freeze-drying process (lyophilisation) does not lead to loss of valuable components.

Zinc is an important component of carotenoids. Costa et al. (2011) have published that no significant variation was found in the Zn, P, Na and K concentrations during the tomato ripening after harvest, but have indicated that zinc concentration were higher in red tomatoes than in green ones. The increase in zinc content and reached a peak at the ripe stage was noted in banana *Musa* ssp.

(Adeyemi and Oladiji 2009). During ripening after harvest, green pigment of unripe banana is converted to carotenoids in ripe banana. Zinc requirements for most young domestic animals and poultry range from approximately 40 to 100 ppm in the diet (McDowell 1992). In our study all rats were fed basal diet containing 45 mg/kg zinc. The other four experimental diets with 1% of cholesterol and 5% of fruits in different stage of maturity were not significantly different ( $P > 0.05$ ) in zinc content and amounted 44.2, 47.1, 47.3 and 48.0 mg/kg in ch, chDM, ch DR and chDOR diet, respectively. Various dietary components may change zinc absorption. Puls (1994) indicates that increased dietary cholesterol reduces serum zinc and high density lipoproteins which may result in development of coronary heart disease. Most zinc is in the brain, muscle, bones, kidney and liver, with the highest concentrations in the prostate and parts of the eye (Osredkar and Sustar 2011). The concentrations of zinc in most mammalian tissues ranged from 10 to 100  $\mu\text{g}/\text{g}$  fresh weight (30–250  $\mu\text{g}/\text{g}$  dry weight), with little variation among species (McDowell 1992). The liver plays a central role in zinc homeostasis and vice versa, zinc plays an important role in the therapy for several liver diseases (Sidhu et al. 2004, Tian et al. 2014). In our previous work (Leontowicz et al. 2011) we concluded that durian at different stages of ripening, especially ripe one, constitutes an excellent source of effective natural compounds with antioxidant and health-protective activity in general, and liver and heart-protective effect in cholesterol fed rats in particular. Hepatic tissue morphology of rats fed basal diet with 1% of cho-

lesterol and with unripe durian revealed high contents of fat inside hepatic cells in contrast to rats fed diets with ripe durian, where hepatic tissue shows small number of large fat droplets. Furthermore somatic index of liver increased significantly in all rats receiving diet supplemented with cholesterol. This stress could affect zinc levels, despite the fact that durian fruits are rich in various minerals and other bioactive compounds (Leontowicz et al. 2011). The conducted research was designed to answer if diet supplementation with durian at different stages of maturity influences the liver zinc content in rats loaded with cholesterol. As shown at Figure 1, despite the highest feed intake in the control group (C), the zinc content is not reflected in the liver.

The content of zinc in the liver in all animal groups was within the normal limits and amounted from 25.0 to 33.0 mg/kg fresh weight. The lowest zinc content and feed intake were noticed in control rats (ch) fed diet with cholesterol. Rats receiving atherogenic diet with ripe durian have the highest content of zinc in the liver, significantly different from C and ch group. The various components of the diet and the durian fruit itself may change absorption of zinc and affect its bioavailability. Durian fruit is a good source of Fe, Mn and Cu, which may compete or/and inhibit of Zn uptake into intestinal cells. This fruit is also rich in dietary fiber that binds cholesterol and decreases its content in the blood. It is important to mention that the amount of



a-b – columns marked with different letter differ at  $P \leq 0.05$ ;

values are means  $\pm$ SD (n = 6);

C – control group; ch – control with 1% of cholesterol; chDM – group with 5% of mature durian and cholesterol; chDR – group with 5% of ripe durian and cholesterol; chDOR – group with 5% of ripe durian and cholesterol durian fruits.

FIGURE 1. Zinc content in the liver (fresh weight) and total feed intake of rats fed diets with durian in different stages of maturity and with cholesterol

alanine and aspartate aminotransferases were in normal range in blood plasma of rats fed atherogenic diet supplemented with durian (Leontowicz et al. 2011). Koo et al. (1986) have reported that nutritional status of zinc is an important factor influencing the intestinal absorption and subsequent metabolism of dietary cholesterol.

## CONCLUSIONS

Diet supplemented with durian fruits at different stages of ripening increases accumulation of zinc in the liver of rats loaded with cholesterol. Durian fruits, especially ripe ones, could be used as a natural supplementation of Zn in the diet and might be helpful in prevention of diseases or disorders related to zinc deficiency.

## REFERENCES

- ADEYEMI O.S., OLADIJI A.T., 2009: Compositional changes in banana (*Musa* ssp.) fruits during ripening. *Afr. J. Biotechnol.* 8: 858–859.
- ARANCIBIA-AVILA P., TOLEDO F., PARK Y.-S., JUNG S.-T., KANG S.-G., HEO B.-G., LEE S.-H., SAJEWICZ M., KOWALSKA T., GORINSTEIN S., 2008: Antioxidant properties of durian fruit as influenced by ripening. *LWT – Food Sci. and Technol.* 41: 2118–2125.
- BHOWMIK D., CHIRANJIB K.P., KUMAR S., 2010: A potential medicinal importance of zinc in human health and chronic disease. *Int. J. Pharm. Biomed. Sci.* 1(1): 5–11.
- CHAROENSIRI R., KONGKACHUICHAI R., 2006: abstract of research project: Analysis of beta-carotene, lycopene, minerals, sugars, insoluble and soluble dietary fiber contents in fresh fruits and fruits products. Mahidol University.
- COSTA F., BAETA M., SARAIVA D., VERISSIMO M.T., RAMOS F., 2011: Evolution of mineral contents in tomato fruits during the ripening process after harvest. *Food Analytical Methods* 4(3): 410–415.
- HARUENKIT R., POOVARODOM S., LEONTOWICZ H., LEONTOWICZ M., SAJEWICZ M., KOWALSKA T., DELGADO-LICON E., ROCHA-GUZMAN N.E., GALLEGOS-INFANTE J.-A., TRAKHTENBERG S., GORINSTEIN S., 2007: Comparative study of health properties and nutritional value of durian, mangosteen, and snake fruit: experiments in vitro and in vivo. *J. Agric. Food Chem.* 55: 5842–5849.
- HARUENKIT R., POOVARODOM S., VEARASILP S., NAMEŚNIK J., SŁIWKA-KASZYŃSKA M., PARK Y.-S., HEO B.-G., CHO J.-Y., JANG H.G., GORINSTEIN S., 2010: Comparison of bioactive compounds, antioxidant and antiproliferative activities on Mon Thong durian during ripening. *Food Chem.* 118: 540–547.
- IMSABAI W., KETSA S., van DOORN W.G., 2002: Effect of temperature on softening and the activities of polygalacturonase and pectinesterase in durian fruit. *Postharvest Biol. and Technol.* 26: 347–351.
- KETSA S., DAENGGANIT T., 1998: Physiological changes during postharvest ripening of durian fruit (*Durio zibethinus* Murray). *J. Hort. Sci. and Biotech.* 73: 575–577.
- KETSA S., PANGKOOL S., 1994: The effect of humidity on ripening of durians. *Post. Biol. Tech.* 4: 159–165.
- KETSA S., PANGKOOL S., 1995: Ripening behaviour of durians (*Durio zibethinus* Murray) at different temperatures. *Tropical Agriculture* 72: 141–145.
- KOO S.I., NORVELL J.E., ALGILANI K., CHOW J., 1986: Effect of marginal zinc deficiency in the lymphatic absorption of cholesterol. *J. Nutr.* 2: 2364–2371.
- LEONTOWICZ H., LEONTOWICZ M., HARUENKIT R., POOVARODOM S.,

- JASTRZEBSKI Z., DRZEWIECKI J., AYALA A.L.M., JESION I., TRAKHTENBERG S., GORINSTEIN S., 2008: Durian (*Durio zibethinus* Murr.) cultivars as nutritional supplementation to rat's diets. *Food and Chem. Toxicol.* 46: 581–589.
- LEONTOWICZ H., LEONTOWICZ M., JESION I., BIELECKI W., POOVERODOM S., VEARASILP S., GONZALEZ-AGUILAR G., ROBLES-SANCHEZ M., TRAKHTENBERG S., GORINSTEIN S., 2011: Positive effects of durian fruit at different stages of ripening on the hearts and livers of rats fed diets high in cholesterol. *Eur. J. Integr. Med.* 3: 169–181.
- MCDOWELL L.R., 1992: Minerals in animal and human nutrition. Academic Press, San Diego.
- OSREDKAR J., SUSTAR N., 2011: Copper and zinc, biological role and significance of copper/zinc imbalance. *J. Clin. Toxicol.* 53: 1–18.
- POOVARODOMS., HARUENKITR., VEARASILP S., NAMIESNIK J., CVIKROVA M., MARTINCOVA O., EZRA A., SUHAJ M., RUAMSUKE P., GORINSTEIN S., 2010: Comparative characterization of durian, mango and avocado. *Int. J. Food Sci. and Technol.* 45: 921–929.
- PULS R., 1994: Mineral levels in animal health: diagnostic data. 2<sup>nd</sup> edition. Sherpa, Clearbrook, BC.
- SIDHU P., GARG M.L., DHAWAN D.K., 2004: Protective effects of zinc on oxidative stress enzymes in liver of protein deficient rats. *Nutr. Hosp.* XIX (6): 341–347.
- TIAN X., ZHENG Y., LI Y., SHEN Z., TAO L., DOU X., QIAN J., SHEN H., 2014: Psychological stress induced zinc accumulation and up-regulation of ZIP 14 and metallothionein in rat liver. *BMC Gastroenterology* 14: 32.
- TOLEDO F., ARANCIBIA-AVILA P., PARK Y.-S., JUNG S.-T., KANG S.-G., HEO B.G., DRZEWIECKI J., ZACHWIEJA Z., ZAGRODZKI P., PASKO P., GORINSTEIN S., 2008: Screening of the antioxidant and nutritional properties, phenolic contents and proteins of five durian cultivars. *Int. J. Food Sci. and Nutr.* 59(5): 415–427.
- YAACOB O., SUBHADRABANDHU S., 1995: The production of economic fruits in South-East Asia. Oxford University Press, Kuala Lumpur.
- Streszczenie:** Wpływ różnych faz dojrzałości owocu durianu *Durio zibethinus* na zawartość cynku w wątrobie szczurów obciążonych cholesterolem. Durian *Durio zibethinus* to egzotyczny, klimakteryczny owoc, bogaty we włókno pokarmowe, polifenole, oligosacharydy, witaminy i minerały. Zmiany następujące po zbiorze owoców durianu wpływają na ich fizyczne i chemiczne parametry determinujące ich właściwości zdrowotne – przeciwutleniające i hypocholesterolemiczne. Durian jest cennym źródłem cynku. Celem badania było określenie zawartości cynku w owocach durianu, w dietach z dodatkiem durianu w różnych fazach jego dojrzałości. Badanie *in vivo* miało na celu określenie wpływu tych diet na zawartość cynku w wątrobie szczurów obciążonych cholesterolem. Zawartość cynku w owocach durianu, w dietach i wątrobach oznaczono metodą płomieniowej absorpcji atomowej (Perkin-Elmer 1100B). Zawartość cynku w liofilizacie owoców durianu mieściła się w zakresie 8,1–12,3 mg/kg. Zawartość cynku w dietach: C, ch, chDM, ch DR and chDOR wynosiła odpowiednio 45,0, 44,2, 47,1, 47,3 i 48,0 mg/kg. W wątrobie szczurów wszystkich grup zawartość cynku mieściła się w zakresie wartości referencyjnych i wynosiła od 25,0 do 33,0 mg/kg świeżej masy. Diety z dodatkiem owoców durianu w różnej fazie dojrzałości zwiększają akumulację cynku w wątrobie szczurów obciążonych cholesterolem. Owoce durianu, w szczególności dojrzałe, mogą być stosowane jako naturalny suplement cynku w diecie i być pomocne w prewencji chorób lub stanach związanych z niedoborem cynku.

MS. received November 2014

**Authors' address:**

Iwona Jesion  
Wydział Nauk o Zwierzętach SGGW  
Katedra Biologii Środowiska Zwierząt  
ul. Ciszewskiego 8, 02-786 Warszawa, Poland  
e-mail: iwona\_jesion@sggw.pl



## Diets supplemented with *Mytilus galloprovincialis* from polluted and non-polluted waters and their influence on zinc content in liver of rats loaded with cholesterol

IWONA JESION<sup>1</sup>, MARIA LEONTOWICZ<sup>2</sup>, HANNA LEONTOWICZ<sup>2</sup>,  
MARTA KOŁNIERZAK<sup>1</sup>, MIKOŁAJ A. GRALAK<sup>2</sup>, YONG-SEO PARK<sup>3</sup>,  
SHELA GORINSTEIN<sup>4</sup>

<sup>1</sup> Department of Biology of Animal Environment, Warsaw University of Life Sciences – SGGW

<sup>2</sup> Department of Physiological Sciences, Warsaw University of Life Sciences – SGGW

<sup>3</sup> Department of Horticultural Science, Mokpo National University

<sup>4</sup> Department of Medicinal Chemistry and Natural Products, The Hebrew University – Hadassah Medical School

**Abstract:** Diets supplemented with *Mytilus galloprovincialis* from polluted and non-polluted waters and their influence on zinc content in liver of rats loaded with cholesterol. Mussels may reflect the environmental problems that the ecosystem faces. *Mytilus galloprovincialis* is widely used as an indicator of water pollution in biological monitoring studies. These mussels are filter feeders and may accumulate important ecosystem pollutants what in turn may pose a potential risk to other organisms in the food chain. The aim of the study was to determine zinc content in the diets supplemented with mussels from polluted and non-polluted areas and in the livers of rats fed these diets for 30 days. The zinc content in the diets with mussels from contaminated and non-contaminated waters and in rat livers was examined using a flame atomic absorption spectrophotometry (Perkin-Elmer 1100B). The slight increase in zinc content from 73.2 ± 1.2 mg/kg in ch diet (control with 1% of cholesterol) to 77.2 ± 0.5 mg/kg in the diet with mussels from polluted site was found, but zinc content in all diet was within the normal limits reviewed by McDowell (1992) – 40 to 100 ppm. Zinc content in the diet supplemented with mussels from non-polluted site (73.7 ± 3.7 mg/kg) was similar to that found in ch diet. It can be concluded that cholesterol does not affect zinc bioaccumulation. However, prolonged consumption of mussels from contaminated sites may increase accumulation of zinc in the liver of rats.

**Key words:** mussels, polluted, non-polluted, zinc, rat, liver

## INTRODUCTION

The mussels are invertebrates of great importance for the economy and the environment. They have a high nutritional value (Leontowicz et al. 2008). The popularity, availability and consumption of seafood has increased (Gorinstein et al. 2008). Several reports indicate that mussels possess high level of proteins, vitamins, minerals, polyphenols and other antioxidants (Gorinstein et al. 2008, Leontowicz et al. 2008, Namiesnik et al. 2008). However, these marine organisms are filter feeders and may uptake from the water organic pollutants, heavy metals and accumulated them in the body (Potrykus et al. 2003, Spada et al. 2013). This physiological ability makes mussels – *Mytilus galloprovincialis* – biomarker of seawater pollution. Metal accumulation and rate of metal uptake in *Mytilus galloprovincialis* has been presented in

many reports (Machado et al. 1999, Adami et al. 2002, Kanduc et al. 2011, Marone et al. 2011, Ergul and Aksan 2013, Spada et al. 2013). Some authors (Lopez et al. 2001, Mosquera et al. 2003) present genetic variability and differences in protein expression between intertidal and cultured *Mytilus galloprovincialis*. It has been shown that the habitat of mussels may affect changes in heavy metals, antioxidant compounds, free radical scavengers in their tissues (Gorinstein et al. 2006, Leontowicz et al. 2008). It was decided to investigate the concentration of zinc in the diets with mussels from polluted and non-polluted areas and also evaluate the influence of these diets on zinc content in the liver of rats. Zinc is released to the environment from both natural and anthropogenic sources, however, releases from the second sources are greater. Severe zinc pollution tends to be confined to areas near emission sources, like ports, where zinc is deposited primarily in sediments through adsorption and precipitation. Large intake of foods high in zinc content, such as oysters and mussels, may be unsafe and may lead to metal bioaccumulation.

## MATERIAL AND METHODS

### Samples and sites of collection

Mussels were collected in two regions of Mokpo coast (Republic of Korea):

- non-polluted area: out of the port, 20 mi. North-West from Mokpo bay;
- polluted site: the Mokpo port, in the bay of Halla Ship large scale construction company which belongs to Hyundai group) at sea depth of 3–4 m (Leontowicz et al. 2008).

The collected mussels from both sites length  $4.37 \pm 0.5$  cm and it was 75–85 % of the maximum size. This approach allowed to assume that mussels were at the age of the similar metabolism (Gorinstein et al. 2006). Whole soft tissue ( $n = 30$  of each population) were frozen and storage at  $-80^{\circ}\text{C}$ . Then the samples were freeze dried in glass flasks on Finn-Aqua, Lyovac GT-2 equipment for 36 h and transported to Department of Physiological Sciences (Warsaw, Poland), where were waiting for further analysis.

### Rats, diets and management

The study was conducted in the Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW and lasted 30 days. The results of feed intake, body gains, plasma lipids and antioxidant activity were published previously (Leontowicz et al. 2008). The mean body weight of the male Wistar rats ( $n = 28$ ) at the beginning of the experiment was  $111 \pm 3.0$  g. The animals were randomly divided into 4 groups. The control (C) receiving basal diet (BD) (Leontowicz et al. 2011) only, and the diets of the other three groups were supplemented with 1% of cholesterol (ch). Two cholesterol diets contained 5.6% of mussel dry matter from polluted (chMP) and non-polluted areas (chMNP). All rats were fed *ad libitum* and had free access to water. The diets were offered once a day. Before the section the rats were not fed for 24 h. At the end of the experiment, the rats were anaesthetized using Narcotan® (Zentiva) for inhalation, and from the liver of each experimental rats left lateral lobe were dissected. The Animal Care Committee of the Warsaw University of Life

Sciences – SGGW, Poland approved this study (No. 10/2007).

### Zinc determination

Approximately 0.5 g of four diets: control (C), control with cholesterol (ch) and with lyophilised mussels from polluted (chMP) and non-polluted areas (chMNP) and 1.0 g of liver were mineralised in microwave oven (Milestone Ethos 900) with 5 ml 65% HNO<sub>3</sub> (Merck 1.00441) and 1 ml 30% H<sub>2</sub>O<sub>2</sub> (Merck 1.07298) and the content of zinc was determined using a flame atomic absorption spectrophotometer (Perkin-Elmer 1100B), at 210 nm. The method provides a linearity in the concentration range of 0–1 mg/l with a detection limit of 1 µg/l. The standards of 0.5 and 1.0 mg/l were prepared using 9953 Titrisol Zinc standard (Merck).

### Statistical analysis

One-way ANOVA analysis of variance (Duncan's test) was performed using Statistica 9 software. Differences were considered significant at  $P \leq 0.05$ . Data are presented in figure as means  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

Zinc is capable to form complexes with a variety of organic and inorganic groups. The environmental parameters like temperature, salinity and turbidity may influence zinc bioaccumulation. Zinc bioconcentrates moderately in aquatic organisms. The crustaceans and mussels species accumulate more heavy metals and other chemical contaminations than fish living in open water (Namiesnik et

al. 2008, Ergul and Aksan 2013). The reason for this may be less mobile lifestyle and often limiting to the bottom of the sea. The biota contain relatively little zinc compared to the sediments. In addition, the zinc content in sediment closely correlated with the depth, organic matter content, and clay content of the sediments. Mussels may reflect the environmental problems that an ecosystem faces. *Mytilus galloprovincialis* are widely used as an indicator of water pollution in biological monitoring studies. In young and small-sized individuals trend to accumulate heavy metals is reduced. The concentration of toxic elements is connected to the dry mass of tissue and that is why the adult mussels are preferable. This allows also the measurement of trace elements, even if their contents in environment are low (Ravera et al. 2003). In our experiment, mussels were characterised by a similar maximum length and size reached within each population. This approach makes that physiological differences between two populations would be less pronounced.

*M. galloprovincialis* process large volume of the water they live in to obtain food. At the same time they uptake other substances like: polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organotins, heavy metals presented in contaminated waters (Namiesnik et al. 2008). This may lead to concentrate important ecosystem pollutants in the mussel tissues and be a potential risk to other organisms in the food chain. Gorinstein et al. (2006) have found that the amounts of zinc and copper were significantly higher in the mussel proteins from the polluted than from the non-polluted sites of the Bulgarian

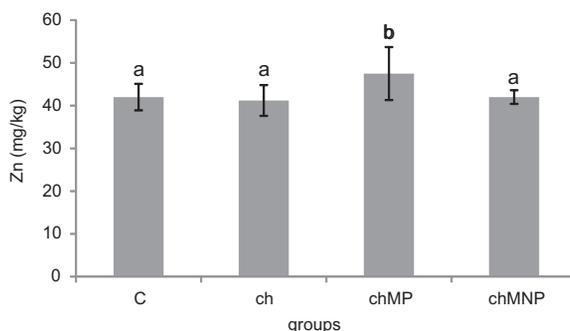
Black Sea coast. It could be concluded that the high content of zinc in the water and good physical and chemical environmental conditions for zinc accumulation influence zinc bioaccumulation in marine organisms and may contribute to higher risk of exposure to it. The diets except the mussels collected from areas with different levels of contamination, included wheat starch, casein, soybean oil, vitamin and mineral mixtures (Leontowicz et al. 2011). The inclusion rate of mussels in the diet (chMP and chMNP) was 5.6% and no significant differences were noted in zinc content between all experimental diets. However, slight increase in zinc content from  $73.2 \pm 1.2$  in ch diet to  $77.2 \pm 0.5$  mg/kg in the diet with mussels from polluted site (chMP) was found, but zinc content in all diet was within the normal limits reviewed by McDowell (1992) – 40 to 100 ppm. Zinc content in the diet supplemented with mussels from non-polluted site ( $73.7 \pm 3.7$  mg/kg) was similar to that found in ch diet.

Most of *in vivo* studies dedicated to mussels concern their effects on body weight gains of animals fed diets supplemented with different species of mussels and in different forms – raw, dry, as mixed commercial feed (Mao et al. 1999, Jönsson and Holm 2011, Anagnostidis et al. 2013). These authors concluded that mussels are a good source of protein and various biologically active substances. The results showed by Mao et al. (1999) indicate that selenium in mussel extract could be better absorbed in the rats and effectively promote their growth. Jönsson and Holm (2011) conducted in preliminary study on 12 hens, which were fed diets with 15% addition of normal or

15% addition of toxic mussels, that mussel meal could be a novel protein source for laying hens and toxic mussels at determined level may be included in the feed without negative effects on health and egg production.

Conducted research was designed to answer if supplementation with mussels from polluted and non-polluted areas influences liver zinc content in rats loaded with cholesterol. Several reports noted that mussels can be used as nutritional supplement in diet for animals like hens, fish and could improve feed and protein efficiency of commercial feeds (Anagnostidis et al. 2013). However, studies on the toxicity of mussels from contaminated sites on the accumulation of heavy metals in the body of animals that consume them are not so numerous. The content of zinc in most mammalian tissues ranged from 10 to 100  $\mu\text{g/g}$  fresh weight which equals 30–250  $\mu\text{g/g}$  dry weight (McDowell 1992). As shown at Figure 1, zinc content in the rat livers ranged from  $41.2 \pm 3.6$  in ch group to  $47.5 \pm 6.2$  mg/kg fresh weight in chMP group; was within the normal limits. It can be concluded that cholesterol does not affect zinc accumulation. The zinc content in the liver of rats receiving diet with mussels from polluted waters increase significantly ( $P \leq 0.05$ ) in related to other three groups, although there was no difference ( $P > 0.05$ ) in feed intake between animal groups (Leontowicz et al. 2008) and zinc content in the experimental diets also did not differ ( $P > 0.05$ ).

This small increase in the content of zinc in the diet with mussels from polluted waters could affect its accumulation in the liver of rats. Animals had received



a–b – columns marked with different letter differ at  $P \leq 0.05$ ; values are means  $\pm$ SD (n = 6); C – control group; ch – control with 1% of cholesterol; chMP – group with 5.6% of mussels from polluted site and cholesterol; chMNP – group with 5.6% of mussels from non-polluted site and cholesterol.

FIGURE 1. Zn content in the liver (fresh weight) of rats fed diets with mussels from polluted and non-polluted areas and with cholesterol

this diet for 30 days and it could be the cause of increase liver zinc content. Another cause could be diet components and/or heavy metals from contaminated waters, which could influence the absorption of zinc and its bioavailability. Bat et al. (2012) concluded that the high copper and zinc content in mussels reduces the absorption of toxic metals such as cadmium and lead. However, it should be noted, that zinc and copper in excessive amounts are also harmful. On the other hand supplementation of diets, containing cholesterol, with mussels from both contaminated and non-contaminated areas may improve animal protein metabolism and positively affect plasma lipid profile and plasma antioxidant activity (Gorinstein et al. 2008, Leontowicz et al. 2008).

## CONCLUSIONS

Prolonged consumption of mussels from contaminated sites may increase accumulation of zinc in the liver of rats.

## REFERENCES

- ADAMI G., BARBIERI P., FABIANI M., PISELLI S., PREDONZANI S., REISENHOFER E., 2002: Levels of cadmium and zinc in hepatopancreas of reared *Mytilus galloprovincialis* from the Gulf of Trieste (Italy). *Chemosphere* 48: 671–677.
- ANAGNOSTIDIS A., MICHAILEDIOU M., VATSON I.N., TSOPELAKOS A., MILIOU H., ANGELIDIS P., 2013: Use of frozen mussel (*Mytilus galloprovincialis*) and mussel meal in the diet of sea bass (*Dicentrarchus labrax* Linnaeus, 1758) and sea bream (*Sparus aurata* Linnaeus, 1758) fingerlings – a preliminary study. *Aquacult. Res.* 1: 5.
- BAT L., USTUN F., BAKI O., 2012: Trace element concentration in the Mediterranean mussels *Mytilus galloprovincialis* caught from Sinop Coast of the Black Sea, Turkey. *Open Mar. Biol. J.* 6: 1–5.
- ERGUL H.A., AKSAN S., 2013: Evaluation of non-essential element and micronutrient concentrations in seafood from the Marmara and Black Seas. *J. Black Sea/Mediterranean Environment.* 19, 3: 312–330.
- GORINSTEIN S., LEONTOWICZ M., LEONTOWICZ H., NAMIESNIK J.,

- JASTRZEBSKI Z., DRZEWIECKI J., PARK Y.-S., HAM K.-S., HEO B.-G., TRAKHTENBERG S., 2008: Influence of mussels (*Mytilus galloprovincialis*) from polluted and non-polluted areas on some atherosclerosis indices in rats fed cholesterol. *Food Chem.* 111: 381–386.
- GORINSTEIN S., MONCHEVA S., TOLEDO F., ARANCIBIA-AVILA P., TRAKHTENBERG S., GORINSTEIN A., GOSHEV I., NAMIESNIK J., 2006: Relationship between seawater pollution and qualitative changes in the extracted proteins from mussels *Mytilus galloprovincialis*. *Sci. Total Environ.* 364: 251–259.
- JÖNSSON L., HOLM L., 2011: Effects of toxic and non-toxic blue mussel meal on health and product quality of laying hens. *J. Anim. Physiol. Anim. Nutr.* 94: 405–412.
- KANDUC T., MEDAKOVIĆ D., HAMER B., 2011: *Mytilus galloprovincialis* as a bioindicator of environmental conditions: the case of the eastern coast of the Adriatic Sea. *Isot. Environ. Health.* 47: 42–61.
- LEONTOWICZ H., LEONTOWICZ M., JESION I., BIELECKI W., POOVERODOM S., VEARASILP S., GONZALEZ-AGUILAR G., ROBLES-SANCHEZ M., TRAKHTENBERG S., GORINSTEIN S., 2011: Positive effects of durian fruit at different stages of ripening on the hearts and livers of rats fed diets high in cholesterol. *Eur. J. Integr. Med.* 3: 169–181.
- LEONTOWICZ H., LEONTOWICZ M., NAMIESNIK J., DRZEWIECKI J., PARK Y.-S., ZACHWIEJA Z., ZAGRODZKI P., GORINSTEIN A., TRAKHTENBERG S., GORINSTEIN S., 2008: Nutritional properties of mussels *Mytilus galloprovincialis*. *Eur. Food Res. Technol.* 227: 1251–1258.
- LOPEZ J.L., MOSQUERA E., FUENTES J., MARINA A., VAZQUEZ J., ALVAREZ G., 2001: Two-dimensional gel electrophoresis of *Mytilus galloprovincialis*: differences in protein expression between intertidal and cultured mussels. *Mar. Ecol. Prog. Ser.* 224: 149–156.
- McDOWELL L.R., 1992: Minerals in animal and human nutrition. Academic Press, San Diego.
- MACHADO L., BEBIANNO M., BOSKI T., MOURA D., 1999: Trace metals on the Algarve coast, II: Bioaccumulation in mussels *Mytilus galloprovincialis*. *Biol. Inst. Esp. Oceanogr.* 15,1–4: 465–471.
- MAO W.-J., LI Y., LI B.-F., LIU Y.-H., 1999: Study on biological activity ofelenium-enriched mussel extract. *Chin. J. Oceanol. Limnol.* 15, 4: 1–4.
- MARRONE R., CAROSIELLI L., CHIARAVALLE A., MIEDICO O., VOLLANO L., DELLAROTONDA M., 2011: Trace elements and radionuclides in *Mytilus galloprovincialis* from Campania region (Italy). *Ital. J. Food Saf.* 1,1: 233–236.
- MOSQUERA E., LOPEZ J.L., ALVAREZ G., 2003: Genetic variability of the marine mussels *Mytilus galloprovincialis* assessed using two-dimensional electrophoresis. *Heredity* 90: 432–442.
- NAMIESNIK J., MONCHEVAS., PARKS.-Y., HAM K.-S., HEO B.-G., TASHMA Z., KATRICH E., GORINSTEIN S., 2008: Concentration of bioactive compounds in mussels *Mytilus galloprovincialis* as an indicator of pollution. *Chemosphere* 73: 938–944.
- POTRYKUS J., ALBALAT A., PEMP-KOWIAK J., PORTE C. 2003: Content and pattern of organic pollutants (PAHs, PCBs and DDT) in blue mussels (*Mytilus trossulus*) from the southern Baltic Sea. *Oceanologia* 45, 1, 337–355.
- RAVERA O., CENCI R., BEONE G., DANTAS M., LODIGIANI P., 2003: Trace element concentrations in freshwater mussels and macrophytes as related to those in their environment. *J. Limnol.* 62: 61–70.
- SPADAL., ANNICCHIARICOC., CARDELLICCHIO N., GIANDOMENCIO S., DI LEO A., 2013: Heavy metal monitoring in the mussels *Mytilus galloprovincialis* from the Apulian coast (Southern Italy). *Medit. Mar. Sci.* 14, 1: 99–108.

**Streszczenie:** *Diety z dodatkiem Mytilus galloprovincialis z wód niezanieczyszczonych i zanieczyszczonych oraz ich wpływ na zawartość cynku w wątrobie szczurów obciążonych cholesterolem.* Małże mogą odzwierciedlać problemy środowiskowe, przed którymi stoi ekosystem. *Mytilus galloprovincialis* jest szeroko wykorzystywany w biomonitoringu. Małże te są filtratorami i mogą kumulować w tkankach ważne zanieczyszczenia z ekosystemu, co z kolei może stanowić potencjalne zagrożenie dla innych organizmów w łańcuchu troficznym. Celem badania było określenie zawartości cynku w dietach z dodatkiem małży z obszarów niezanieczyszczonych i zanieczyszczonych oraz w wątrobach szczurów żywionych tymi dietami przez 30 dni. Zawartość cynku w dietach z małżami z zanieczyszczonych i niezanieczyszczonych wód i wątrobach szczurów oznaczono metodą płomieniowej absorpcji atomowej (Perkin-Elmer 1100B). Wykazano nieznaczny wzrost zawartości cynku w die-

cie z małżami z wód zanieczyszczonych ( $77,2 \pm 0,5$  mg/kg) względem grupy ch – kontrola z 1% udziałem cholesterolu ( $73,2 \pm 1,2$  mg/kg), ale zawartość cynku we wszystkich dietach mieściła się w granicach normy podanych przez McDowell (1992) – 40–100 ppm. Zawartość cynku w diecie z małżami z obszarów niezanieczyszczonych ( $73,7 \pm 3,7$  mg/kg) była zbliżona do zawartości w diecie grupy ch. Podsumowując wyniki, można stwierdzić, że cholesterol nie wpływa na bioakumulację cynku. Jednak, długotrwałe spożywanie małży z wód zanieczyszczonych może zwiększyć akumulację cynku w wątrobie szczurów.

*MS. received November 2014*

**Authors' address:**

Iwona Jesion  
Wydział Nauk o Zwierzętach SGGW  
Katedra Biologii Środowiska Zwierząt  
ul. Ciszewskiego 8, 02-786 Warszawa, Poland  
e-mail: iwona\_jesion@sggw.pl



## **Effect of copper nanoparticles and copper sulfate administered *in ovo* on copper content in breast muscle, liver and spleen of broiler chickens**

NATALIA MROCZEK-SOSNOWSKA<sup>1</sup>, MONIKA ŁUKASIEWICZ<sup>1</sup>,  
AGNIESZKA WNUK<sup>1</sup>, EWA SAWOSZ<sup>2</sup>, JAN NIEMIEC<sup>1</sup>

<sup>1</sup>Department of Animal Breeding and Production, <sup>2</sup>Department of Animal Nutrition and Biotechnology  
Warsaw University of Life Sciences – SGGW

**Abstract:** *Effect of copper nanoparticles and copper sulfate administered in ovo on copper content in breast muscle, liver and spleen of broiler chickens.* The initial experimental material included 300 hatching eggs of Hubbard Flex chickens. The eggs were divided into three groups: control, NanoCu and CuSO<sub>4</sub>. Eggs from groups NanoCu and CuSO<sub>4</sub> were subjected to *in ovo* injection to the air cell of egg. Experimental solutions were administered by *in ovo* injection using a sterile needle and a 0.3 mL syringe as follows: NanoCu – colloid of copper nanoparticles, concentration 50 ppm; and CuSO<sub>4</sub> – colloid of copper sulfate, concentration 50 ppm. The eggs were incubated under standard conditions. After hatching, 50 chicks were selected from each group for 42-day rearing. The birds were fed standard feed concentrates for broilers. In that last day of rearing (42 day), 12 females and 12 males were selected from each group. The right part of their breast muscle, their liver and spleen were collected for copper content determinations. Results of this experiment confirm previous scientific reports which demonstrate that the greatest accumulation of copper is observed in soft organs like liver or spleen.

**Key words:** nanoparticles, *in ovo*, copper, copper sulfate, egg

### **INTRODUCTION**

Researches concerning content of mineral elements in tissues of farm animals are interesting both from the point of view of the good of animals and people's health

as people use animal products. These researches include not only exposition to heavy metals, such as cadmium and lead, but also concentration and transferring in the food chain of indispensable elements, such as zinc, copper and magnesium. Metals which are the components of animal tissues are consumed by people and therefore they directly affect the condition of public health (Nriagu et al. 2009).

Particular ability to accumulate some of the elements has animal liver which is commonly used in cooking. Liver is widely considered among numerous societies as the source of macro- and microelements and vitamins soluble in fats. Kunachowicz et al. (1998), report that the highest content of copper in chicken meat and edible products is in liver (0.3 mg/100 g). Due to this fact, liver is one of the best indicators showing demand for particular elements in organism (Dorton et al. 2003).

Although meat is a significant component of human diet, it can also accumulate heavy metals which in concentrations exceeding reference values may pose toxic risk. Level of trace elements in meat and in meat products depends on different factors, such as condition of environment, type of grazing land and

genetic features of organisms (Demirezen and Aksoy 2004). Rays et al. (1994) underline that toxicity can be harmful even in small intensity upon long-term consumption, owing to its capability to accumulate in human and animal bodies. Although copper has significant role in proper functioning of the organism, its high intake can cause health problems and lead to damage of liver and kidneys (Beneddouché et al. 2014).

Currently, copper is added as copper sulphate to pre-mixes of blends for broiler chicken due to its anti-bacterial properties and to promote the effect of growth (Pesti and Bakalii 1996). Owing to the fact that copper has anti-bacterial properties and it can become an alternative to antibiotics, growing interest has been observed in this element used in production of poultry. Different sources and forms of copper have also diverse bio-availability and influence on animals. Du et al. (1996) claim that the most assimilable copper is organic one and nanoparticles of copper. However, still the most widely used form of copper is copper sulphate due to its lower price and easy access.

The aim of this experiment was to determine the effect of copper nanoparticles and copper sulfate administered *in ovo* on the content of copper in breast muscle, liver and spleen of broiler chickens.

## MATERIAL AND METHODS

### **In ovo injection and incubation of hatching eggs**

300 hatching eggs of Hubbard Flex broiler chickens (average weight 62.25 ± 2.2 g) were the experimental material

in the first stage of the study. The eggs were stored for 4 days at a temperature of 12°C and humidity of 73%. The eggs were weighed and divided into three experimental groups: C, NanoCu, CuSO<sub>4</sub>, 100 eggs each.

Experimental solutions were administered by *in ovo* injection to the air cell of egg using a 0.3-mL sterile syringe with a needle in the following doses for particular groups: NanoCu (colloid of copper nanoparticles, concentration 50 ppm) and CuSO<sub>4</sub> (copper sulfate colloid, concentration 50 ppm). Holes after injection were sterile-tightened and the eggs were placed in an incubator and hatched under standard conditions (temperature 37.8°C, humidity 60%, eggs rotation per hour by 90° angle for 18 days). The eggs were hatched in an incubator by a German company Heka equipped in temperature, air humidity and egg rotation controlling module. During incubation, the eggs were two-fold light-exposed on 6<sup>th</sup> and 18<sup>th</sup> day, and weighed in order to determine egg weight loss. On 19<sup>th</sup> day of incubation, the eggs were transferred to a hatching compartment with a temperature of 37.0–37.5°C and relative humidity of 75–80%. After hatching, one-day chicks were evaluated and healthy chicks with healed navels were selected for further rearing experiment.

### **Rearing, housing conditions and feeding**

Further stage of the study included 150 Hubbard Flex broiler chickens (50 birds in each group), that were kept on litter until 42 days of age under standard animal husbandry conditions, in a room without the access of daylight. One-day chicks after weighing and tagging with individ-

ual tags were divided into three groups (C – control, NanoCu, CuSO<sub>4</sub>), in two replications, 25 birds each. Stock density in a poultry house was 11 birds per 1 m<sup>2</sup>. Immediately after introduction to the poultry house, the chickens from all groups were vaccinated against Marek's disease, infectious bronchitis and coccidiosis.

A three-stage feeding program was applied during rearing: starter (crumb), grower and finisher (granulate) – Table 1; the birds were fed *ad libitum*. Body weight of the birds (1st, 14th, 35th and 42th), mortality rate and feed intake were controlled throughout the rearing period.

TABLE 1. Feed mixture composition and nutritional value according to producer's

Specification	Starter (1–14 days)	Grower (15–35 days)	Finisher (36–42 days)
Diet component [%]			
Corn	10.00	11.40	10.00
Wheat	53.00	55.00	60.80
Soybean meal	30.60	27.40	21.60
Feeding limestone	1.19	1.20	0.97
Sodium bicarbonate	0.20	0.14	0.16
NaCl	0.24	0.28	0.26
Stimulant	0.01	0.01	0.01
Dicalcium phosphate	1.18	0.78	0.64
Soybean oil	2.10	2.40	4.40
Methionine 84% calcium salt	0.48	0.42	0.28
Lysine	0.36	0.34	0.28
Threonine	0.14	0.13	0.10
Premix C196 PX05802 0.5%	0.50	0.50	0.50
Nutritional value			
ME [kcal]	2 990.20	3 047.19	3 217.10
Fat [%]	3.67	4.00	5.92
Protein [%]	21.99	20.78	18.51
Fiber [%]	3.60	2.55	2.41
Ash [%]	5.83	5.35	4.67
Lysine [%]	1.38	1.28	0.97
Methionine + cystine [%]	1.08	1.01	0.76
Available phosphorus [%]	0.45	0.38	0.35

Provided per kilogram of diet: STARTER: vitamin A 11.00 K UL; organic phosphorus 0.59%; calcium 0.98%; phosphorus available 0.45%; calcium chloride 0.24%; sodium 0.15%; chlorine 0.27%; potassium 0.90%; magnesium 0.17%; manganese 142.32 mg; copper 31.59 mg; selenium 0.41 mg; iron 191.51 mg; sulfur 0.34%; zinc 116.80 mg; lysine 1.36%; methionine 0.31%; GROWER: vitamin A 11.00 K UL; organic phosphorus 0.51%; calcium 0.87%; phosphorus available 0.38%; calcium chloride 0.28%; sodium 0.15%; chlorine 0.29%; potassium 0.85%; magnesium 0.16%; manganese 141.84 mg; copper 30.82 mg; selenium 0.41 mg; iron 174.55 mg; sulfur 0.32%; zinc 115.03 mg; lysine 1.26%; methionine 0.30%; FINISHER: vitamin A 11.00 K UL; vitamin D3 3.00 K UL; vitamin E; organic phosphorus 0.73%; calcium 0.35%; calcium chloride 0.26%; sodium 0.15%; chlorine 0.27%; potassium 0.74%; magnesium 0.15%; manganese 140.80 mg; copper 29.92 mg; selenium 0.40 mg; iron 159.92 mg; sulfur 0.28%; zinc 113.14 mg; lysine 1.06%; methionine 0.27%.

### Slaughter and collection of material for analyses

In the 6th week of rearing, 12 cocks and 12 hens, with body weights similar to the average body weight in a group, were selected from each group. After 12-hour fasting, the chickens were transported to a poultry slaughter house. After 24 h of air-chilling of the carcasses at 4°C, slaughter traits were analyzed and contents of breast muscles, leg muscles, adipose fat and giblets in the carcass were calculated. For determinations of copper content, the right part of breast muscle, liver and spleen were collected from each bird.

### Determination of copper content in breast muscles, liver and spleen

Samples to be analyzed were subjected to microwave mineralization in a Milestone 1200 mineralizer equipped in high-pressure mineralization vessels. The size of the weighed portion depended on the character of the samples and ranged from ca. 0.5 to 1.0 g. The samples were mineralized with 7 mL of concentrated (69%) nitric acid V of spectral purity and 1.1 mL of concentrated (30%) hydrogen peroxide also of spectral purity. The optimized controlling programme of the

TABLE 2. Optimized controlling programme of the mineralization process

Stage	Time [min]	Power [W]
1	1	250
2	1	0
3	5	250
4	5	400
5	5	650

mineralization process was presented in Table 2.

The content of copper was determined with the method of atomic emission spectrometry with inductively-coupled plasma (ICP-AES) using an iCAP 6500 spectrometer by Thermo Scientific company. Optimized parameters of spectrometer work were presented in Table 3. The spectrometer was calibrated with multi-element standard solutions prepared from an ICP Multi element standard solution IV CertiPUR by Merck company. A few analytical lines (presented in Table 3) were collected for copper determinations. The final result of the analysis represented the arithmetic mean of partial results obtained for selected analytical lines devoid of interference. The quality of study results was confirmed with the use of reference material, i.e. lyophilized chicken meat NCS ZC73016 with a certified content of copper.

TABLE 3. Optimized parameters of spectrometer work

Parameters	Conditions of iCAP 6500 spectrometer work
Power generator [W]	1 150
Atomizer	type Meinhard
Mist chamber	cyclone
Plasma gas [l/min]	12
Supporting gas [l/min]	0.5
Gas flow rate in atomizer [l/min]	0.5
Length of copper line [nm]	324.7
	327.3
	224.7
	219.9

### Statistical analysis

The data obtained were analyzed statistically using a multi-factor analysis of variance (least squares) using SPSS 21.0 software (SPSS, Chicago, IL, USA). Only significant interactions between factors ( $P \leq 0.01$  or  $P \leq 0.05$ ) were considered in the study. The level of significance was determined after performing preliminary statistical analyses

### RESULTS AND DISCUSSION

Akan et al. (2010), who determined concentration of heavy metals (Cu, Zn, Co, Mn, Mg, Fe, Cr, Cd, As, Ni and Pb) in internal organs and meat of selected species of farm animals, conclude that the highest concentration of these elements is in liver and kidneys, whereas the lowest in meat. It also confirms (Tekin-Özan 2008) that metals accumulate in the biggest amount in tissues and soft organs. Similar effect were confirmed in our

research (Table 4) in which content of copper was the highest in liver and next in spleen, whereas the lowest in breast muscle. Niedziółka et al. (2007) in their researches aiming to determine the rate of selected elements in meat, liver of kid and ram fed full portions of blend with 10% of linseed observed that liver was the organ of concentration of copper, zinc and manganese. Although the amount of copper in muscular tissue of analysed animals was small, the authors concluded statistically vital differences ( $P < 0.01$ ) (kid 0.08 mg/100 g; ram 0.07 mg/100 g).

The *in ovo* injection of copper colloids applied in the study caused a significant ( $P < 0.05$ ) decrease in copper content in breast muscles of the experimental chickens only in male species NanoCu (0.31 mg/100 g) and CuSO<sub>4</sub> (0.30 mg/100 g) compared to control group (0.39 mg/100 g). The concentration of copper in muscular tissue in female species was on the same level and no significant differences were observed.

TABLE 4. Content of copper in breast muscle, liver and spleen of broiler chickens (mg/100 g product)

Sex	Group	Breast muscle	Liver	Spleen
♂♂	C	0.39 ±0.04 <sup>a</sup>	3.20 ±0.46 <sup>Bb</sup>	0.65 ±0.07
	NanoCu	0.31 ±0.03 <sup>b</sup>	4.34 ±0.37 <sup>A</sup>	0.75 ±0.08
	CuSO <sub>4</sub>	0.30 ±0.03 <sup>b</sup>	4.14 ±0.46 <sup>a</sup>	0.68 ±0.07
♀♀	C	0.27 ±0.03	4.12 ±0.45	0.64 ±0.07
	NanoCu	0.26 ±0.03	3.77 ±0.41	0.73 ±0.08
	CuSO <sub>4</sub>	0.26 ±0.03	3.98 ±0.44	0.72 ±0.08

<sup>A,B</sup> Mean values within a sex group differ significantly at  $P < 0.01$ ;

<sup>a,b</sup> mean values within a sex group differ significantly at  $P < 0.05$ .

Didanto and Sarkar (1997) claim that absorbing of copper occurs mostly in stomach and small intestine of most of animal species. Thornburg (2000) in his research on dogs demonstrated that absorption of copper occurred mostly in the upper part of the small intestine, and that liver was the main organ of copper capture. After absorption in intestine and penetration to blood serum, copper ions bind with albumins. Within 2 h, they are inbuilt to liver cells. Herein copper is either stored (in liver liposome) or is bound with apoceruloplasmin forming ceruloplasmin which then is secreted to blood plasma. Ceruloplasmin is the main factor of copper transport from liver to other tissues and acts as a donor of this metal in production of copper-dependant enzyme (Kochanowska et al. 2008).

Our research conducted on three types of tissues proves that liver has the biggest tendency to accumulate copper. The level of copper in birds liver in research conducted after the injection of colloids both with nanoparticles of copper and with copper sulphate had a significant effect on increasing of this element content in both experimental groups of male species NanoCu (4.34 mg/100 g) and CuSO<sub>4</sub> (4.14 mg/100 g) compared to control group (3.20 mg/100 g). Accumulation of copper in liver of females has had a reverse tendency. In spite of lack of significant differences, liver of female group of experimented animals characterizes itself by smaller content of copper compared to females in control group.

Doudi and Setorki (2014) took liver and lungs to histological observation aiming to evaluate toxicity of nanoparticles of copper in vivo conditions. The histology of the hepatic tissues showed vasculature

in central veins and portal triad vessels in all three treatment groups. Histology of lungs showed air sac wall thickening and increased fibrous tissue in all three groups with nano-copper. The results of research conducted by Chen et al. (2006) demonstrated that nanoparticles of copper cause severe toxicological effects and serious damage of kidneys, liver and spleen in experimental group of mice.

Content of copper in spleen of birds in our research did not differ between the groups of two sexes of birds. Marcinkowska and Dobicki (2014) confirmed higher capability to accumulate copper in liver and spleen than in gonads, gills, muscles and gastrointestinal track of perches from Barycz river, what is beneficial due to these fish use for consumption purposes. Nanoparticles of copper, as well as other nanoparticles of metals, get to the environment and human body in different ways, i.e. through sewerage or leak during transportation. However, research concerning activeness of nanoparticles of copper administrated subcutaneously showed that operating of nanoparticles in organisms depends mostly on applied dosage (Glushchenko et al. 1989). Lei et al. 2008 in histological image of liver and kidneys of rats treated with nanoparticles of copper on different level (50, 100 and 200 mg/kg daily through 5 days) showed that the effects caused by nanoparticles of copper in dosage of 100 or 50 mg/kg daily were slighter than when bigger dosage was applied. Sizova et al. (2012) underline that nanoparticles of copper are located in organs and tissues of the whole organism causing particular structural changes. According to the research, increase of nanoparticles of copper in organism up

to the verge of toxicity (maximum dosage tolerable by organism) will cause necrosis of tissues and dystrophy.

## CONCLUSIONS

Results of this experiment confirm previous scientific reports which demonstrate that the greatest accumulation of copper is observed in soft organs like liver or spleen. Moreover, the research conducted showed that male species accumulate copper in liver better. The differences which appeared during research between groups show that different forms of copper have different bioavailability, assimilability and ability to accumulate in organism. Simultaneously, it can be claimed that application of copper sulphate or nanoparticles of copper at the stage of developing chicken embryo do not pose threats of extensive accumulating of this element in organs and muscles of birds in the age at slaughter. As a result, meat and internal organs do not pose a threat to consumers' health.

## REFERENCES

- AKAN J.C., ABDULRAHMAN F.I., SODIPO O.A., CHIROMA Y.A., 2010: Distribution of Heavy Metals in the Liver, Kidney and Meat of Beef, Mutton, Caprine and Chicken from Kasuwan Shanu Market in Maiduguri Metropolis, Borno State, Nigeria. *Res. J. Appl. Sci. Eng. Technol.* 2 (8): 743–748.
- BENDEDDOUCHE B., ZELLAGUI R., BENDEDDOUCHE E., 2014: Levels of Selected Heavy Metals in Fresh Meat from Cattle, Sheep, Chicken and Camel Produced in Algeria. *Annu. Res. Rev. Biol.* 4 (8): 1260–1267.
- CHEN Z., MENG H., XING G., CHEN C., ZHAO Y., JIA G., 2006: Acute toxicological effects of copper nanoparticles in vivo. *Toxicol Lett.* 163 (2): 109–120.
- DEMIREZEN D., AKSOY A., 2004: Accumulation of heavy metals in *Typha angustifolia* (L.) and *Potamogeton pectinatus* (L.) living in Sultan Marsh (Kayseri, Turkey). *Chemosphere* 56, (7): 685–696.
- DIDONATO M., SARKAR B., 1997: Copper transport and its alterations in Menkes and Wilson diseases. *Acta Biochim. Biophys.* 1360: 3–16.
- DORTON K.L., ENGLE T.E., HAMAR D W., SCILIANO P.D., YEMN R.S., 2003: Effects of copper source and concentration on copper status and immune function in growing finishing steer. *Anim. Feed Sci. Technol.* 110: 31–44.
- DOUDI M., SETORKI M., 2014: Acute effect of nano-copper on liver tissue and function in rat. *J. Nanomed.* 1 (5): 331–338.
- DU Z., HEMKEN R.W., JACKSON J.A., TRAMMELL D.S., 1996: Utilization of copper in copper proteinate, copper lysine and cupric sulfate using the rat as an experimental model. *J. Anim. Sci.* 74: 1657–1663.
- GLUSHCHENKO N.N., OLKHOVSKAYA I.P., PLETENEVA T.V., FATKULLINA L.D., ERSHOV Y.U. A., FEDOROV Y.U.I., 1989: The Biological Effect of Superfine Metal Powders. *Izvestiya AN* 3: 415–418.
- KOCHANOWSKA I., HAMPEL-OSIPOWICZ E., WALOSZCZYK P., 2008: Menkes disease – genetic defect in copper metabolism. *Praca przeglądowa. PTND.* 1, 7 (33): 63–67.
- KUNACHOWICZ H., NADOLNA I., PRZYGODA B., IWANOW K., 1998: Tabele wartości odżywczej. [Food Composition Tables]. IŻŻ, Warszawa.
- LEI R., WU C., YANG B., MA H., SHI C., WANG Q., WANG Q., YUAN Y., LIAO M., 2008: Integrated metabolomic analysis of the nano-sized copper particle-induced hepatotoxicity and nephrotoxicity in rats: a rapid in vivo screening method for nanotoxicity. *Toxicol. Appl. Pharmacol.* 15, 232 (2): 292–301.

- MARCINKOWSKAM., DOBICKI W., 2014: Bioakumulacja metali ciężkich w tkankach ryb z rzeki Baryczy. Interdyscyplinarne zagadnienia w inżynierii i ochronie środowiska. Tom 4. T.M. Traczewska, B. Kaźmierczaka (Eds). Oficyna Wydawnicza PWR, Wrocław.
- NIEDZIÓŁKA R., PIENIAK-LENDZION K., HOROSZEWICZ E., 2007: Concentration of Cd and Pb in the muscle, liver and kidney lambs and goat kids fattened in the Podlasie montains. Br. Soc. Anim. Sci. 1: 98–99.
- NRIAGU J., BOUGHANEN M., LINDERA., HOWE A., GRANT C. H., RATTRAY R., VUTCHOV M., LALOR G., 2009: Levels of As, Cd, Pb, Cu, Se and Zn in bovine kidneys and livers in Jamaica. Ecotox. and Environ. Safety 72 (2): 564–571.
- PESTI G.M., BAKALLI R.I., 1996: Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Poult. Sci. 75: 1086–1091.
- RAYS S., KICENIUK J.W., RAYS E., 1994: Analysis of contaminants in edible aquatic resources. VCH Publishers, Inc.: 91–113.
- SIZOVA E., MIROSHNIKOV S., POLYAKOVA V., GLUSCHENKO N., SKALNYA., 2012: Copper Nanoparticles as Modulators of Apoptosis and Structural Changes in Tissues. J. Biomed. Nanotechnol. 3: 97–104.
- TEKIN-ÖZAN S., 2008: Determination of heavy metals levels in water, sediment and tissues of techn. (*Tinca tinca* L., 1758) from Beysehir Lake (Turkey). Environ. Monit. Assess. 145: 295–302.
- THORNBURG L.P., 2000: Perspective on Copper and Liver Disease in the Dog. J. Vet. Diagn. Invest. 12: 101–110.
- Streszczenie:** Wpływ nanocząstek miedzi i siarczanu miedzi podawanych *in ovo* na zawartość miedzi w mięśniach piersiowych, wątrobie i śledzionie kurcząt brojlerów. Materiał doświadczalny początkowo stanowiło 300 jaj wylęgowych kurcząt Hubbard Flex. Jaja podzielono na trzy grupy: kontrola, NanoCu i CuSO<sub>4</sub>, z czego jaja z grupy NanoCu i CuSO<sub>4</sub> poddano zabiegowi iniekcji *in ovo* do komory powietrznej. Eksperymentalne roztwory podano poprzez wstrzyknięcie *in ovo* przy użyciu sterylnej igły i strzykawki 0,3 ml kolejno do grup: NanoCu (koloid nanocząstek miedzi, stężenie 50 ppm), CuSO<sub>4</sub> (koloid siarczanu miedzi, stężenie 50 ppm). Jaja inkubowano w standardowych warunkach. Po wykluciu z każdej grupy wybrano po 50 piskląt do odchowu trwającego 42 dni. Ptaki żywiono standardowymi mieszankami pełnoporcjowymi dla brojlerów. W ostatnim 42. dniu odchowu z każdej grupy wybrano po 12 samic i 12 samców, od których pobrano prawą część mięśnia piersiowego, wątrobę i śledzionę celem określenia zawartości miedzi. Wyniki doświadczenia potwierdzają wcześniejsze doniesienia naukowe, że największą kumulację miedzi obserwuje się w narządach miękkich, takich jak wątroba czy śledziona.

MS. received November 2014

**Authors' address:**

Natalia Mroczek-Sosnowska  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Drobiu  
ul. Ciszewskiego 8  
02-786 Warszawa, Poland  
e-mail: nataliamroczek1@wp.pl

## Study on the relation between an accipiter bird and man

RENÁTA TOBOLOVÁ<sup>2</sup>, PIOTR WIETROW<sup>2</sup>, PAWEŁ KOSIARZ<sup>2</sup>,  
ANNA GŁOWACKA<sup>2</sup>, MONIKA ŁUKASIEWICZ<sup>1</sup>

<sup>1</sup> Department of Animal Breeding, <sup>2</sup> „Aves” Scientific Circle  
Warsaw University of Life Sciences – SGGW

**Abstract:** *Study on the relation between an accipiter bird and man.* The aim of this study was to learn relations between accipitrids and men working with them. The study consisted in observations of trained accipitrids – common buzzard *Buteo buteo* and Northern goshawk *Accipiter gentili*, during everyday trainings, with special attention devoted to the role of sight in their life. As a result of observations made in the study, it was concluded that the visual and audio signal as well as knowing each other and common trust were important factors during man's work with a raptor. Common buzzard turned out to be a calmer bird, its responses were less rapid compared to Northern goshawk. This raptor did not pay attention to the presence of unfamiliar persons, but was vulnerable to objects emitting signals unfamiliar to it (buses or moving trolleys). None of the hunting birds allowed to be touched by an unfamiliar person, which is a natural behavior of raptors. Northern goshawk was responding significantly faster to the sight of an approaching person and was flaying away almost immediately.

*Key words:* common buzzard *Buteo buteo*, Northern goshawk *Accipiter gentili*, man-bird relations

## INTRODUCTION

For ages, relations between man and animals have been accomplished on the plane of production and coexistence. Since ancient times, a man has been remaining in close relations with birds that apart from keeping company to man,

were also trained for work or for sports. A currently popular method of training is the so-called positive reinforcement which consists in the strengthening of positive behaviors by the use of, e.g. rewards and behavioral markers. Before such training, an appropriately strongly motivating reward is selected depending on species preferences.

Northern goshawk *Accipiter gentili* is a timid and secretive bird, difficult to observe (Pielowski 1996). It is a common, though not numerous, species of forest interior (Tomiałojć and Stawarczyk 2003, Anderwald 2013). It is unsociable, impetuous, wild and very bold. It eagerly preys in the afforsted rural and field landscape. The spectrum of its preys is very wide because the trophic niches of male and female hardly coincide. The female is remarkably larger than the male and its prey may be that of the size of a hare, whereas male prey does not exceed the size of a pigeon. Recent goshawk expansion (Rutz et al. 2006b) has been attributable to a continuous increase in the population number of pigeons as a result of transformations in agriculture (Rutz et al. 2006a). Goshawk is reluctant to carrion and has a strong hunting instinct, therefore it is not the ally to pigeon and poultry producers. Many goshawks end their lives in traps set nearby dovecotes

(Kruszewicz 2010). Perceived as a vermin, this raptor has been for ages exterminated also by hunters who were blaming it for losses in populations of hares and game birds.

The situation of common buzzard *Buteo buteo* is much more favorable. It is the most numerous accipiter bird of Poland and Europe (Tomiałojć and Stawarczyk 2003). A high population number, small habitat requirements and “open lifestyle” enable its easy observation outside cities (Pielowski 1996). It nests in forests and large tree complexes. As it feeds with rodents, the biotopes of this raptor include rural and field landscapes (Perrins 1998). Birds constitute the second in line component of its diet, although some scientists claim that the share of birds in its prey may be significantly greater (Goszczyński and Piłatowski 1986, Jędrzejewska and Jędrzejewski 2001, Reif et al. 2001, Goszczyński et al. 2005, Skierczyński 2006). As common buzzard feeds with field pests, a man has no reason for exterminating this raptor.

These birds are used in falconry to, e.g. hunt with a raptor for game (most frequently with goshawk). The widely understood falconry has been popular in many parts of the world inhabited by the birds of prey. It includes both hunting with raptors of the falcon family (Falconidae) *sui generis*, but also hawking with various species of accipitrids belonging to the Accipitridae family including eagles and their subfamily – sea eagles, e.g. white-tailed eagle *Haliaeetus albicilla* or fish hawks *Pandion haliaeetus* – although they all belong to the order Falconiformes (all contemporary ornithological and hunting data come from:

Korbel 1983, Dudziński 1988, Havet 1994, Godlewski 1997, Elphick 2003, Hayman 2007, Nüsslein 2008).

Falconry involves not only preying, but also cooperation between a raptor and a man. Many falconers take care over their raptors for pure pleasure. In general, falconry involves taming (manning) of a raptor and teaching it to fly to the falconer. After appropriate training, the birds equipped in claws and excellent senses are able to capture a prey. A falconer takes a great pleasure in possessing a “winged pet” and in the possibility of observing its flights, whereas the preying itself is a wonderful spectacle and, presumably, the most natural way of practicing hunting.

The aim of this study was to learn relations between accipitrids and men working with them. The study consisted in observations of trained accipitrids – common buzzard *Buteo buteo* and Northern goshawk *Accipiter gentili*, during everyday trainings, with special attention devoted to the role of sight in their life.

## MATERIAL AND METHODS

Observations involved two species of accipitrids: the female common buzzard *Buteo buteo* aged 6 years and male Northern goshawk *Accipiter gentili* 1 year of age. Observations of bird trainings were recorded using a stationary digital camera and a flying platform, in the form of short videos presenting behaviors of birds in different situations (Fig. 1): (1) calling the bird to a falconry glove with meat hidden inside; (2) calling the bird to a falconry glove without feed; (3) calling

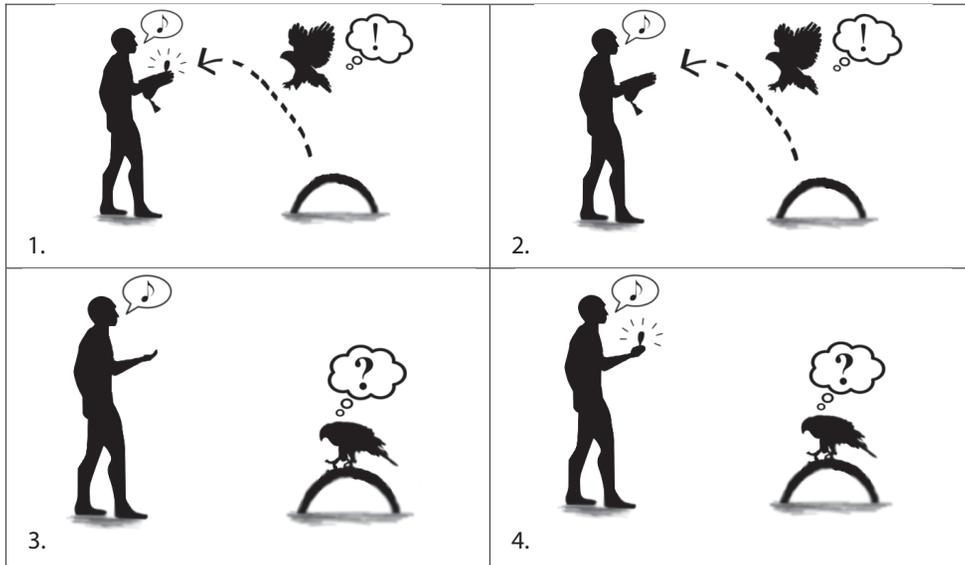


FIGURE 1. Various scenarios of bird training

the raptor to a falconer's hand without a glove and meat; (4) calling the raptor to a falconer's hand without a glove, but with meat; and summoning the birds with the use of different types of obstacles, situations non-specific to raptors' environment and training spaces, including the stress-inducing ones. Training of birds have made their owners. Training the birds took three hours a day through September, October and November. All observations were meticulously described.

## RESULTS AND DISCUSSION

Training of the birds of prey is aimed at improving their physical performance and at developing habits indispensable during cooperation of a falconer and his raptor. The basic training method includes the calling of a bird to a glove. Af-

ter each flight, the bird is rewarded with a piece of meat. Depending on physical conditions, the bird performs from a few to several dozens of flights in a single training session. Hence, the stimulus the bird is responding to is the falconry glove.

The first stage of the study included the identification of a stimulus which triggered the accipitrid's response in the form of flight to the trainer. The experiment was carried out in a few variants. The first consisted in calling the bird to a falconry glove with meat hidden inside. The response of the raptor was practically instantaneous. Another variant included calling the bird to a glove without feed. In this case, the response of the raptor was also appropriate. The third variant included an attempt of calling the raptor to a falconer's hand without glove and meat. It resulted in the indifference as well as lack of response and interest

of the bird. The final variant included an attempt of calling the bird to a falconer's hand with feed. Despite reward being noticeable to the raptor, this attempt also ended with bird's indifference towards man. It may, therefore, be concluded that it was the sight of the glove (which is associated by the bird with reward from the beginning of the training) and not of meat that was the stimulus which determined raptor's flight to the falconer. With time and with growing trust in bird-owner relation, some modifications may be introduced into trainings. The falconer is able to teach the raptor flying through between legs of another person. The raptor is attempting to execute this unnatural to it command when it sees the appropriate signal given by the falconer, and its action is motivated by a double reward. The bird would not execute this task without being sure that it may trust its trainer. Another modification of the training is the thrust of a piece of meat into the air and its catch by the bird. Seeing a characteristic pose assumed by the trainer, the raptor is preparing to catch the feed in the air.

Another stage of the study was aimed at confirming the thesis that the sight is the main sense organ of accipitrids in their everyday training. The first experimental variant consisted in observations of bird behavior during calling it to a glove when the owner (with a glove) was hidden behind an obstacle. The goshawk's owner was hiding behind a tree, which resulted in bird's jumping from branch to branch to spot the falconer. Coming out of the owner from behind the tree and showing the glove with feed resulted in the immediate response and flight of the raptor. A similar situation

was observed in the experiment with common buzzard. When the owner was calling the bird from behind a building, the raptor who could not see the glove was not responding to signals. When the falconer emerged from behind the building, the response was instantaneous (as in the case of goshawk) and the bird was flying to the trainer. The second variant of the experiment consisted in examining the function of sight of common buzzard in conditions unknown to the bird, i.e. stress-inducing conditions. Initially, the bird was left on the bus stop at a high-traffic street. Seeing the approaching cars and buses, the raptor was showing stress responses, which included rapid movements of head in various directions and attempts of escape. Once the hood was placed on bird's head, despite sounds of buses and cars, it was no longer showing any signs of anxiety. A similar experiment was carried out when a trolley was moving in common buzzard's environment. It was causing anxiety of the bird which was attempting fly out of the glove. However, once the hood was put onto its head, the bird did not pay attention to even such characteristic sounds as those made by a moving trolley.

The next stage of the study was aimed at observing an important factor in the cooperation of bird and man – namely: trust. A few experiments were carried out that were based on checking raptor's response to its owner and to unfamiliar persons. The first experiment consisted in calling goshawk by the falconer when unfamiliar persons were freely strolling in the field in different directions. The raptor could not decide to fly over the field, was intimidated and anxious with the presence of other people. Only

when the unfamiliar persons dispersed and made a gap, the bird could freely fly through, the raptor took the occasion immediately and returned to its owner. In turn, when the persons unfamiliar to the goshawk were coming together with the falconer (as persons known to the owner), the bird was flying to the glove without hesitation. With this behavior, the bird showed trust to the owner. In the case of common buzzard, the outcome of the experiment was slightly different. In the first and the second situation, the bird did not hesitate and was landing on the glove almost immediately. Without any greater reservations, the raptor was flying through between the persons and was calmly reaching its owner at the moment when the unfamiliar persons were freely strolling in various directions as well as when the unfamiliar persons were coming together with the falconer. Another experiment consisted in bird's flights from one glove to another, from the owner to the unfamiliar person and vice versa. In the case of goshawk, some complications occurred. The raptor did not want to fly to the unfamiliar person. But without any reservations, was returning to its owner. In contrast, common buzzard was flying to both its owner and to unfamiliar persons. The last experiment investigating the bird-owner trust consisted in checking raptor's behavior that was sitting on the ground toward familiar and unfamiliar persons coming by. Seeing its owner, the goshawk was practically staying in place; it was not running away, showed no fear nor anxiety – just the opposite, the bird was shaking its feathers off, showing rest and comfort. When the unfamiliar person was approaching, the bird was immediately frightened and flew away.

In the case of the common buzzard, the response to the owner was the same – the bird was calmly sitting in once place and showed no fear. Once the unfamiliar person was approaching, the bird showed no response and was staying in place, while too close contact with the strange person resulted in the raptor's escape.

In his book, Mieczysław Mazaraki (1977) describes old methods of falcons training for hunting, applied when the concept of “positive reinforcement” had not been introduced into training methods. However, even then falconers were using the system of rewards as well as simple commands and signals to develop a desired behavior in a bird. The training of falcons included four basic stages. It started from the so-called manning, i.e. taming of the bird; elimination of its wildness and strive for freedom. The bird was not allowed to fall asleep for 3–4 days. Afterwards, it was numb and much calmer, and this enabled starting the second stage of the training when the bird was accustomed to the touch of the falconer, to wearing a hood and to peaceful sitting on the glove. The falcon had to earn every piece of meat it had been given for the appropriate behavior. In turn, the bird was not given the feed to atone disobedience. “Methods currently used by falconers are not that drastic. Just the opposite, they are humanitarian and show full understanding to the responses of the trained bird, and most of all show the knowledge of the theory of conditioned reflexes, which automatically eliminates cruelty and excessive violence from training” (Mazaraki 1977). Next, there came the time for the principal stage of learning, i.e. for beyond manning. The bird had to learn to fly to the falconer at a given signal, which could

include e.g. name of the bird or sound of a whistle. Afterwards, the bird had to land on the glove and eat a piece of meat prepared for it. It was important to teach the falcon to restrain from the attempt of escaping with the feed. At this stage of training, the bird should be calm and tamed with horses, dogs, tools used for hunting like, e.g. nets, but most of all it shall not be afraid of the game it would be pointing in the future. The fourth stage of the training, i.e. setting, was began when the falconer was sure that the bird might be left free, and that the bird would not escape. Initially, the falcon's "prey" included dummy or carrion. With time, the raptor was attacking weak animals, to beat them easily and to gain confidence. Courage and experience gained in exercises of this type made that during hunting the falcon could prey animals significantly greater than it would normally attack in natural conditions. An important element of the training was to teach the bird to give away its prey. Instead, it was given a reward, e.g. a sparrow or a bovine liver. Although the training itself was somehow cruel, at least at the first stages of bird taming, the best results were achieved by falconers who were gentle to their raptors. A key to success was intermittent spending time together with the falcon during training, feeding the falcon from hand, patience and delicacy as well as caring for its health (Mazaraki 1977).

## SUMMARY

As a result of observations made in the study, it was concluded that the visual and audio signal as well as knowing each other and common trust were important

factors during man's work with a raptor. Common buzzard turned out to be a calmer bird, its responses were less rapid compared to Northern goshawk. This raptor did not pay attention to the presence of unfamiliar persons, but was vulnerable to objects emitting signals unfamiliar to it (buses or moving trolleys). It results from the biology of this bird and its prey preferences, as well as from direct causes that include the nervous system and the hormonal system that control animals behaviors. These systems receive information from the outside environment through sense organs. Usually, the nervous system mediates in more detailed and instantaneous responses, whereas the hormonal system monitors the slower and more general responses.

None of the hunting birds allowed to be touched by an unfamiliar person, which is a natural behavior of raptors. Northern goshawk was responding significantly faster to the sight of an approaching person and was flaying away almost immediately.

Training a raptor is not easy and requires devoting a significant amount of time as well as possessing sound knowledge and competencies. Even under man's care, this bird remains wild and free, and each mistake made by man may cause that it will chose life in the natural environment.

## REFERENCES

- ANDERWALD D., 2013: Drapole w Lasach Państwowych, CI LP, Warszawa.  
 DUDZIŃSKI W., 1988: Ptaki łowne. PWRiL, Warszawa.

- ELPHICK J., WOODWARD J., 2003: Kieszonkowy atlas ptaków. Transl. A. Borucka-Rasińska. Solis, Warszawa.
- GODLEWSKI S., 1997: Vademecum myśliwego. Wyd. Bellona, Warszawa.
- GOSZCZYŃSKI J., PIŁATOWSKI T., 1986. Diet of common buzzards (*Buteo buteo* L.) and goshawks (*Accipiter gentilis* L.) in the nesting period. *Ekologia Polska* 34: 655–667.
- GOSZCZYŃSKI J., GRYZ J., KRAUZE D., 2005: Fluctuations of a Common Buzzard *Buteo buteo* population in Central Poland. *Acta Ornithologica* 40: 75–78.
- HAVET P., 1994: Łowiectwo. Larousse Encyklopedia. Transl. M., I. Szmurło. Delta W-Z, Warszawa.
- HAYMAN P., ROB H., 2007: Ptaki drapieżne. Transl. A. Kruszewicz i A. Czujkowska, Muza S.A., Warszawa
- JĘDRZEJEWSKA B., JĘDRZEJEWSKI W. 2001. *Ekologia Zwierząt Drapieżnych Puszczy Białowieskiej*. Wydawnictwo Naukowe PWN, Warszawa.
- KORBELL. (Ed.), 1983: Świat zwierząt. Transl. M. Cymerman. PWRiL, Warszawa.
- KRUSZEWICZ A.G., 2010: Ptaki Polski. Multico, Warszawa.
- MAZARSKI M. 1977. Z sokołami na Łowy. *Sport i Turystyka* 63-72, Warszawa.
- NÜSSEIN F., 2008: Łowiectwo. Galaktyka, Łódź,
- PERRINS C., 1998: The Complete Birds of the Western Palearctic [CD-ROM]. Oxford University Press, Oxford.
- PIELOWSKI Z., 1996: Ptaki drapieżne.
- REIF V., TORNBORG R., JUNGELL S., KORPIMÄKI E., 2001: Diet variation of common buzzards in Finland supports the alternative prey hypothesis. *Ecography* 24: 267–274.
- RUTZ C., BIJLSMA R.G., MARQUISS M., KENWARD R.E., 2006a: Population Limitation in the Northern Goshawk in Europe: a Review with Case Studies. *Studies in Avian Biology* 31: 158–197.
- RUTZ C., WHITTINGHAM M. J., NEWTONI., 2006b: Age-dependent diet choice in an avian top predator. *Proceedings of the Royal Society B: Biological Sciences* 273: 579–586.
- SKIERCZYŃSKI M., 2006: Food niche overlap of three sympatric raptors breeding in agricultural landscape in Western Pomerania region of Poland. *Buteo* 15: 17–22.
- TOMIAŁOJC L., STAWARCZYK S., 2003: Awifauna Polski. Rozmieszczenie, liczebność i zmiany. PTPP “pro Natura”, Wrocław.

**Streszczenie:** *Badanie relacji ptaka szponiastego z człowiekiem.* Celem przeprowadzonych badań było poznanie relacji między ptakami szponiastymi i ludźmi, którzy z nimi pracują. Prowadzono obserwacje ułożonych ptaków drapieżnych – myszołowa zwyczajnego *Buteo buteo* oraz jastrzębia gołębiarza *Accipiter gentili*, podczas codziennych treningów ze szczególnym uwzględnieniem roli, jaką w życiu tych ptaków pełni ich wzrok. W wyniku obserwacji stwierdzono, że podczas pracy człowieka z ptakiem łowczym ważne są sygnały wizualny i słuchowy oraz wzajemne poznanie i zaufanie. Myszołów zwyczajny jest ptakiem spokojniejszym, jego reakcje są mniej gwałtowne w porównaniu do jastrzębia gołębiarza. Drapieżnik ten nie zwraca uwagi na obecność obcych ludzi, natomiast jest wrażliwy na obiekty emitujące nienaturalny dla niego dźwięk (autobusów czy poruszających się wózki). Żaden z ptaków łowczych nie pozwolił się dotknąć obcej osobie, co jest zachowaniem naturalnym dla drapieżników. Jastrząb gołębiarz reagował znacznie wcześniej na widok zbliżającego się do niego człowieka i odlatywał niemal natychmiastowo.

MS. received November 2014

**Authors' address:**

Monika Łukasiewicz  
Koło Naukowe „Aves”  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Drobiu  
ul. Ciszewskiego 8,  
02-786 Warszawa, Poland



## Characteristics of muscle fibers of breast and leg muscles of grey partridges *Perdix perdix*

AGNIESZKA WNUK<sup>1</sup>, MONIKA ŁUKASIEWICZ<sup>1</sup>, NATALIA MROCZEK-SOSNOWSKA<sup>1</sup>, JAN NIEMIEC<sup>1</sup>, BARTŁOMIEJ POPCZYK<sup>2</sup>, MAREK BALCERAK<sup>1</sup>, DOBROCHNA ADAMEK<sup>3</sup>, MACIEJ KAMASZEWSKI<sup>3</sup>

<sup>1</sup> Department of Animal Breeding and Production, <sup>2</sup> Department of Animal Environment Biology, <sup>3</sup> Laboratory of Ichthyobiology and Fisheries  
Warsaw University of Life Sciences – SGGW

**Abstract:** *Characteristics of muscle fibers of breast and leg muscles of grey partridges *Perdix perdix*.* The experimental material included grey partridges *Perdix perdix* L. planned for re-introduction into the natural habitat, reared at the Game Breeding Center on the area of Mazovia Province. 10 females and 10 males were selected for slaughter. The birds were slaughtered in poultry abattoir and specimens of their pectoral superficial muscle (*pectoralis superficialis*) and biceps femoris muscle (*biceps femoris*) were sampled. A greater diameter of fibers was determined for breast muscles. The study did not show any effect of sex on muscle fiber diameter of breast and leg muscles of grey partridges *Perdix perdix*.

**Key words:** grey partridge, muscle fiber, game

### INTRODUCTION

Muscle fibers are the main components of skeletal muscle tissue. According to Damez and Clerjon (2008), the number, size and type of muscle fibers as well as their biochemical, physiological and histological characteristics may lead to changes in muscle quality. Many authors emphasize that the histological structure is reflected in the sensory assessment of meat products made by consumers (Ozawa et al. 2000, Nam et al. 2009, Lee et al. 2012). In turn, Sajdakowska et

al. (2011) emphasize that the quality of food is not an easy notion to define and depends, to a large extent, on its subjective evaluation. In addition, food quality means meeting expectations of consumers, bearing in mind that contemporarily consumers are increasingly demanding. Their growing requirements regarding food refer not only to its nutritional and health-promoting value, but also its sensory traits, which extorts the improvement of food products quality (Krupiński et al. 2011). Increasingly often, the anxious consumers not only express their interest in foods produced under the extensive conditions (Połtowicz et al. 2003, Tuytens et al. 2005, Hughner et al. 2007, Pouta et al. 2010, Napolitano et al. 2013), but also search for alternative species of meat (Nuernberg 2011). Meat of the game have for ages played the most important role among meat courses. According to Hoffman and Wiklund (2006), consumers are aware that game meat is healthy and characterized by low fat content. In some countries, courses prepared from wild fowl are a local delicacy, are perceived as a luxury course often claimed a tourist attraction. They are, at the same time, a valuable dietary

complement and dietetic variety (Wójcik et al. 2010). Undoubtedly, game meat of grey partridge is highly appreciated by consumers as the most delicate and the most tasty meat of all game birds (Łebkowska and Łebkowski 1995). According to Choi and Kim (2009), a high number of fibers with small and medium diameters affects meat quality improvement.

The aim of study was to characterize muscle fibers of breast and leg muscles of grey partridge *Perdix perdix*.

## MATERIAL AND METHODS

The experimental material included grey partridges *Perdix perdix* L. planned for re-introduction into natural habits, reared at the Game Breeding Centre on the area of Mazovia Province.

Complete the feed mixtures were applied in the rearing period. In the first 4 weeks of birds life, the mixtures contained: 29% of total protein, 11.5 MJ of metabolizable energy and 3.6% of crude fiber, whereas since the 6<sup>th</sup> till the 10<sup>th</sup> week of birds life the mixtures contained 23% of total protein, 11.5 MJ of metabolizable energy and 4% of crude fiber. From the 10<sup>th</sup> week of birds life till the end of rearing, the birds were fed diets, with a daily feed ration including up to 50 g of wheat and maize grain and ad libitum grass which included a mixture of maize, sunflower, alfalfa, grasses and marrows stem kale.

For the first 4 weeks, 3500 partridges were kept indoors. Since the 4<sup>th</sup> week of age, they had free access to rearing aviaries that were partly roofed, with the roof covered with an electric cord to pro-

tect against predators. The aviaries with gravel-sand bottom were planted with vegetation and possessed natural hide-aways in the form of rootstocks and large stones.

10 females and 10 males with body weight about average in 14-weeks old were selected for slaughter. The birds were slaughtered in poultry abattoir and the specimens of their pectoral superficial muscle (*pectoralis superficialis*) and biceps femoris muscle (*biceps femoris*) were sampled. Samples in size of 0.5 × 0.5 × 1 cm were collected within 15 min since slaughter after appropriate exsanguination of the birds and subsequently subjected to 24-hour fixation (in a Bouin solution). The samples were then washed in ethanol to remove the fixing agent and dehydrated by a series of increasing ethyl alcohol concentrations. Dehydrated samples were saturated with paraffin. Paraffin saturation was carried out in the incubator at the melting point of paraffin. Saturation duration was adapted to muscle samples collected and amounted to a few hours. Paraffin blocks were formed after completion of the saturation process. Microtome Leica RM 2265 (Leica Microsystems, Nussloch, Germany) was used to cut paraffin sections. Muscle cross sections had the thickness of 5 μm. Standard H&E staining was performed. The diameter and area of 200 muscle fibers was measured in each slide using a Nikon Ellipse E200 light microscope equipped with a Nikon DS-Fi2 camera and COOL view 2.7 software.

Results achieved were elaborated statistically with the use of Student's t-test in SPSS 19.0 PL software IBM Corp. Released (2010). Differences were found significant at  $p \leq 0.05$  and  $p < 0.01$ .

**RESULTS AND DISCUSSION**

As reported by Tumova and Teimouri (2009) and by Lefaucheur (2010), the diameter of muscle fibers may range from 10 to 100  $\mu\text{m}$ . According to Papinaho et

al. (1996) and Geyikoglu et al. (2005), in the case of chickens, the mean thickness of muscle fibers depends on the type of muscle and reaches 60.0  $\mu\text{m}$  for *m. pectoralis major*, 51.6  $\mu\text{m}$  for *m. biceps femoris*, 59.8  $\mu\text{m}$  for *m. extensor*

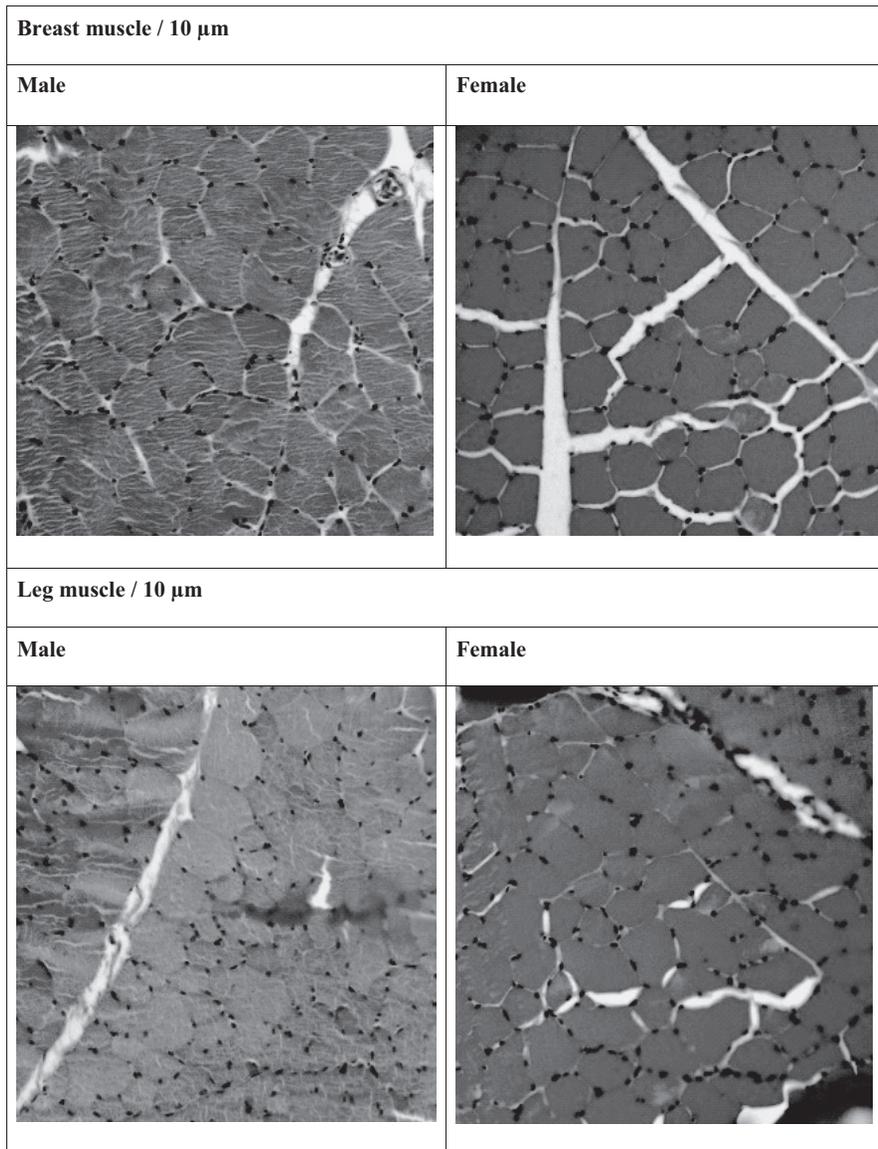


FIGURE 1. Cross-section of breast and leg muscles of grey partridges

TABLE 1. Fiber diameter of breast and leg muscles of grey partridges

Group	Muscles			
	breast		leg	
	$\bar{x}$	SE	$\bar{x}$	SE
♂♂	60.71	14.54	45.69	10.67
♀♀	56.22	12.35	40.16	5.00

*hallucis longus* and 60.45  $\mu\text{m}$  for *m. gastrocnemius*. In the case of grey partridge, the diameter of muscle fibers ranges from 25.7 to 97.95  $\mu\text{m}$  (58.53 on average) for pectoral muscles as well as from 19.33 to 68.18  $\mu\text{m}$  (42.93  $\mu\text{m}$  on average) for leg muscles (Wnuk et al., 2013b). Similarly small diameters of breast muscle fibers accounting for 37.86–39.11  $\mu\text{m}$  and these of leg muscle fibers accounting for 63.55–66.54  $\mu\text{m}$  were reported for Białe Kofudzkie geese (Biesiada-Drzazga et al. 2006) as well as for Silkie hens, i.e. 32.23 and 28.73  $\mu\text{m}$ , respectively (Łukasiewicz et al. 2013). Along with the selection of livestock for a high growth rate, problems emerge with their muscle fibers which result in hyperplasia and the appearance of the so-called giant fibers, the diameter of which is usually threefold greater than that of normal fibers (Dransfield and Sosnicki 1999).

No giant fibers were observed in the analyzed muscles of grey partridges, that were characterized by a high contribution of fibers with small and medium diameters (Fig. 1). In addition, greater diameters of fibers of breast muscles (60.71  $\mu\text{m}$ ) and leg muscles (45.69  $\mu\text{m}$ ) were determined in the case of roosters compared to hens (Table 1). In the group of males, diameters of breast muscle fibers were relatively equal, 70–90% of the fibers in particular bundles had diameters

in the range of 50–60  $\mu\text{m}$ , and 10–30% of the fibers had diameters in the range of 25–40  $\mu\text{m}$ . In the group of females, diameters of breast muscle fibers were more equalized and reached 55–60  $\mu\text{m}$  in ca. 80–90% of the fibers and less than 25  $\mu\text{m}$  in 10–15% of the fibers.

The same tendency was observed in case of leg muscles – larger diameters were demonstrated in males. Diameters of leg muscle fibers in the group of males were relatively equalized, i.e. 80–90% of fibers in particular bundles had diameters of 45  $\mu\text{m}$ , and 10–30% of fibers had diameters in the range of 25–40  $\mu\text{m}$ . In the group of females, the diameters of leg muscle fibers, likewise these of breast muscles, were more equalized compared to the males because 80–90% of fibers in particular bundles had diameters in the range of 35–40  $\mu\text{m}$ , and in 10% of the fibers diameters were lesser than 20  $\mu\text{m}$ .

Similar tendencies were observed for Hubbard JA 957 and Ayam Cemani chickens and their hybrids (Łukasiewicz et al. 2014), as well as for Cobb  $\times$  Zk hybrids (Wnuk et al. 2013a), which simultaneously indicated the effect of sex on muscle fiber diameter and thereby confirmed results of earlier study by Khoshooi et al. (2013). According to Candek-Potokar et al. (1998), sex is one of the factors which affect the histological structure of muscles. A completely different opinion was expressed by Mobini et al. (2013) who did not demonstrate any effect of sex on muscle fiber diameter. It does not change the fact, however, that the presence of a high number of fibers with a small diameter may be indicative of meat tenderness. Fanatico et al. (2007) found that the selection for fast growth and high yield

have negatively impacted the sensory and functional qualities of the meat, pushing muscle fibers to their maximum functional size constraints.

## CONCLUSIONS

The study showed no effect of sex on the diameter of muscle fibers of breast and leg muscles of grey partridge *Perdix perdix*. Generally, greater fiber diameters were determined in breast muscles. Worthy of notice is a small diameter of the fibers of the analyzed muscles, which is indicative of a delicate structure of grey partridge meat.

## REFERENCES

- BIESIADA-DRZAZGA B., GÓRSKI J., GÓRSKA A., 2006: Analysis of slaughter value and muscle fiber thickness of selected muscles in geese broilers as related to feeding applied during the period. *Anim. Sci. Pap. Rep.* 24(2): 37–44.
- CANDEK-POTOKAR M., ZLENDER B., LEFAUCHEUR L., BONNEAU M., 1998: Effects of age and/or weight at slaughter on longissimus dorsi muscle: biochemical traits and sensory quality in pigs. *Meat Sci.* 48: 287–300.
- CHOI Y. M., KIM B. C., 2008: Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. *Livest. Sci.* 122, 105–118.
- DAMEZ J. L., CLERJON S., 2008: Meat quality assessment using biophysical methods related to meat structure. *Meat Sci.* 80, 132–149.
- DRANSFIELD E., SOSNICKI A. A., 1999: Relationships between muscle growth and poultry quality. *Poultry Sci.* 78: 743–746.
- FANATICO A. 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: 2245–2255.
- GEYIKOGLU F., VURALER Z., TEMEL-LU A., 2005: The histochemical and ultrastructural structures of avian latissimus dorsi muscle fiber types and changes in them caused by water cooper level. *Turk. J. Vet. Anim. Sci.* 29: 131–138.
- HOFFMAN L. C., WIKLUND E., 2006: Game and venison for the modern consumer. *Meat Sci.* 74: 197–208.
- HUGHNER R. S., MCDONAGH P., PROTHERO A., SHULTZ C. J., STANTON J., 2007: Who are organic food consumers? A compilation and review of why people purchase organic food. *J. Consum. Behav.* 6: 94–110.
- KHOSHOOI A. A., MOBINI B., RAHIMI E., 2013: Comparison of chicken strains: muscle fiber diameter and number in Pectoralis superficialis muscle. *Global Vet.* 11(1): 55–58.
- KRUPIŃSKI J., HORBAŃCZUK J. O., KOŁACZ R., LITWIŃCZUK Z., NIEMIEC N., ZIĘCIK A., 2011: Strategiczne kierunki rozwoju produkcji zwierzęcej uwarunkowane oczekiwaniem społecznym, ochroną środowiska i dobrostanem zwierząt. *Polish J. Agron.* 7: 59–67.
- ŁEBKOWSKA D., ŁEBKOWSKI D., 1995: *Dziczyzna. Tenten*, Warszawa: 1–93.
- LEE S. H., CHOE J. H., CHOI Y. M., JUNG K. C., RHEE M. S., HONG K. C., LEE S. K., RYU Y. C., KIM B. C., 2012: The influence of pork quality traits and muscle fiber characteristics in the eating quality of pork from various breeds. *Meat Sci.* 90: 284–291.
- LeFAUCHEUR L., 2010: A second look into fiber typing – Relation to meat quality. *Meat Sci.* 84: 257–270.
- ŁUKASIEWICZ M., MROCZEK-SOSNOWSKA N., WNUK A., KAMASZEWSKI M., ADAMEK D., TARASEWICZ L., ZUFFA P., NIEMIEC J., 2013: Histological profile of breast and leg muscles of Silkies chickens and of slow-growing

- Hubbard JA 957 broilers. Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 52: 113–120.
- ŁUKASIEWICZ M., NIEMIEC J., WNUK A., MROCZEK-SOSNOWSKA N., 2014: Meat quality and the histological structure of breast and leg muscles in Ayam Cemani chickens, Ayam Cemani × Sussex hybrids and slow-growing Hubbard JA 957 chickens. J. Sci. Food Agric. 23. doi: 10.1002/jsfa.6883.
- MOBINI B., KHOSHOOI., 2013: A comparative histomorphometrical study of Quadriceps femoris muscle fibers between commercial broiler and domestic flows. WASJ 22 (10): 1506.
- NAM Y.J., CHOI Y.M., LEE S.H., CHOE J.H., JEONG D.W., KIM Y.Y., 2009: Sensory evaluations of porcine longissimus dorsi muscle: Relationships with postmortem meat quality traits and muscle fiber characteristics. Meat Sci. 83: 731–736.
- NAPOLITANO F., CASTELLINI C., NASPETTI S., PIASENTIER E., GIROLAMI A., BRAGHIER I., 2013: Consumer preferences for chickens breast May be more affected by information on organic production than by product sensory properties. Poultry Sci. 92: 820–826.
- NUERNBERG K., SLAMECKA J., MOJTO J., GASPARIK J. NUERNBERG G., 2011: Muscle fat composition of pheasants (*Phasianus colchicus*) and black coots (*Fulica atra*). Eur J. Wildlife Res. 57: 795–803.
- OZAWA S., MITSUHASHI T., MITSUMOTO M., MATSUMOTO S., ITOH N., ITAGAKI K., KOHNO Y., DOHGO T., 2000: The characteristics of muscle fiber types of longissimus thoracic muscle and their influences on the quantity and quality of meat from Japanese Black steers. Meat Sci. 83: 731–736.
- PAPINAHO P.A., RUUSUNEN M.H., SUUROMENT T., FLETCHER D.L., 1996: Relationship between muscle biochemical and meat quality properties of early deboned broiler beasts. J. Appl. Poultry Res. 5: 126–133.
- POŁTOWICZ K., WEŻYK S., CYWA-BENKO K., 2003: Wykorzystanie rodzinnych ras kur w produkcji mięsa bezpiecznego dla zdrowia konsumenta. Praca zbiorowa. Zakrzewo: 21–32.
- POUTA E., HEIKKILÄ J., FORSMAN-HUGG S., MÄKELÄ J., 2010: Consumer choice of broiler meat. The effects of country of origin and production methods. Food Qual Prefer. 21: 539–546.
- SAJDAKOWSKA M., GUTKOWSKA K., ŻAKOWSKA-BIEMANS S., KOWALCZUKI., 2011: Postrzeganie konsumencie jakości produktów żywnościowych pochodzenia zwierzęcego na podstawie wyników badań jakościowych. ZPPNR 269: 209–218.
- TUMOVA E., TEIMOURI., 2009: Chicken muscle fiber characteristics and meat quality: A review. SAB 40 (4): 253–258.
- TUYTTENS F., HEYNDRIKX M., BOECK M., MOREELS A., NUFFEL A., POUCKE E., COILLIE E., DONGEN S., LENS L., 2005: Comparison of broiler chicken health and welfare in organic versus traditional production systems. Anim. Sci. Pap. Rep. 23 (Suppl. 1): 217–222.
- WNUK A., MROCZEK-SOSNOWSKA N., ADAMEK D., KAMASZEWSKI M., ŁUKASIEWICZ M., NIEMIEC J., 2013a: Effect of rearing system and gender on histological profile of chicken breast and leg muscles in hybrid (Coob × Zk) Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 52: 219–225.
- WNUK A., MROCZEK-SOSNOWSKA N., ŁUKASIEWICZ M., POPCZYK B., NIEMIEC J., 2013b: Histological characteristics of breast and leg muscles partridges (*Perdix perdix* L.) Science for Sustainability, International Scientific Conference for PhD Students. University of West Hungary, Győr, 19–20.03.2013: 79–80.
- WÓJCIK K., SOBCZAK M., ŻOCHOWSKA-KUJAWSKA J., ZIELIŃSKI K., 2010: Porównanie tekstury i struktury oraz podatności na proces masowania mięśni danieli (*Dama dama*). ŻNTJ 1(68): 93–104.

**Streszczenie:** Charakterystyka włókien mięśniowych mięśni piersiowych i nóg kuropatwy polnej *Perdix perdix*. Materiał badawczy stanowiły kuropatwy polne *Perdix perdix* L. przeznaczone do wsiedlenia do środowiska naturalnego, odchowywane w Ośrodku Hodowli Zwierzyny na terenie województwa mazowieckiego. Do uboju wybrano po 10 kur i 10 kogutów. Ptaki ubito, a następnie pobrano próbki mięśni piersiowych (*pectoralis superficialis*) oraz mięśni nóg (*biceps femoris*). Większą średnicą włókien charakteryzowały się mięśnie piersiowe. Nie wykazano wpływu płci na średnicę włókien mięśni piersiowych i mięśni nóg kuropatwy polnej *Perdix perdix*.

*MS. received November 2014*

**Authors' address:**

Agnieszka Wnuk  
Wydział Nauk o Zwierzętach SGGW  
Katedra Szczegółowej Hodowli Zwierząt  
Zakład Hodowli Drobiu  
ul. Ciszewskiego 8  
02-786 Warszawa, Poland  
e-mail: aga.m.wnuk@gmail.com

