ISSN 1898-8830

Annals of Warsaw University of Life Sciences – SGGW

Animal Science No 56 (1) 2017

Annals of Warsaw University of Life Sciences – SGGW Animal Science No 56 (1) 2017

Agriculture
(Agricultural and Forest Engineering)
Animal Science
Forestry and Wood Technology
Horticulture and Landscape Architecture
Land Reclamation

Distribution

Annals are distributed by the Bookshop of Warsaw University of Life Sciences Press, 166 Nowoursynowska St., Warsaw, Poland.

WARSAW UNIVERSITY
OF LIFE SCIENCES PRESS

166 Nowoursynowska St., 02-787 Warsaw
Poland, tel. (48 22) 593 55 20
e-mail: wydawnictwo@sggw.pl
www.wydawnictwosggw.pl

Annals of Warsaw University of Life Sciences – SGGW

Animal Science No 56 (1)
Warsaw 2017

Contents

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 (2014 – 83.35; 2015 – 78.24), CAB Direct, CEON, ARIANTA, ePNP, PBN, POL-INDEX, POLON
Bazy: AGRO, Index Copernicus (2014 – 83,35; 2015 – 78,24), CAB Direct, CEON – Biblioteka Nauki, ARIANTA, e-Publikacje Nauki Polskiej, PBN, POL-INDEX, POLON

WARSAW UNIVERSITY
OF LIFE SCIENCES PRESS

ISSN 1898-8830

EDITORIAL STAFF
Anna Dołomisiewicz
Elżbieta Wojnarowska

PRINT: POLIMAX s.c., 161 L Nowoursynowska St.,
02-787 Warsaw

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 5–13
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.1

Effect of birth weight of piglets on growth rate and rearing performance up to 8 weeks of age

EMILIA AMBROZIAK, ANNA REKIEL
Department of Animal Breeding and Production, Warsaw University of Life Sciences – SGGW

Abstract: *Effect of birth weight of piglets on growth rate and rearing performance up to 8 weeks of age.* The aim of the experiment was to determine the effect of the birth weight of piglets on their rearing results up to 56th day of age, as expressed by growth rate and survival. Observations were made on 277 crossbred piglets from 22 litters of F1 sows (Polish Landrace × Polish Large White) derived from crossbred boars (Duroc × Pietrain), which were kept and fed the same way. Piglets were reared with mothers for 5 weeks and observed for 8 weeks. At 1st, 7th, 21st and 56th day of age, piglets were individually weighed. The body weight on day 1 of age served as a basis for dividing the piglets into groups I, II, III and IV (≤1.2; 1.21–1.39; 1.40–1.59; and ≥1.60 kg body weight, respectively). Coefficients of correlation were estimated between body weight on day 1 of age and at day 7, 21 and 56 of life, and daily gains. In the subsequent rearing periods, daily gains in groups I–IV increased and the differences between the groups showed similar relationships. Differences between groups II and III were small (P >0.05), and those between groups I and IV considerable and highly significant. The coefficients of correlation for piglets from groups I (the lightest at birth) and IV (the heaviest at birth) confirm the relationship between birth weight and body weight at 7th (P ≤0.01), 21st (P ≤0.01) and 56th day of age (P ≤0.05), with a downward tendency for the calculated relationships. Furthermore, in group I piglet birth weight was correlated with daily gains from 1st to 7th day (r = +0.365, P ≤0.01) and from 1st to 56th day of age (r = +0.291, P ≤0.05). With the increasing mean body weight at birth, piglet survival increased and was higher in group IV vs I by 13.64 percentage points. The birth weight ≥1.60 kg ensured the best growth rate and survival of the piglets.

Key words: piglets, body weight, daily gains, survival

INTRODUCTION

The biological potential of the species (18 piglets born per litter and 44 piglets per sow per year) is not used to the full, but the performance parameters continue to increase. Improvements in reproductive traits of pigs have been reported in many countries of Europe, including Poland. Over the last three decades of the 20th century, the number of piglets reared per sow per year increased on well-managed farms from 16 to 22, currently standing at 28–30 (Orzechowska and Mucha 2010, Blicharski et al. 2016). Modern sows are characterized by high fertility, prolificacy and milk yield, which has been achieved due to breeding work, selection programmes, crossbreeding, as well as improvements in feeding programmes and management (Baxter et al. 2013, Rutherford et al. 2013, Douglas 2015). After substantial progress in litter size was made, neonatal weight was

observed to decrease (Škorjanc et al. 2007, Wolf et al. 2008, Beaulieu et al. 2010, Douglas 2015, Hales et al. 2015). This is associated with impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy – IUGR (Wu et al. 2004, Wang et al. 2005, Rekiel et al. 2014b). This phenomenon concerns not only multifetal and multiparous species, such as pigs, rabbits, mink, chinchillas, dogs, cats, mice or rats (Dzierżanowska et al. 2014, Rekiel et al. 2014b, Święcicka et al. 2016), but also those giving birth to one or 2–3 young, such as sheep, goats, cattle, and horses (Wang et al. 2005, Rekiel and Królewska 2014). Realized fertility of sows was found to be conducive to reducing neonatal weight, as confirmed by the correlation $r = -0.46$, estimated by Milligan et al. (2002). According to Foxcroft et al. (2006), this relationship may be due to limited nutrient and oxygen transfer to the fetus. Prenatal nutritional deficiencies during myogenesis are conducive, according to many authors (Bee 2004, Wang et al. 2005, Królewska et al. 2014, Rekiel et al. 2014b), to reducing birth weight of piglets and slowing postnatal growth and development. In the offspring born to sows underfed during gestation, Bee (2004) observed not only lower body weight, but also increased mortality during the first days of life. Highly significant positive coefficients of correlation between the body weight of suckling piglets and the growth rate of young pigs were reported by Bocian et al. (2011). These relationships are supported by the findings of Václavková et al. (2012). In the context of the presented subject matter, it is important to cite Rehfeldt et al. (2012), who believe that despite the limited compensatory growth of piglets after birth, it may occur later on in pigs.

The effect of the birth weight of piglets on rearing performance and survival during the suckling period is economically important and so has been the subject of many analyses (Bocian et al. 2011, Królewska et al. 2014, Rekiel et al. 2015). It also influences the rate of growth later in the rearing period (i.e. after weaning and during fattening), as well as the quality of slaughter material (Gondret et al. 2005, Bocian et al. 2011, Václavková et al. 2012, Rekiel et al. 2014a, b). Fatteners with low birth weights were characterized by increased deposition of fatty tissue, which is unfavourable for processors and consumers (Gondret et al. 2005, Rekiel et al. 2014a).

The aim of the experiment was to determine the effect of the birth weight of piglets on their rearing results up to 56 days of age, as expressed by growth rate and survival.

MATERIAL AND METHODS

The experiment was conducted on a commercial farm located in the Mazowieckie province. Subjects were 277 crossbred piglets from 22 litters of F1 sows (Polish Landrace × Polish Large White) derived from crossbred boars (Duroc × Pietrain). Piglets were reared with mothers for 35 days and observed until 56th day of age. All the experimental piglets were subjected to routine veterinary and management procedures, such as: marking, tail docking, teeth clipping, iron supplementation, preventive vaccinations; the young boars were castrated. Sows were moved into farrowing pens with crates

7 days before predicted parturition. Sows and their offspring were kept on partially slatted floor, and piglets were warmed with heating mats and infrared heaters. After weaning, piglets from two litters were placed into groups of around 20 and maintained in slatted floor pens without bedding. From the first day of life, all piglets were allowed continuous access to water (nipple drinkers), and from 6–7 days to solid feed (ad libitum feeding). Bonni-M Forte (Sano) was used as the first feed, and a week after weaning (day 42) a farm-produced feed based on cereals and Piglet concentrate (Josera) was introduced; it was fed from automatic feeders for 2 weeks, up to the end of observations at 56th day of age.

At 1st, 7th, 21st and 56th day of age, all piglets were individually weighed. The body weight (b.w.) on first day of age served as a basis for dividing the piglets into quartile groups, with 66, 73, 72 and 66 piglets in groups I (b.w. ≤1.20 kg), group II (b.w. 1.21–1.39 kg), group III (b.w. 1.40–1.59 kg) and group IV (b.w. ≥1.60–2.51 kg), respectively. The effect of piglet birth weight on growth rate and survival to 56nd day of rearing was monitored.

The results were statistically analysed using one-way analysis of variance (IBM SPSS Statistics 24). Pearson's coefficients of correlation were estimated within groups between piglet body weight on first day of age, subsequent body weights, and daily gains.

RESULTS AND DISCUSSION

In our study, the number of piglets born per litter averaged 12.59, which is considered satisfactory. The increase in litter size at birth results from the selection for prolificacy, the creation of lines with very good reproductive traits, and the widespread use of maternal heterosis, which is found in two-breed cross sows. The increases in sow fertility but also piglet mortality have been reported in many European herds of pigs (Boulot et al. 2008, Orzechowska and Mucha 2010). Quiniou et al. (2002) report that when litter size increased from 11 to 16 piglets, the mean birth weight of piglets decreased by around 330 g and the proportion of light piglets (weighing less than 1 kg) increased by 16%. The percentage of stillborn animals increased among newborn piglets with such a low birth weight. There was also an increase in mortality on first day of age. The increased differences in neonatal body weight, similar to the body weight itself, may influence the productive traits, including the growth rate during rearing and fattening, as well as carcass muscling and fatness; low body weight has a slowing effect on weight gains and reduces carcass slaughter value (Milligan et al. 2002, Tribout et al. 2003, Gondret et al. 2005, Boulot et al. 2008, Beaulieu et al. 2010).

The mean body weight of piglets obtained in our study (Table 1) is considered satisfactory (Rekiel et al. 2015). According to Quiniou et al. (2002), heavier newborn piglets are more viable and adapt more rapidly to the extrauterine environment. Share of 35% of piglets weighing less than 0.8 kg are stillborn, and those whose birth weight averages between 1.2 and 1.4 kg, account for only 4% of stillbirths.

Daily gains during the first week of life were greater in groups II and III

TABLE 1. Characteristics of experimental animals

Trait	Descriptive statistics	Total	Group I	Group II	Group III	Group IV
Body weight of piglet at day 1 of age (kg)	*n*	277	66	73	72	66
	mean	1.40	1.04	1.30	1.49	1.79
	min	0.58	0.58	1.21	1.40	1.60
	max	2.51	1.20	1.39	1.59	2.51
	SD	0.30	0.16	0.05	0.06	0.19
Body weight of piglet at day 7 of age (kg)	*n*	258	58	68	68	64
	mean	2.54	2.01	2.40	2.60	3.09
	min	1.12	1.12	1.47	1.76	2.36
	max	4.43	2.69	3.08	3.27	4.43
	SD	0.53	0.38	0.35	0.30	0.46
Body weight of piglet at day 21 of age (kg)	*n*	253	56	65	68	64
	mean	6.10	5.46	5.90	6.09	6.86
	min	3.54	3.54	4.21	4.72	4.64
	max	9.76	7.62	7.80	7.70	9.76
	SD	0.96	0.79	0.80	0.62	1.03
Body weight of piglet at day 56 of age (kg)	*n*	247	53	65	67	62
	mean	17.30	15.33	16.89	17.53	19.16
	min	9.84	9.84	11.92	11.76	12.34
	max	24.74	20.66	24.74	23.02	24.46
	SD	2.82	2.16	2.72	2.35	2.68

vs I ($P \leq 0.05$), and in group IV vs I, II, III ($P \leq 0.01$) – Table 2. The differences between groups II and III were not significant ($P > 0.05$). Weight gains in group IV vs I, II and III were greater by 50.3 g (37.1%), 29.8 g (19.1%) and 27.9 g (17.7%), respectively. From 7th to 21st day of rearing, weight gains differed significantly between groups I and III vs IV ($P \leq 0.01$) and between group II vs IV ($P \leq 0.05$). Weight gains in group IV, in relation to groups I, II and III were greater by 24 g (9.8%), 19.3 g (7.7%) and 20.1 g (8.1%), respectively. From 22nd to 56th day of age, daily gains differed significantly between group I vs II, III, IV ($P \leq 0.01$), and between groups II and III vs IV ($P \leq 0.05$). In group IV compared to groups I, II and III, piglet weight gains were greater by 69.8 g (24.8%), 37.1 g (11.8%) and 23.8 g (7.5%), respectively. Daily gains from birth to 56 days of age showed significant differences between groups I vs II, III, and IV, and between groups II and III vs IV ($P \leq 0.01$); no statistically significant differences were found between groups II and III ($P > 0.05$). The mean daily gains were greater in group IV compared to groups I, II and III by 55.7 g (21.9%), 31.9 g (11.5%) and 23.9 g (8.3%), respectively. The results obtained between 8th and 21st day of age appear to be indicative of the preliminary stage of compensatory growth in piglets from groups I, II and III (Rehfeldt et al. 2012). The coef-

TABLE 2. Daily gains of piglets

Trait	Total		Group I b.w. ≤1.20		Group II b.w. 1.21–1.39		Group III b.w. 1.40–1.59		Group IV b.w. ≥1.60	
	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*
Daily gains from day 1 to 7 (g)	159.4	3.212	135.6 Aa	6.645	156.1 Ab	6.000	158.0 Ab	5.910	185.9 B	6.144
Daily gains from day 8 to 21 (g)	253.6	3.137	245.2 A	6.675	250.0 a	6.027	249.2 A	5.937	269.3 Bb	6.172
Daily gains from day 22 to 56 (g)	319.9	4.436	281.3 A	9.030	314.0 Ba	8.154	327.3 Ba	8.032	351.1 Bb	8.349
Daily gains from day 1 to 56 (g)	283.4	3.052	254.6 A	6.073	278.3 B	5.484	286.4 B	5.401	310.3 C	5.615

b.w. – body weight of piglet at day 1 of age (kg).
A, B – means in rows with different capital letters differ significantly at $P \leq 0.01$.
a, b – means in rows with different small letters differ significantly at $P \leq 0.05$.

ficients of correlation for piglets from groups I (the lightest at birth) and IV (the heaviest at birth) confirm the relationship between birth weight and body weight at 7th ($P \leq 0.01$), 21st ($P \leq 0.01$) and 56th day of age ($P \leq 0.05$), with a downward tendency for the calculated relationships (Table 3). Furthermore, in group I piglet birth weight was correlated with daily gains from 1st to 7th day ($r = +0.365$, $P \leq 0.01$) and from 1st to 56th day of age ($r = +0.291$, $P \leq 0.05$). Nissen et al. (2004) (citing Rekiel et al. 2015) demonstrated positive coefficients of correlation between birth weight and weaning weight ($r = +0.53$), as well as between birth weight, weight at slaughter, and mean gain to slaughter weight ($r = +0.29$ and $r = +0.24$). Highly significant correlations of birth weight with body weight measured during maternal nursing and after weaning were also reported by Škorjanc et al. (2007) and Bocian et al. (2011). The coefficient of correlation estimated by Canario et al. (2010) between the number of piglets born alive and their weight on day 21 was $r = +0.40$. The correlation obtained by the authors cited above between neonatal weight and body weight at 21 days was ($r = +0.59$). These relationships show the need to increase the mean body weight of the piglets as well as litter weight at birth, and to make it uniform. This is possible through hormonal and dietary treatment of pregnant sows during intensive fetal myogenesis (Rekiel et al. 2015).

The increasing liter size is paralleled by the increasing variation in neonatal weight, which clearly shows that piglets in the litter are becoming less uniform. The low birth weight combined with high within-litter variation is negatively cor-

TABLE 3. Coefficients of correlation

Correlation between body weight on day 1 of age	Group I b.w. ≤1.20		Group II b.w. 1.21–1.39		Group III b.w. 1.40–1.59		Group IV b.w. ≥1.60	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Body weight on day 7	+0.639	0.001	+0.136	0.270	+0.102	0.406	+0.517	0.001
Body weight on day 21	+0.449	0.001	–0.052	0.682	+0.215	0.078	+0.332	0.007
Body weight on day 56	+0.342	0.012	+0.074	0.556	–0.006	0.961	+0.312	0.014
Weight gains from day 1 to 7	+0.365	0.005	–0.010	0.933	–0.092	0.457	+0.123	0.332
Weight gains from day 8 to 21	+0.194	0.152	–0.109	0.386	+0.199	0.103	+0.120	0.343
Weight gains from day 22 to 56	+0.173	0.173	+0.102	0.420	–0.063	0.611	+0.195	0.129
Weight gains from day 1 to 56	+0.291	0.035	+0.055	0.662	–0.031	0.806	+0.245	0.055

b.w. – body weight of piglet at day 1 of age (kg).

related with piglet survival. In our study, mortality was 19.70, 10.96, 6.94 and 6.06% in groups I, II, III and IV, averaging 10.83% for the investigated group of piglets. Piglet survival in the groups is presented in the figure. The results obtained in groups III and IV vs I were better by about 13 percentage points and show that the neonatal weight of piglets should be optimized to about 1.4 kg.

According to Quiniou et al. (2002), postnatal survival decreases by 95 to 15% when birth weight of piglets is reduced by 1.80 kg to <0.61 kg. In herds with medium fertility and neonatal weights, the losses do not exceed 6–8%, but an increase in litter size from 11 to 16 was found to result in mortality of 28%. Data confirming the high losses among piglets born to high-fertility sows, are also provided by Polish researchers. According to Jarczyk et al. (2009), depending on farm and production cycle, mortality ranged from 3–5 to 14–17%. Mortality was 14–16% in litters of 13 to 15 piglets, and from 24 to over 30% in litters with more than 16 piglets. Jarczyk et al. (2009) also found a relationship between litter size and birth weight of piglets. The greater the litter size, the lower the neonatal weight: when sow fertility was ≥16 and ≤9, the mean birth weight of piglets was 1.27 and 1.73 kg, respectively (difference of 0.46 kg). This had an effect on the growth rate and body weight at days 21 and 82. The body weight of piglets from small litters was higher than for piglets from very large litters, by 0.60 kg ($P \leq 0.05$) and 1.02 kg ($P \leq 0.01$) on the above days of rearing, respectively. Similar results were obtained by Boulot et al. (2008). When litter size increased from 7 to 16 piglets, Quiniou et al. (2002) found

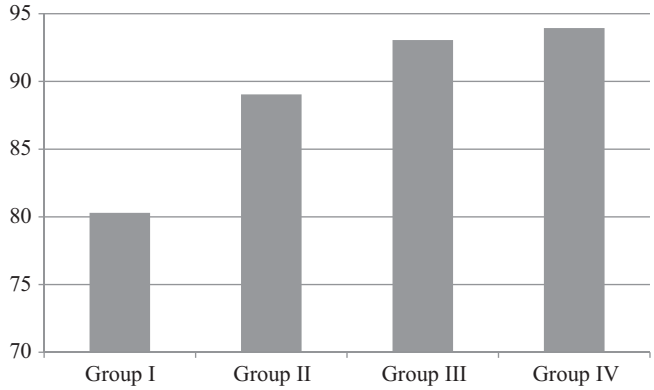

FIGURE. Survivability (%) of piglets (age of 1–56 days)

greater differences in neonatal weight, lower litter uniformity, and 5 times as many stillborn piglets. The proportion of the lightest piglets (weighing <1 kg at birth) and the heaviest piglets (weighing >1.8 kg) changed in the litters from 3 and 63 to 15 and 13%, respectively. Quiniou et al. (2002) and Wu et al. (2004) hold the view that in practice, about 15–20% of piglets weigh 1 kg or less at birth, which considerably reduces their survival.

CONCLUSION

It is concluded based on the findings of the present study that the mean body weight of piglets at 7th, 21st and 56th days of age differed between groups I, II, III and IV. Daily gains in groups I–IV increased with each rearing period (day 1–7, 8–21, 22–56). The differences between groups II and III were small (P >0.05), and those between groups I and IV considerable and highly significant. The coefficients of correlation for piglets from groups I (the lightest at birth) and IV (the heaviest at birth) confirm the relationship between birth weight and body weight at 7th ($P \leq 0.01$), 21st ($P \leq 0.01$) and 56th days of age ($P \leq 0.05$), with a downward tendency for the calculated relationships. Furthermore, in group I piglet birth weight was correlated with daily gains from 1st to 7th day ($r = +0.365$, $P \leq 0.01$) and from 1st to 56th day of age ($r = +0.291$, $P \leq 0.05$). Mortality among neonatal piglets with low birth weight (≤1.20 kg) was high (19.7%). With an increasing mean body weight at birth, piglet survival increased and in group IV it was higher by 13.64 percentage points in relation to group I. The birth weight of piglets ≥1.60 kg ensures the best growth rate and survival of the piglets.

REFERENCES

BAXTER E.M., RUTHERFORD K.M.D., D'EATH R.B., ARNOTT G., TURNER S.P., SANDOE P., MOUSTSEN V.A., THORUP F., EDWARDS S.A., LAWRENCE A.B., 2013: The welfare implications of large litter size in the domestic pig II: management factors. Anim. Welfare 22: 219–238. http://dx.doi.org/10.7120/09627286.22.2.199.

BEAULIEU A.D., AALHUS J.L., WILLIAMS N.H., PATIENCE J.F., 2010: Impact of piglet birth weight, birth order, and litter size on subsequent growth performance, carcass

quality, muscle composition and eating quality of pork. J. Anim. Sci. 88: 2767–2778. doi:10.2527/jas.2009-2222.

BEE G., 2004: Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. J. Anim. Sci. 82: 826–836.

BLICHARSKI T., HORBAŃCZUK J., ŁUKASZEWICZ M., PIERZCHAŁA M., PTAK J., SNOPKIEWICZ M., 2016: Performance results 2015. Pigs. PZHiPTCh „POLSUS". Warszawa: 1–64.

BOCIAN M., GRAJEWSKA S., KAPELAŃSKA J., WIŚNIEWSKA J., WŁODARSKI W., 2011: Wpływ masy ciała prosiąt przy urodzeniu na efekty ich odchowu i wyniki tuczu. Roczn. Nauk. Zoot. 38 (2): 189–195.

BOULOT S., QUESNEL H., QUINIOU N., 2008: Management of high prolificacy in French herds: can we alleviate side effects on piglet survival? Adv. Pork Prod. 19: 213–220.

CANARIO L., LUNDEHEIM N., BIJMA P., 2010: Pig growth is affected by social genetic effects and social litter effects that depend on group size. Proceedings of the 9th World Congress on Genetic Applied to Livestock Production (WCGALP), 1–6 August 2010, Leipzig, Germany.

DOUGLAS S.L., 2015: Management and nutritional strategies to improve the postnatal performance of light weight pigs. PhD. School of Agriculture Food and Rural Development. Newcastle University: 1–141.

DZIERŻANOWSKA-GÓRYŃ D., BRZOZOWSKI M., GÓRAL-RADZISZEWSKA K., 2014: Young chinchillas weight gain, depending on their body mass at birth. Ann. Warsaw Univ. Life Sci. – SGGW, Anim. Sci. 53: 95–101.

FOXCROFT G.R., DIXON W.T., NOVAK S., PUTMAN C.T., TOWN S.C., VINSKY M.D., 2006: The biological basis for prenatal programming of postnatal performance in pigs. J. Anim. Sci. 84 (E Suppl.): 105–112.

GONDRET F., LEFAUCHEUR L., LOUVEAU I., LEBRET B., PICHODO 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.

HALES J., MOUSTSEN V.A., NIELSEN M.B.F., HANSEN C.F., 2015: Temporary confinement of loose-housed hyperprolific sows reduced piglet mortality. J. Anim. Sci. 93: 4079–4088. doi:10.2527/jas2015-8973.

JARCZYK A., MILEWSKA W., WINIARSKI K., KOBAK K., SITKIEWICZ D., 2009: Reproductive performance on Pen Ar Lan sows in the small and large farms. Roczn. Nauk. PTZ 5 (4): 145–154.

KRÓLEWSKA B., REKIEL A., WIĘCEK J., 2014: Effect of birth body weight of piglets on their rearing up to the age of 10 weeks. Ann. Warsaw Univ. Life Sci. – SGGW, Anim. Sci. 53: 21–28.

MILLIGAN B.N., FRASER D., KRAMER D.L., 2002: 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.

ORZECHOWSKA B., MUCHA A., 2010: Ocena użytkowości rozpłodowej loch. Stan hodowli i wyniki oceny świń. Wyd. IZ, Kraków, 3–19.

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., STABENOW B., PFUHL R., BLOCK J., NÜRNBERG G., OTTEN W., METGES C.C., KALBE C., 2012: Effects of limited and excess protein intakes of pregnant gilts on carcass quality and cellular properties of skeletal muscle and subcutaneous adipose tissue in fattening pigs. J. Anim. Sci. 90: 184–196.

REKIEL A., BARTOSIK J., WIĘCEK J., BATORSKA M., KUCZYŃSKA B., ŁOJEK A., 2014a: Effect of piglet birth weight on selected characteristics of pork. Ann. Anim. Sci. 14 (4): 967–975. doi: 10.2478/aoas-2014-0033.

REKIEL A., KRÓLEWSKA B., 2014: Wewnątrzmaciczne zahamowanie wzrostu u zwierząt – syndrom IUGR. Przegl. Hod. 81 (5): 10–12.

REKIEL A., WIĘCEK J., BATORSKA M., KULISIEWICZ J., 2014b. Effect of sow prolificacy and nutrition on pre- and postnatal growth of progeny: a review. Ann. Anim. Sci. 14 (1): 3–15. doi: 10.2478/aoas-2013-0060.

REKIEL A. WIĘCEK J., SOŃTA M., 2015: Naukowe i praktyczne aspekty optymalizacji urodzeniowej masy ciała prosiąt. Przegl. Hod. 4: 15–17.

RUTHERFORD K.M.D., BAXTER E.M., D'EATH R.B., TURNER S.P., ARNOTT G., ROEHE R., ASK B., SANDOE P., MOUSTSEN V.A., THORUP F., EDWARDS S.A., BERG P., LAWRENCE A.B., 2013: The welfare implications of large litter size in the domestic pig I: biological factors. Anim. Welfare 22: 199–218. http://dx.doi.org/10.7120/09627286.222.199.

ŠKORJANC D., BRUS M., POTOKAR M.C., 2007: Effect of birth weight and sex on pre-weaning growth rate of piglets. Arch. Tierz. Dummerstorf 50, 5: 476–486.

ŚWIĘCICKA N., BERNACKA H., ZAWIŚLAK J., 2016: Mean body weight gains in standard mink depending on the number of kits in a litter. Ann. Warsaw Univ. Life Sci. – SGGW, Anim. Sci. 55 (2): 293–300.

TRIBOUT T., CARITEZ J.C., GOGUÉ J., GRUAND J., BILLON Y., BOUFFAUD M., LAGANT H., Le DIVIDICH J., THOMAS F., QUESNEL H., GUÉBLEZ R., BIDANEL J.P., 2003: Estimation, par utilisation de semence congelée, du progrès génétique réalisé en France entre 1977 et 1998 dans la race porcine Large White: Résultats pour quelques caractères de reproduction femelle. Journ. Rech. Porc. Fr. 35: 285–292.

VÁCLAVKOVÁ E., DANĚK P., ROZKOT M., 2012: The influence of piglet birth weight on growth performance. Res. Pig Breed. 6 (1): 59–61.

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. doi. 10.1159/000084645.

WU G., BAZER F.W., CUDD T.A., MEININGER C.J., SPENCER T.E., 2004: Maternal nutrition and fetal development. J. Nutr. 134: 2169–2172.

WOLF J., ZAKOVA E., GROENEVELD E., 2008: Within-litter variation of birth weight in hyperprolific Czech Large White sows and its relation to litter size traits, stillborn piglets and lossed until weaning. Livest. Sci. 115: 195–205.

Streszczenie: *Wpływ masy ciała prosiąt przy urodzeniu na tempo wzrostu i wyniki odchowu do wieku 8 tygodni*. Celem badań było określenie wpływu masy ciała prosiąt przy urodzeniu na wyniki ich odchowu do 56. dnia życia, wyrażone tempem wzrostu i przeżywalnością. Obserwacjami objęto 277 prosiąt mieszańców z 22 miotów od loch F1 (polska biała zwisłoucha × wielka biała polska) po knurach krzyżówkowych (Duroc × Pietrain), jednakowo żywionych i utrzymywanych. Odchów potomstwa przy matkach trwał 5 tygodni a obserwacje 8 tygodni. W 1., 7., 21., oraz 56. dniu życia prosięta ważono indywidualnie. Masa ciała w 1. dniu życia była podstawą do podziału prosiąt na grupy: I, II, III, IV, odpowiednio: ≤1,2; 1,21–1,39; 1,40–1,59; ≥1,60 kg m.c. Oszacowano współczynniki korelacji między masą ciała prosiąt w 1. dniu życia a w 7., 21. i 56. dniu życia oraz przyrostami dobowymi. W kolejnych okresach odchowu przyrosty dobowe w grupach I–IV zwiększały się, przy czym ich zróżnicowanie pomiędzy grupami wykazywało zbliżone zależności. Między grupami II i III różnice były niewielkie (P >0,05), między grupami I i IV znaczne i wysoko istotne statystycznie. Obliczone dla prosiąt z grupy I (najlżejsze przy urodzeniu) i z grupy IV (najcięższe przy urodzeniu) współczynniki korelacji potwierdzają zależność między masą ciała przy urodzeniu a masą ciała w 7. (P ≤0,01), 21. (P ≤0,01) i 56. dniu życia (P ≤0,05), z tendencją malejącą dla obliczonych zależności. Ponadto, w grupie I stwierdzono zależność między masą ciała prosiąt przy urodzeniu a przyrostami dobowymi od 1. do 7. dnia (r = +0,365, P ≤0,01) oraz od 1. do 56. dnia życia (r = +0,291, P ≤0,05). Przy zwiększającej się średniej masie ciała przy urodzeniu przeżywalność prosiąt zwiększała się, była większa w grupie IV w porównaniu z grupą I o 13,64 punktów procentowych. Masa ciała przy urodzeniu ≥1,60 kg gwarantowała najlepsze tempo wzrostu i przeżywalność prosiąt.

Słowa kluczowe: prosięta, masa ciała, przyrosty dobowe, przeżywalność

MS received 21.02.2017
MS accepted 03.04.2017

Authors' address:
Anna Rekiel
Katedra Szczegółowej Hodowli Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8, 02-786 Warszawa
Poland
e-mail: anna_rekiel@sggw.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 15–21
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.2

Cross-species hybridizations *in situ* for identification of Robertsonian translocation in wild boar

MAREK BABICZ[1], BARBARA DANIELAK-CZECH[2], ANNA KOZUBSKA-SOBOCIŃSKA[2]

[1]Department of Pig Breeding and Production Technology, University of Life Sciences in Lublin

[2]Department of Animal Genomics and Molecular Biology, National Institute of Animal Production, Balice/Kraków

Abstract: *Cross-species hybridizations* in situ *for identification of Robertsonian translocation in wild boar.* Homologies and homeologies between human and pig chromosomes enabled human painting probes to be used for identification of chromosomes involved in homozygous centric fusion in the wild boar (*Sus scrofa scrofa*) with karyotype 36,XY,rob(15;17), which had been provisionally determined on the basis of G-bands (GTG technique). For interspecies hybridizations two commercial differently labelled human painting probes for chromosome pairs 2 and 20 were used. FISH with the human WCP 20 probe revealed green fluorescence signals on the short arms of the 15;17 translocated chromosomes, and after hybridization with the WCP 2 yellow signals were observed along the long arms of these rearranged acrocentric autosomes as well as on small fragments of the SSC3q arms. The results of cross-species hybridizations *in situ* have confirmed preliminary cytogenetic evaluation of the karyotype of the Robertsonian translocation-carrying wild boar as well as numerous homologies and homeologies between chromosomes of human and species (*Sus scrofa domestica* and *Sus scrofa scrofa*) belonging to the Suidae family. The results obtained have confirmed also the usefulness of commercial human painting probes for identification of chromosome rearrangements in other species that received little study.

Key words: wild pig, Robertsonian translocation, karyotype 36,XY,rob(15;17), ZOO-FISH

INTRODUCTION

The normal karyotype of the domestic pig (*Sus scrofa domestica*) contains 2n = 38 chromosomes (involving 24 meta- and submetacentric autosomes, 12 acrocentric chromosomes and two XX and XY heterosomes) is almost identical to that of the wild boar (*Sus scrofa scrofa*) (Gustavsson 1988, 1990).

However, populations of the wild boar frequently demonstrate chromosome number polymorphism caused by chromosome rearrangements of the centric fusion type (Robertsonian translocation), which may lead to a reduction of the chromosome number, but not their arm number (NF), which is characteristic for a given species. The investigations performed on domestic pigs and wild boars showed variation of diploid chromosome numbers of 36-37-38 (Gustavsson et al. 1973, Bosma 1976, Sysa et al. 1984, Troshina et al. 1985, Rejduch et al. 2003, Wnuk et al. 2005).

In pigs, the Robertsonian translocations decrease carriers' fertility by approx. 5–22%, without any visible phenotypic changes. Therefore, such karyotype defects can be widespread in many populations, especially as a result of intensive use of sires affected in artificial insemination (AI) and cause considerable economic losses to breeding organizations (Gustavsson 1990). For this reason, several European countries, including Poland, have developed chromosomal screening programmes involving hypoprolific sires and, recently, young AI boars analysed before reproduction (Danielak-Czech and Słota 2008, Ducos et al. 2008). In cytogenetic monitoring of breeding pigs performed recently in the French and Polish specialist laboratories, in addition to new reciprocal translocations, also rare centric fusions (13;17, 14;15, 14;17 and 15;18) and the unique tandem fusion der(14;17)(14q29;17q10) were identified (Pinton et al. 2012, Danielak-Czech et al. 2016).

In order to predict breeding consequences and prevent the occurrence with early diagnosis, translocations need to be characterized precisely using not only classical cytogenetic techniques but also molecular methods, particularly fluorescence *in situ* hybridization (Rubes et al. 2009, Słota and Danielak 2010, Danielak-Czech et al. 2013a, 2016). Where commercial pig-specific chromosome probes are not available, non-species--specific probes (most often commercial -human probes) could be used (Danielak-Czech et al. 2010, 2013b, Rejduch et al. 2010b).

The aim of the present study was identification of Robertsonian translocation in wild boar, using two commercial human painting probes in cross-species hybridizations *in situ*.

MATERIAL AND METHODS

Sus scrofa scrofa has been obtained (after culling) under the planned wildlife management in the Lublin region (Act of 13 November 1995 Hunting Laws, Dz.U. [Official Journal] 1995 No 147, item 713, as amended Dz.U. of 2015, item 2168, of 2016 item 1082). Metaphase chromosome preparations of wild boar studied, were derived from the classical peripheral blood lymphocyte culture. For chromosome staining, conventional Giemsa staining and the standard protocol of GTG-banding technique were applied. The karyotypes were prepared according to the recommendation of the Committee for the Standardized Karyotype of Domestic Pig (Gustavsson 1988).

For interspecies hybridizations *in situ* two differently labelled commercial human whole-chromosome painting probes (WCP) for chromosome pairs 2 and 20 (Cambio: Human WCP FITC Chromosome 2 – Cat. No. 1083-2F-01 and Human WCP Cy3 Chromosome 20 – Cat. No. 1153-20Cy3-01) were used. The FISH technique was performed according to the manufacturer's procedure. Hybridization signals were observed under a Nikon epifluorescence microscope equipped with appropriate set of filters (single filters for FITC and TexasRed fluorochromes and a triple band pass filter FITC/TR/DAPI). Selected cells were recorded and evaluated using the Cyto Vision Imaging System (Applied Imaging, Newcastle upon Tyne, UK).

RESULTS AND DISCUSSION

Microscope analysis of Giemsa stained metaphase spreads revealed that the wild boar studied had 36 chromosomes (2n = 36), of which 26 were meta- and submetacentric chromosomes, 8 acrocentric autosomes and XY heterosomes. The GTG-banding technique performed in this animal proved the existence of the two additional submetacentric chromosomes in his chromosome set, resulting from a centric fusion between acrocentric chromosomes of the pairs 15 and 17 (Fig. 1). Based on this procedure, the karyotype of the studied wild boar with Robertsonian translocation in homozygotic form was defined as 36,XY,rob(15;17) – Figure 2. The diagnosis was unequivocally evidenced by FISH technique with human whole chromosome painting probes. Interspecific hybridizations *in situ* with the human WCP 20 probe showed green fluorescence signals on the short arms of the 15;17 translocated chromosomes, and after hybridization with the WCP 2 yellow signals were observed along the long arms of these rearranged acrocentric autosomes and on small fragments of the SSC3q arms (Fig. 3).

It is worth noting that similarities between chromosomes of different Suidae species were shown many times in hybridizations *in situ* using probes obtained from *Sus scrofa domestica* chromosomes by flow-sorting and microdissection of whole chromosomes or their fragments as well as by PCR from genomic DNA using appropriate primers (Słota and Danielak-Czech 2010, Doležel et al. 2012, Danielak-Czech et al. 2013a, 2016). On the other hand, the phenomenon of genetic

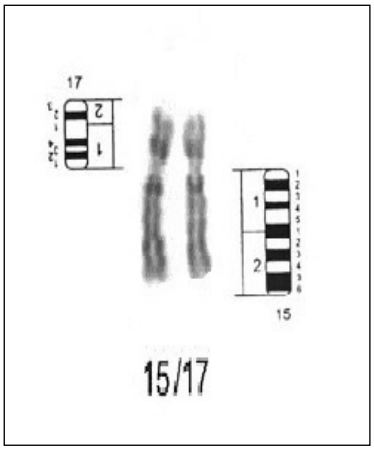

FIGURE 1. GTG-banded 15;17 translocated chromosomes in the wild boar with Robertsonian translocation in homozygous form and ideograms of the acrocentic autosomes 15 and 17 involved in this centric fusion

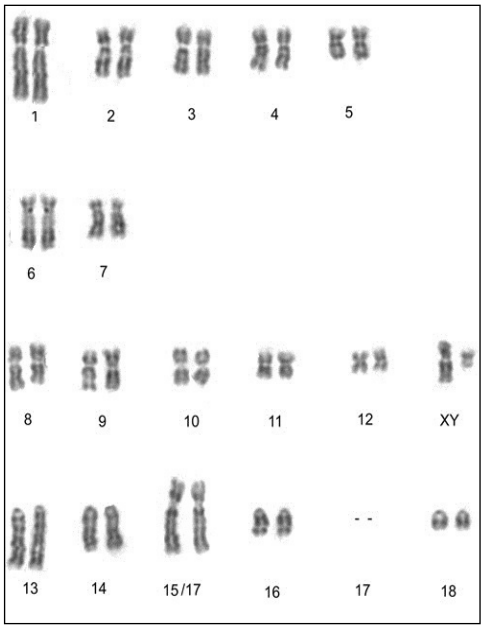

FIGURE 2. GTG-banded karyotype (36,XY, rob15;17) of the wild boar (*Sus scrofa scrofa*) carrier of the 15;17 Robertsonian translocation in homozygous form

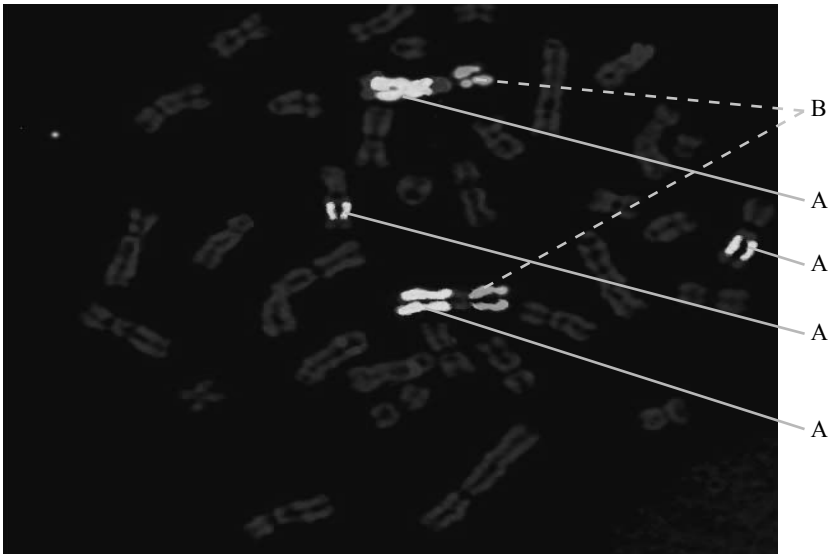

FIGURE 3. The cross-species hybridizations *in situ* with human painting probes (WCP 2 and WCP 20) for identification of Robertsonian translocation in wild boar with karyotype 36,XY,rob15;17. Yellow fluorescence signals (A) after hybridization with the WCP 2 probe were observed along the long arms of the 15;17 translocated chromosomes and on small fragments of the SSC3q arms. Green signals (B) after hybridization with the WCP 20 probe were observed on the short arms of the 15;17 rearranged acrocentric autosomes

conservation between human and pig chromosomes repeatedly enabled human painting probes to be used for identification of particular chromosomes and chromosome rearrangements in Suidae species (Rettenberger et al. 1995, Frönicke et al. 1996, Jiang and Rothschild 2007, Danielak-Czech et al. 2010, Rejduch et al. 2010b, Kociucka et al. 2014, Kozubska-Sobocińska et al. 2014, 2015). Our study showed that the chromosome painting method could effectively supplement classical banding techniques in diagnosis of chromosomal rearrangements.

However, it must be stated that the use of molecular methods for karyotype evaluation is still little developed in pigs because the commercial painting probes for this species are almost not available (Rubes et al. 2009, Barasc et al. 2014, Danielak-Czech et al. 2016). For this reason it is often necessary to perform interspecies hybridization *in situ*, especially with human chromosome probes, because the pig genome is of similar size, complexity and genetic information as the human genome. Although some discrepancies exist among the human and pig genome maps, they have contributed to an identification of over 170 conserved segments between genomes of these two species, which have helped to further determine the evolutionary relationship between them (Goureau et al. 1996, Frönicke et al. 1996, Yerle et al. 1996, Jiang and Rothschild 2007).

It should be added that the numerous comparative mapping studies (genome

linkage and radiation hybrid maps or ZOO-FISH) also definitely proved that porcine karyotype was nearly completely covered with homologous human segments (Danielak-Czech et al. 2010, 2013b, Rejduch et al. 2010a, Kozubska-Sobocińska et al. 2014, 2015). The results obtained in our comparative studies showed conserved segments between human autosome 2 and porcine SSC15, SSC3, as well as between HSA20 and SSC17. Interspecies homologies concerning the largest of these segments served as a basis for choosing human WCP 2 and 20 probes for our experiment, with the aim of molecular identification of the Robertsonian translocation in the wild boar with karyotype 36,XY,rob(15;17) – Figure 3.

In general, our results illustrate how comparative study based on different modern techniques (as well as FISH) and carried out on different species can add power to precise interpretation of genome rearrangements, including structural changes like centric fusion described here. Moreover, our cross-species hybridization *in situ* experiments substantially prove conservation of the linkage groups and high degree of homology and homeology of chromosome regions in human and the domestic and wild pig genomes.

CONCLUSION

The results of FISH analysis have confirmed preliminary cytogenetic evaluation of the karyotype of the Robertsonian translocation carrying wild boar as well as numerous homologies and homeologies between human and *Sus scrofa scrofa* chromosomes.

REFERENCES

BARASC H., FERCHAUD S., MARRY N., CUCCHI M.A., LUCENA A.N., RAYMOND-LETRON I., CALGARO A., BONNET N., DUDEZ A.M., YERLE M., DUCOS A., PINTON A., 2014. Cytogenetic analysis of somatic and germinal cells from 38,XX/38,XY phenotypically normal boars. Theriogenology 81: 368–872.

BOSMA A.A., 1976: Chromosomal polymorphism and G-banding patterns in the wild boar (*Sus scrofa* L.) from the Netherlands. Genetica 46: 391–399.

DANIELAK-CZECH B., KOZUBSKA-SOBOCIŃSKA A., REJDUCH B., 2010: Diagnosis of tandem fusion translocation in the boar using FISH technique with human painting probes. Ann. Anim. Sci. 10: 361–366.

DANIELAK-CZECH B., KOZUBSKA-SOBOCIŃSKA A., REJDUCH B., 2013a: Diagnostyka cytomolekularna w nowoczesnej hodowli zwierząt gospodarskich. Wiad. Zoot. LI 4: 58–64.

DANIELAK-CZECH B., KOZUBSKA-SOBOCIŃSKA A., REJDUCH B., 2016: Molecular cytogenetics in the diagnostics of balanced chromosome mutations in the pig (*Sus scrofa*) – a review. Ann. Anim. Sci. 16 (3): 1–21.

DANIELAK-CZECH B., REJDUCH B., KOZUBSKA-SOBOCIŃSKA A., 2013b: Identification of telomeric sequences in pigs with rearranged karyotype using PRINS technique. Ann. Anim. Sci. 13 (3): 495–502.

DANIELAK-CZECH B., SŁOTA E., 2008: Karyotype control system of AI boars in Poland: the current survey. Ann. Anim. Sci. 3: 255–262.

DOLEŽEL J., VRÁNA J., SAFÁŘ J., BARTOŠ J., KUBALÁKOVÁ M., SIMKOVÁ H., 2012. Chromosomes in the flow to simplify genome analysis. Funct. Integr. Genomics 12: 397–416.

DUCOS A., REVAY T., KOVACS A., HIDAS A., PINTON A., BONNET-GARNIER A., MOLTENI L., SŁOTA E., ŚWITOŃSKI M., ARRUGA M.V., VAN HAERINGEN W.A., NICOLAE I., CHAVES R., GUEDES-PINO H., ANDERSSON M., IANNUZZI L., 2008: Cytogenetic screening of livestock populations in Europe: an overview. Cytogenet. Genome Res. 120: 26–41.

FRÖNICKE L., CHOWDHARY B.P., SCHERTHAN H., GUSTAVSSON I., 1996: A comparative map of the porcine and human genomes demonstrates ZOO-FISH and gene mapping-based chromosomal homologies. Mamm. Genome 7: 285–290.

GOUREAU A., YERLE M., SCHMITZ A., RIQUET J., MILAN D., PINTON P., FRELAT G., GELLIN J., 1996: Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. Genomics 3: 252–262.

GUSTAVSSON I., 1988: Standard karyotype of domestic pig. Hereditas 109: 151–157.

GUSTAVSSON I., 1990: Chromosomes of the pigs. Adv. Vet. Sci. Comp. Med. 34: 7–107.

GUSTAVSSON I., HAGELTORN M., ZECH L., REILAND S., 1973: Identification of the chromosomes in a centric fusion/fission polymorphic system of the pig (*Sus scrofa* L.). Hereditas 75: 153–155.

JIANG Z., ROTHSCHILD M.F., 2007: Swine genome science comes to age. Int. J. Biol. Sci. 3: 129–131.

KOCIUCKA B., SZCZERBAL I., BUGAJ S., ORSZTYNOWICZ M., ŚWITOŃSKI M., 2014. A high incidence of adjacent-1 meiotic segregation pattern, revealed by multicolor sperm FISH, in a carrier boar of a new reciprocal translocation t(6;16)(p13;q23). Cytogenet. Cell Genet. 142: 21–27.

KOZUBSKA-SOBOCIŃSKA A., DANIELAK-CZECH B., BĄK A., BABICZ M., REJDUCH B., 2014: Comparative physical mapping of genes associated with meat production traits in the wild pig genome. Chromosome Res. 22: 414.

KOZUBSKA-SOBOCIŃSKA A., DANIELAK-CZECH B., BABICZ M., 2015: Cross-species hybridizations *in situ* of genes associated with meat production traits in the wild pig genome. Ann. UMCS, sect. EE, Zootechnica. 33 (3): 31–36.

PINTON A., CALGARO A., BONNET N., MARY N., DUDEZ A.M., BARASC H., PLARD C., YERLE M., DUCOS A., 2012: Contrôle chromosomique des populations porcines en France: bilan 2007–2010. Journées Recherche Porcine 44: 43–44.

REJDUCH B., DANIELAK-CZECH B., KOZUBSKA-SOBOCIŃSKA A., 2010a: FISH-based comparative analysis of human and porcine chromosome region involving obesity-related genes. Ann. Anim. Sci. 10: 367–372.

REJDUCH B., KOZUBSKA-SOBOCIŃSKA A., DANIELAK-CZECH B., 2010b: Use of human painting probes for identification of centric fusion in wild boar. Chromosome Res. 18: 727–728.

REJDUCH B., SŁOTA E., RÓŻYCKI M., KOŚCIELNY M., 2003: Chromosome number polymorphism in a litter of European wild boar (*Sus scrofa scrofa* L.). Anim. Sci. Pap. Rep. 21: 57–62.

RETTENBERGER G., KLETT C., ZECHNER U., KUNZ J., VOGL W., HAMEISTER H., 1995: Visualization of the conservation of synteny between humans and pigs by heterologous chromosomal painting. Genomics 26: 372–378.

RUBES J., PINTON A., BONNET-GARNIER A., FILLON V., MUSILOVA P., MICHALOVA K., KUBICKOVA S., DUCOS A., YERLE M., 2009: Fluorescence *in situ* hybridization applied to domestic animal cytogenetics. Cytogenet. Genome Res. 126: 34–48.

SŁOTA E., DANIELAK-CZECH B., 2010: Zastosowanie metod cytogenetyki molekularnej w diagnostyce i profilaktyce wad genetycznych u świń. In: Zastosowanie osiągnięć naukowych z zakresu genetyki i biotechnologii rozrodu w nowoczesnej produkcji trzody chlewnej. Rocz. Nauk Zoot. Monogr. Rozpr. 44: 9–20.

SYSA P.S., SŁAWOMIRSKI J., GROMADZKA J., 1984: Remarks on cytogenetic studies on crossing wild boar (*Sus scrofa fereus*) and domestic pig (*Sus scrofa dom.*). Pol. Arch. Wet. 24: 90–95.

TROSHINA A., GUSTAVSSON I., TIKHONOV V.N., 1985: Investigation of two centric fusion translocations of wild pigs by different banding techniques. Hereditas. 202: 155–158.

WNUK M., KOTYLAK Z., BUGNO M., SŁOTA E., 2005: Karyotype analysis in pigs – hybrids of European wild boar (S*us scrofa scrofa*) and domestic pig (S*us scrofa domestica*). Ann. Anim. Sci. 5: 11–19.

YERLE M., ECHARD G., ROBIC A., MAIRAL A., DUBUT-FONTANA C., RIQUET J., PINTON P., MILAN D., LAHBIB-MANSAIS Y., GELLIN J., 1996: A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. Cytogenet. Cell Genet. 73: 194–202.

Streszczenie: *Międzygatunkowe hybrydyzacje* in situ *do identyfikacji translokacji Robertsonowskiej u dzika*. Homologie i homeologie między chromosomami człowieka i świni domowej umożliwiły zastosowanie ludzkich sond malujących do identyfikacji chromosomów zaangażowanych w homozygotyczną fuzję centryczną u dzika (*Sus scrofa scrofa*) o kariotypie 36,XY,rob(15;17), który został wstępnie zdiagnozowany na podstawie prążków G (technika GTG). Do międzygatunkowych hybrydyzacji wykorzystano dwie komercyjne, różnie znakowane ludzkie sondy malujące dla chromosomów par 2 i 20. FISH z ludzką sondą WCP 20 ujawniła zielone sygnały hybrydyzacyjne na krótkich ramionach translokowanych chromosomów 15;17, a po hybrydyzacji z WCP 2 żółte sygnały obserwowane były wzdłuż długich ramion tych zreorganizowanych akrocentrycznych autosomów. Wyniki międzygatunkowych hybrydyzacji *in situ* potwierdziły wstępną cytogenetyczną ocenę kariotypu dzika – nosiciela translokacji robertsonowskiej, a także liczne homologie i homeologie między chromosomami człowieka i gatunków należących do rodziny Suidae (*Sus scrofa domestica* i *Sus scrofa scrofa*).

Słowa kluczowe: dzik, translokacja Robertsonowska, kariotyp 36,XY,rob(15;17), ZOO-FISH

MS received 23.10.2016

MS accepted 14.03.2017

Authors' address:
Marek Babicz
Katedra Hodowli i Technologii Produkcji Trzody Chlewnej
Uniwersytet Przyrodniczy w Lublinie
Akademicka 13, 20-950 Lublin
Poland
e-mail: marek.babicz@up.lublin.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 23–32
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.3

Carbon nanoparticles as transporters of melittin to glioma grade IV U87 cells in *in vitro* model

PAULINA BINIECKA, SŁAWOMIR JAWORSKI, ŻANETA BUGAJSKA, KAROLINA DANILUK
Department of Animal Nutrition and Biotechnology, Warsaw University of Life Sciences – SGGW

Abstract: *Carbon nanoparticles as transporters of melittin to glioma cells in* in vitro *model.* Substances derived from nature have natural cytotoxic properties, melittin, the main component of bee venom is one of them. It has the ability to destroy any lipid bilayer, therefore to be used in a cancer treatment it needs to be targeted. The aim is to create the drug delivery system, which would efficiently deliver the active substance to glioma cells. Carbon nanoparticles are considered to be a good agent in biomedical applications, due to their biocompatibility and small sizes. In this study five types of nanoparticles were used: pristine graphene (GN), nanographene oxide (nGO), graphite (G), nanodiamond (UDD) and hierarchical nanoporous carbons (HNCs) to target the melittin to cancer cells. The visualization of the drug delivery complexes of melittin and nanoparticles was done with transmission electron microscopy, the influence of the complexes on cell morphology and structure was pictured with scanning electron microscope. Moreover, in order to check the viability of the cells treated with melittin and the complexes of melittin and nanoparticles the PrestoBlue™ assay was done, also to specify the way of the cell death the annexin V/PI assay was carried out. The results indicate that various nanoparticles behave differently in a complex with melittin. The UDD, GN and nGO nanoparticles resulted in higher mortality than the melittin itself. Creating and applying such complexes of melittin with nanoparticles in glioma cancer treatment may be a promising solution in the therapy.

Key words: brain tumor, nanoparticles, melittin, drug delivery system

INTRODUCTION

The use of nanoparticles in cancer treatment has been already widely investigated (Yezhelyev et al. 2006, Peer et al. 2007). There are several different approaches of applying them in *in vitro* experiments. Carbon nanoparticles, such as graphene, nanotubes or nanodiamond, can be applied as a drug itself. Diamond nanoparticles are highly biocompatible and already tested to be effective in inhibition the brain tumor angiogenesis (Grodzik et al. 2011). Nanotubes can be used as both, in detection of cancerous cells and as a drug delivery of small therapeutic molecules to these cells (Ji et al. 2010). Recently, single-walled carbon nanotubes were used in three--dimensional (3D) localization of cancer (Lin et al. 2016). Although the toxicity of graphene nanoparticles is not entirely established, there are some tests already carried out showing that the genotoxicity and cytotoxicity of graphene sheets depends on their concentration and size. The smaller size and the higher

concentration of graphene sheets there is, the higher mortality of the cells occur (Akhavan et al. 2012). Moreover, the influence of reduced graphene oxide was tested on glioma giving the increase in apoptotic cell death (Jaworski et al. 2015). The current research focuses on the most effective treatment, which is targeted drug delivery. Nanoparticles and nanomaterials with the size smaller than 100 nm have high reactivity and can react with other substances practically without complementary energy. A share of surface atoms in nanoparticles is considerably greater than in bulk material and increases with reduction of particle size. Chemical bonds of nanoparticles surface atoms are not compensated and it results in appearance of new electrical, chemical, mechanical, toxic and other properties. That is why, nanoparticles can be easily functionalized with other substances and they seem to be perfect for carrying drugs into the cancer cells (Haley 2008, Blanco et al. 2015). Furthermore, many different substances derived from nature has been investigated due to their toxicity towards cancer cells, e.g. curcumin inhibits pancreatic cancer cells growth (Su et al. 2016). Melittin, the major component of bee venom, containing 25 amino acids, is considered to have lytic properties after spontaneous integration into lipid bilayers (Terwilligert et al. 1982). This small protein has already been tested as anticancer drug, on ovarian cancer cells and in cancer immunotherapy (Jo et al. 2012, Liu et al. 2016), giving the promising results. The aim of the work was to determine the effect of five different carbon nanoparticles as nanocarriers of melittin to glioma cells. The TEM was used to visualize the melittin-nanoparticle complexes and SEM to see the morphology and structure of the exposed cells, as well as the *in vitro* experiments were designed to test the viability and the way of cell death. The results of this study may advance the future application of carbon nanoparticles combined with active substance in cancer treatment.

MATERIAL AND METHODS

Preparation and characterization of nanoparticles-melittin complex

Pure melittin peptide was obtained from Sigma Aldrich (Munich, Germany) in a powder form. Then it was dissolved in 1 ml of Milli-Q water. There were prepared five different complexes from the melittin stock solution of 20 μg/ml and the solution of five different nanoparticles: pristine graphene – GN, nanographene oxide – nGO, graphite – G, nanodiamond – UDD (SkySpring Nanomaterials, Hudson, USA) and hierarchical nanoporous carbons- HNCs (Faculty of Advanced Technologies and Chemistry, Military University of Technologies). Melittin was added to each type of nanoparticle in order to obtain five different complexes in concentration of 20 μg/ml. Then, the complexes were incubated in 37°C and vortexed for 15 min. Previous studies have showed that the concentration of 20 μg/ml of nanoparticles was not toxic to glioma cells, therefore to check the effect of melittin itself the same concentration was used (Jaworski et al. 2015). Melittin, five different nanoparticles and complexes of nanoparticles and melittin were investigated by the transmission electron microscope (TEM) JEM-1220 (JEOL, Tokyo, Japan) at 80 KeV, with

a Morada eleven-megapixel camera (Olympus Soft Imaging Solutions, Münster, Germany). Samples for the TEM were prepared by placing droplets of hydrocolloids on to Formvarcoated copper grids (Agar Scientific, Stansted, UK). Immediately after drying the droplets in the room temperature, the grids were inserted into the TEM.

Cell culture

Human glioma U87 cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). Cell line was cultured in Dulbecco's Modified Eagle's culture Medium containing 10% fetal bovine serum (Life Technologies, Houston, TX, USA) and 1% penicillin and streptomycin (Life Technologies) at 37°C in a humidified air atmosphere containing 5% carbon dioxide in a DH AutoFlow CO_2 air-jacketed incubator (NuAire, Plymouth, MN, USA).

Cell morphology

In order to check the morphology of cells after treatment with tested complexes the SEM examination was done. The U87 cells were seeded in six-well plates ($5 \times \times 10^5$ cells per well) and incubated for 24 h. Then the medium was removed, and the complexes of melittin with different nanoparticles as well as the melittin itself (20 μg/ml) were introduced to the medium. The next day, cells were washed in PBS (0.01 M, pH 7.2, Sigma), fixed in 2.5% glutaraldehyde (Sigma) for 1 h, washed twice in PBS, and placed on aluminum SEM stubs. The SEM stubs were kept in a moist atmosphere for 1 h, washed in PBS, post fixed in 1% osmium tetroxide (Sigma) for 1 h, rinsed in distilled water, and dehydrated with progressive alcohol solutions (30–50–70–90–95–99%). The preparations were further dehydrated with a critical point-dried (Polaron CPD 7501, Quorum Technologies, Newhaven, East Sussex, UK) and covered by a thin layer of gold (JEE-4C, JEOL Ltd., Tokyo, Japan). The samples were inspected by SEM at 1 KeV (FEI Quanta 200, FEI Co., Hillsboro, OR, USA).

Cell viability

Human glioma U87 cells were cultured in 96-well plates (5×10^3 cells per well) and incubated for 24 h. Then the medium was removed, and the complexes of melittin with five different nanoparticles as well as the melittin itself in concentration of 20 μg/ml were introduced to the medium. The blank tests, medium with nanoparticles, were also prepared. After 24 h, 10 μl of PrestoBlue™ reagent was added to each well and incubated for an additional 2 h at 37°C. The optical density of each well was recorded on ELISA reader (Infinite M200, Tecan, Durham, NC, USA). Cell viability was expressed as the percentage (ODtest-ODblank)/ /(ODcontrol-ODblank), where "ODtest" is the optical density of cells exposed to melittin complexes, "ODcontrol" is the optical density of the control sample, and "ODblank" is the optical density of wells without glioma cells. Test was performed in triplicates.

Mode of cell death

Type of cell death was evaluated with an annexin V/PI assay (Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit-Invitrogen, Carlsbad, CA, USA). After 24-hour incubation of U87 glioma cells in 75 ml flasks (1×10^6 cells per flask),

the medium was removed, and the complexes of melittin in concentration of 20 μg/ml and melittin in complexes with different nanoparticles were added at 20 μg/ml. After a further 24-hour incubation, the medium was removed and the cells were washed in ice-cold PBS and trypsinized. Harvested cells were suspended in 100 μl annexin-binding buffer (Invitrogen) and afterwards 5 μl of annexin V linked with Alexa Fluor 488 and 1 μl of PI were added (Invitrogen). Cells were analysed using FACStrak (Becton-Dickinson, Germany; software – SimulSet), measuring the fluorescence emission at 530 and 575 nm using excitation at 488 nm.

Statistical analysis

One-way variance analysis of viability was performed by Tukey's multiple range test. Differences were considered significant at $P \leq 0.05$. For the analysis the Statgraphics Centurion software (StatPoint Technologies, Warrenton, VA, USA) was used.

RESULTS AND DISCUSSION

Drug delivery systems have been lately widely investigated (Torchilin et al. 2010). The observation of connections in tested complexes and cytotoxicity of them is crucial in order to use those complexes in cancer treatment. Moreover, the components have to be easily functionalized. The nanoparticles are such multifunctional agents, which can interact with different type of cells (Singh et al. 2009). When it comes to carbon nanoparticles, they are more biocompatible compared with different materials (Liu et al. 2008, Zhu et al. 2012), accordingly they are broadly used in cancer therapies of cervical cancer cells and glioma cells (Kim et al. 2008, 2011, Grodzik et al. 2011). The nanocarriers with graphene oxide are used to deliver cancer drug, doxorubicin, directly to the nucleus of the cell (Zhou et al. 2014), as well as nanodiamonds-mediated doxorubicin is used to inhibit the lung metastasis of breast cancer (Xiao et al. 2013). In this work the carbon nanoparticles were tested as components of drug delivery system for melittin, the lytic agent, on glioma cells in *in vitro* cell culture. The self-organization of complexes (melittin with different nanoparticles) was checked with TEM. The morphology of cells after treatment was investigated with SEM. Moreover, the viability was tested with PrestoBlue™ reagent and the way of cell death with flow cytometry using an annexin V/PI assay.

The TEM pictures visualize the complexes, which were done through self-organization, connecting the melittin with different nanoparticles: UDD, nGO, GN, G and HNCs (Fig. 1). Self-organization of the melittin in complexes was different with different nanoparticle. In order to compare how nanoparticles look in the complexes and by themselves, the TEM pictures of only nanoparticles are shown on Figure 2. For UDD and nGO this small peptide behaved as a linker, but it also stayed on the outside of the complex what allows it to interact well with cancer cells (Fig. 1B, D). Whereas the HNCs and graphite cover the melittin from every side, what may result in inefficient treatment (Fig. 1E, F). Graphene, which is the one atom-thick layer of bonded carbon (Stankovich et al. 2006), in a complex with melittin resulted in very low viability of glioma cells, the peptide was evenly dispersed on graphene sheet (Fig. 1C).

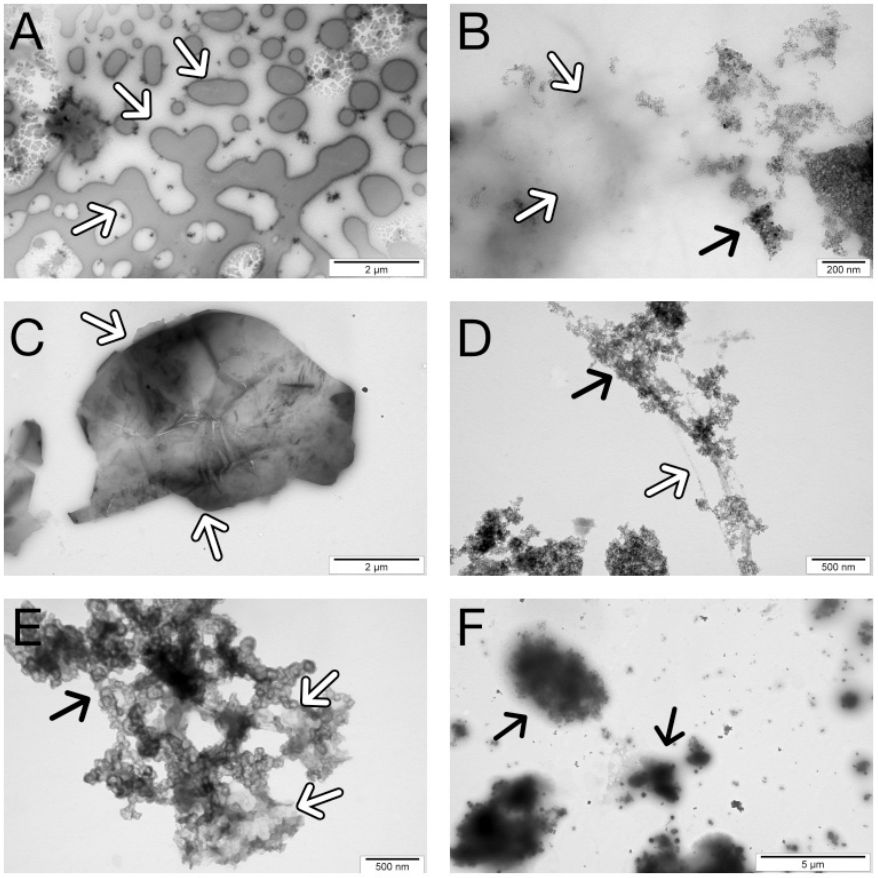

FIGURE 1. Complexes of melittin and nanoparticles. A – melittin, B – melittin with UDD, C – melittin with GN, D – melittin with nGO, E – melittin with HNCs, F – melittin with G (white arrow points the melittin and black arrow points the nanoparticles agglomerates)

In the pictures of glioma cells after treatment with melittin (Fig. 3B) and complexes of melittin with different nanoparticles – UDD, nGO and GN (Fig. 3C, D, E) there were seen the changes, especially in the protrusions of the cells, treated cells have them less and they are thinner. Whereas, cells in a control group are thick and have long protrusions (Fig. 3A). In reference to HNCs and graphite, the morphology of the cells remained unchanged, they looked as in the control group.

Different nanoparticles have diverse effects on *in vitro* cultured cells. What is more, their cytotoxicity depends on a given dose, their size, surface chemistry and also the type of the cells and excretion (Jia et al. 2005, Firme et al. 2010). There was a significant difference between control group and cells treated with melittin in a complex with nanodiamond, where almost the half of the cells died. It was already settled that nanodiamond is the most biocompatible among

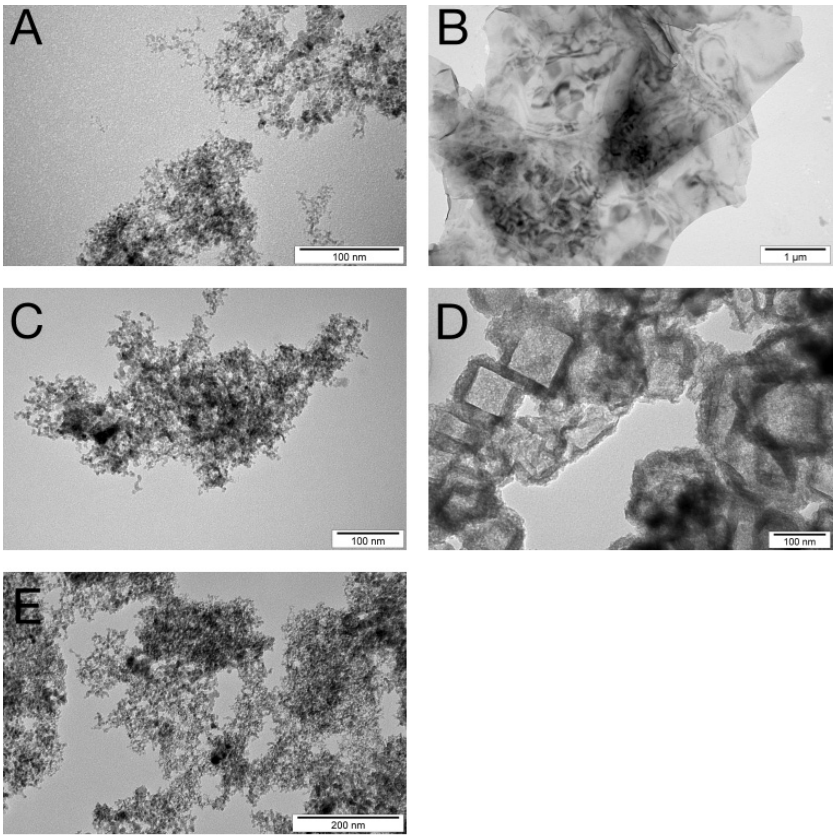

FIGURE 2. Carbon nanoparticles. A – UDD, B – GN, C – G, D – HNCs, E – nGO

other carbon nanoparticles (Zhu et al. 2012). Regarding graphene (GN) and nanographene oxide (nGO) the outcome was also promising, over 40% of dead cells occurred (Fig. 4). Melittin by itself had lower cytotoxicity than in a complex with mentioned above nanoparticles, which indicate their contribution to the more efficient uptake of the component by the cells. The influence of HNCs and graphite in complex with melittin resulted in high viability of glioma cells, what may be caused by their big tendency to agglomeration or their structure (Fig. 1E, F).

Apoptosis is a programmed cell death and it is a natural process, too little apoptosis may result in cancer, autoimmune or inflammatory diseases, the macrophages in apoptosis remove the cells' debris, so that the inflammation does not occur. Hence, the way the cells die is crucial in cancer treatment (Schwartzman et al. 1993). Thus the flow cytometry was used in order to analyze how many cells are apoptotic and how many are necrotic. For this assay only three nanoparticles were chosen (UDD, nGO and GN), which gave the best results in previous tests. The most cells,

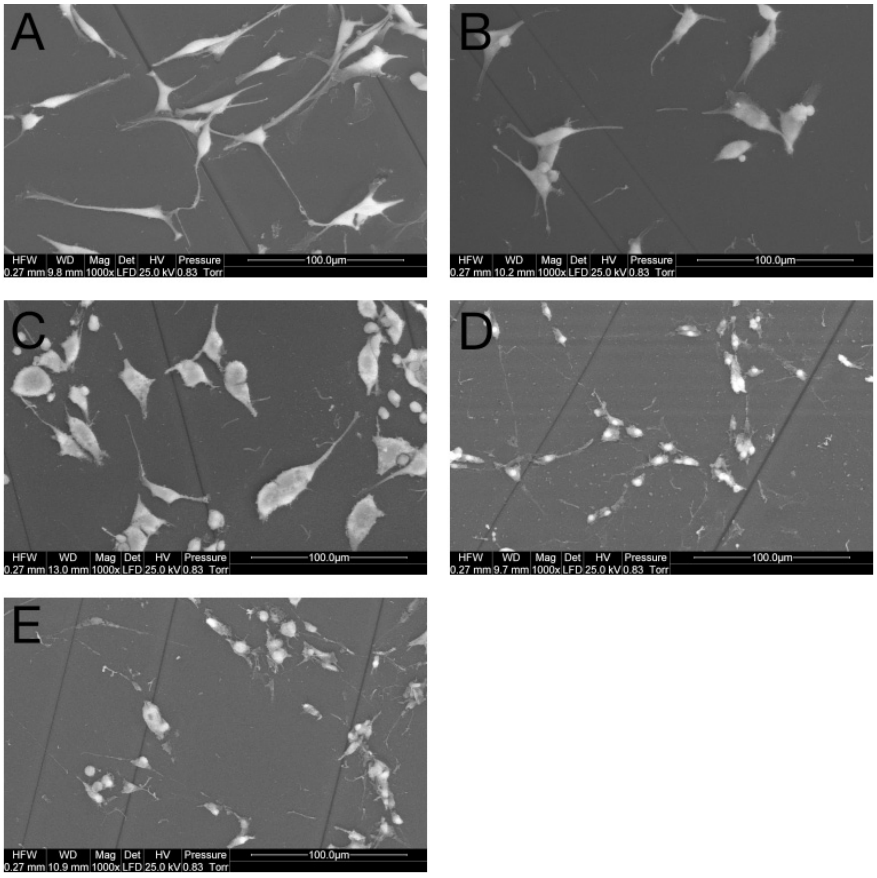

FIGURE 3. Cell morphology after treatment. A – control group, B – cells treated with melittin, C – cells treated with melittin with UDD, D – melittin with GN, E – melittin with nGO

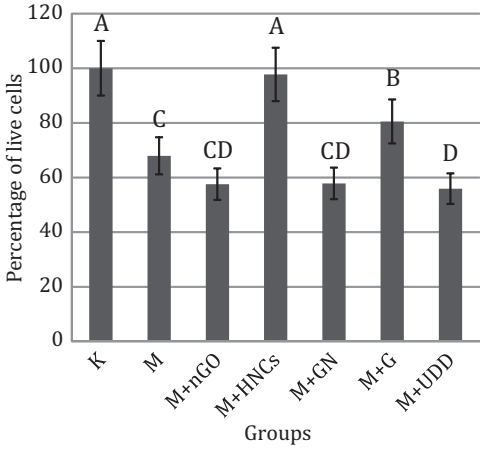

FIGURE 4. Effect of melittin and complexes of nanoparticles with melittin on viability of glioma cells. Columns marked with different letters (A–D) show significant differences between treated groups, they differ at $P = 0.0000$. C – control, M – melittin, M+nGO – melittin with nanographene oxide, M+HNCs – melittin with hierarchical nanoporous carbons, M+GN – melittin with pristine graphene, M+G – melittin with graphite, M+UDD – melittin with ultradispersed diamonds

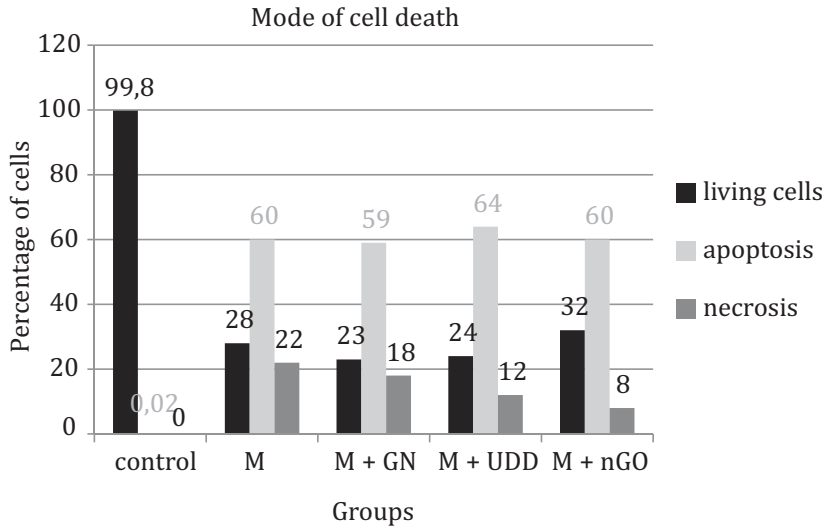

FIGURE 5. Comperation of cell deaths among different groups: C – control, M – melittin, M+GN – melittin with pristine graphene, M+UDD – melittin with nanodiamond, M+nGO – melittin with nanographene oxide

which undergo apoptosis, were in the complex of melittin with UDD, while there was low level of necrosis (12%), what may indicate that UDD could be useful nanocarrier for drug delivery of the substances. The melittin itself added to the cells resulted in more necrotic cells (Fig. 5). The complexes of melittin with GN and nGO gave similar results to UDD. Present results are preliminary and at this stage they indicate that carbon nanoparticles increase the efficiency of absorption of melittin by the cells and cell adhesion. Consequently, a dose of melittin in a drug delivery system may decrease.

CONCLUSION

Results of the work prove the effectiveness of carbon nanoparticles as nanocarriers, especially UDD, GN and nGO. They effectively transport the targeted melittin and help with adhesion of it to the glioma cells. Furthermore, they cause the apoptotic way of cell death. Such complexes can be used in future treatment of cancer, although further *in vivo* experiments are needed.

Acknowledgement

This report was supported by the grant NCN PRELUDIUM 2013/09/N/ /N29/01898.

REFERENCES

AKHAVAN O., GHADERI E., AKHAVAN A., 2012: Size-dependent genotoxicity of graphene nanoplatelets in human stem cells. Biomaterials 33 (32): 8017–8025.

BLANCO E., SHEN H., FERRARI M. 2015: Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nature Biotechnology 33 (9): 941–951.

FIRME C.P. III, BANDARU P.R., 2010: Toxicity issues in the application of carbon nanotubes to biological systems. Nanomedicine 6 (2): 245–256.

GRODZIK M., SAWOSZ E., WIERZBICKI M., ORŁOWSKI P., HOTOWY A., NIEMIEC T., SZMIDT M., MITURA K., CHWALIBOG A., 2011: Nanoparticles of carbon allotropes inhibit glioblastoma multiforme angiogenesis *in ovo*. Int. J. Nanomedicine 6: 3041–3048.

HALEY B., FRENKEL E., 2008: Nanoparticles for drug delivery in cancer treatment Urol. Oncol. 26 (1): 57–64.

JAWORSKI S., SAWOSZ E., KUTWIN M., WIERZBICKI M., HINZMANN M., GRODZIK M., WINNICKA A., LIPIŃSKA L., WŁODYGA K., CHWALIBÓG A., 2015: *In vitro* and *in vivo* effects of graphene oxide and reduced graphene oxide on glioblastoma. Int. J. Nanomedicine 10 (1): 1585–1596.

JI G., WANG H., YAN L., WANG X., PEI R., YAN T., ZHAO Y., GUO X., 2005: Cytotoxicity of Carbon Nanomaterials: Single-Wall Nanotube, Multi-Wall Nanotube and Fullerene. Environ. Sci. Technol. 39 (5): 1378–1383.

JI S.R., LIU C., ZHANG B., YANG F., XU J., LONG J., JIN C., FU D.L., NI Q.X., YU X.J., 2010: Carbon nanotubes in cancer diagnosis and therapy. Biochim. Biophys. Acta 1806 (1): 29–35.

JO M., PARK M.H., KOLLIPARA P.S., AN B.J., SONG H.S., HAN S.B., KIM J.H., SONG M.J., HONG J.T., 2012: Anti-cancer effect of bee venom toxin and melittin in ovarian cancer cells through induction of death receptors and inhibition of JAK2/STAT3 pathway. Toxicol. Appl. Pharmacol. 258 (1): 72–81.

KIM T.W., CHUNG P.W., SLOWING I.I., TSUNODA M., YEUNG E.S., LIN V.S., 2008: Structurally Ordered Mesoporous Carbon Nanoparticles as Transmembrane Delivery Vehicle in Human Cancer Cells. Nano Lett. 8 (11): 3724–3727.

LIN C.W., BACHILO S.M., VU M., BECKINGHAM K.M., BRUCE WEISMAN R., 2016: Spectral triangulation: a 3D method for locating single-walled carbon nanotubes *in vivo*. Nanoscale 8 (19): 10348–10357.

LIU Z., ROBINSON J.T., SUN X., DAI H., 2008: PEGylated nanographene oxide for delivery of water-insoluble cancer drugs. J. Am. Chem. Soc. 130 (33): 10876–10877.

LIU M., WANG H., LIU L., WANG B., SUN G., 2016: Melittin-MIL-2 fusion protein as a candidate for cancer immunotherapy. J. Transl. Med. 14 (1): 155.

PEER D., KARP J.M., HONG S., FAROKHZAD O.C., MARGALIT R., LANGER R., 2007: Nanocarriers as an emerging platform for cancer therapy. Nat. Nanotechnol. 2 (12): 751–760.

SCHWARTZMAN R.A., CIDLOWSKI J.A., 1993: Apoptosis: The Biochemistry and Molecular Biology of Programmed Cell Death. Endocr. Rev. 14 (2): 133–151.

SINGH R., LILLARD J.W jr, 2009: Nanoparticle-based targeted drug delivery. Exp. Mol. Pathol. 86 (3): 215–223.

STANKOVICH S., DIKIN D.A., DOMMETT G.H., KOHLHAAS K.M., ZIMNEY E.J., STACH E.A., PINER R.D., NGUYEN S.T., RUOFF R.S., 2006: Graphene-based composite materials. Nature 442 (7100): 282–286.

SU J., ZHOU X., WANG L., YIN X., WANG Z., 2016: Curcumin inhibits cell growth and invasion and induces apoptosis through down-regulation of Skp2 in pancreatic cancer cells. Am. J. Cancer Res. 6 (9): 1949–1962.

TERWILLIGERT T.C., EISENBERGG D., 1982: The Structure of Melittin, PrinIed in U.S.A. 257 (11): 6016–6022.

TORCHILIN V.P., 2010: Passive and Active Drug Targeting: Drug Delivery to Tumors as an Example. Handb. Exp. Pharmacol. (197): 3–53.

XIAO J., DUAN X., YIN Q., ZHANG Z., YU H., LI Y., 2013: Nanodiamonds-mediated doxorubicin nuclear delivery to inhibit lung metastasis of breast cancer. Biomaterials 34 (37): 9648–9656.

YEZHELYEV M.V., GAO X., XING Y., Al-HAJJ A., NIE S., O'REGAN R.M., 2006: Emerging use of nanoparticles in diagnosis and treatment of breast cancer. Lancet Oncol. 7 (8): 657–667.

ZHOU T., ZHOU X., XING D., 2014: Controlled release of doxorubicin from graphene oxide based charge-reversal nanocarrier. Biomaterials 35 (13): 4185–4194.

ZHU Y., LI J., LI W., YANG X., CHEN N., SUN Y., ZHAO Y., FAN C., HUANG Q., 2012: The biocompatibility of nanodiamonds and their application in drug delivery systems. Theranostics 2 (3): 302–312.

Streszczenie: *Nanocząstki węglowe jako transportery melityny do komórek glejaka IV stopnia linii U87 w modelu* in vitro. Melityna jest jedną z naturalnie występujących substancji w przyrodzie, jest składnikiem jadu pszczelego. Jest cytotoksyczna i ma silne właściwości lityczne, które niszczą każdą błonę komórkową, co może mieć zastosowanie w zwalczaniu komórek nowotworowych. Aby można było ją wykorzystywać w leczeniu, wymagane jest zastosowanie dodatkowego składnika, który pokierowałby ją w odpowiednie miejsce. Celem jest stworzenie systemu kontrolowanego dostarczania leków, z wykorzystaniem nanocząstek węglowych, które mają małe rozmiary oraz są uważane za biokompatybilne. W badaniach użyto pięć rodzajów nanocząstek: grafenu, nanotlenku grafenu, nanodiamentu, grafitu oraz hierarchicznych nanoporowatych nanocząstek. Do wizualizacji powstałego kompleksu nanocząstek z melityną użyto elektronowego mikroskopu transmisyjnego, a do sprawdzenia wpływu melityny oraz jej kompleksu z nanocząstkami na morfologię oraz strukturę komórek użyto elektronowego mikroskopu skaningowego. W celu sprawdzenia żywotności komórek poddanych działaniu melityny oraz jej kompleksów z nanocząstkami wykonano test PrestoBlue™, a w celu specyfikacji drogi śmierci komórek test z jodkiem propidyny i aneksyną V. Wyniki wskazują, że różne rodzaje nanocząstek węglowych mogą w inny sposób oddziaływać z melityną. Kompleksy melityny z nanodiamentem, grafenem oraz nanotlenkiem grafenu spowodowały większą śmiertelność komórek niż sama melityna. Tworzenie oraz zastosowanie w praktyce kompleksów melityny z nanocząstkami węglowymi może skutkować efektywniejszym leczeniem glejaka.

Słowa kluczowe: nowotwory mózgu, nanocząstki, melityna, system kontrolowanego dostarczania leków.

MS received 10.10.2016
MS accepted 31.10.2017

Authors' address:
Paulina Biniecka
Zakład Nanobiotechnologii
Katedra Żywienia i Biotechnologii Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8
02-786 Warszawa
Poland
e-mail: binieckap@gmail.com

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 33–36
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.4

Preliminary studies on the relation between polar vixens' temperament type and the values of selected physiological indicators and cortisol level in the blood serum

MARIAN BRZOZOWSKI[1], LESZEK GACEK[2],
DANUTA DZIERŻANOWSKA-GÓRYŃ[1]
[1] Department of Animal Breeding and Production, Warsaw University of Life Sciences – SGGW
[2] The National Research Institute of Animal Production, Experimental Station Chorzelów

Abstract: *Preliminary study on the relation between polar vixens' temperament type and the values of selected physiological indicators and cortisol level in the blood serum*. The aim of this study was to examine whether there is a correlation between temperament type of polar foxes females, determined with the behavioral tests, and the level of selected physiological indicators and the level of cortisol in blood serum. In the result of conducted studies, there were no differences found between the number of breaths or the number of heartbeats in animals of different temperament types. It was found a higher body temperature in vixens with aggressive temperament compared with the vixens of trustful temperament. The difference was statistically significant (P <0.05). The level of cortisol in the blood serum of aggressive and fearful foxes was higher compared with trustful animals. The observed trend was statistically confirmed (P <0.01). In conclusion it can be said that the study of body temperature and the cortisol level in a blood serum of vixens can be a complementary method to evaluate its suitability as a breeding animals. Full verification of observed dependencies requires confirmation from greater material.

Key words: blue fox, females, temperament, physiological parameters, cortisol

INTRODUCTION

Polar foxes belong to this group of farm animals, which is in the phase of domestication. Lack of full domestication manifest their distrust of human and, consequently, affect the results of use. If we want to achieve better results in this area, we need to know as much about the criteria that allow you to select from breeding individuals guaranteeing the best indicators of use. This criterion is the temperament of animals. Animals trustful, with a balanced temperament are considered best suited to the farm conditions, and the level of physiological parameters (heart rate, respiration rate, body temperature) conforms physiological for this species (Hansen 1993, Pedersen 1996, Mononen et al. 1999). The breeding results of animal distrusted (aggressive, fearful) are worse than trusted (Kaleta 1982, Brzozowski et al. 1999, Gacek 1999, Zoń et al. 1998). On the other hand, Filistowicz et al. (2003) and Przysiecki et al. (2010) in their study found no clear impact of the type of

temperament polar vixen on the results of their reproduction.

The aim of this study was to examine whether there is a correlation between temperament type of polar foxes females, determined with the behavioral tests, and both: the level of selected physiological indicators and the level of cortisol in blood serum. The confirmation of such relationship would give breeders an additional opportunity to identify trustful animals which potentially better suit for breeding herd.

MATERIAL AND METHODS

The study was conducted at The National Research Institute of Animal Production, Experimental Station Chorzelów on polar foxes. Polar fox females at the age of 1–3 years were selected for the study. Using the "test of hand" (Kaleta 1982, Gacek 1999), animals were divided into experimental groups, depending on their temperament:

- A – clear aggressive animals (15 vixens);
- T – clear trustful animals (16 vixens);
- F – clear fearful animals (10 vixens).

The examination was conducted in August during *anestrus* period. The body temperature, the pulse and the number of breaths were measured. The study was conducted on the treatment table. After 3–4 min after the capture of the animal and placed on the treatment table, rectal temperature was measured. The pulse rate, expressed in the number of heart beats per 1 minute, was measured by the touch of the chest under the left front limb. The number of breaths was determined on the movements of the abdominal wall at the inhalation and exhalation of the animal in a minute. Blood samples for determination of cortisol was collected from brachial vein. The concentration of cortisol in the blood serum was tested by radioimmunoassay using a set of cortisol (ORION DIAGNOSTICA) for measurement range of 0–2.000 nmol/l with a sensitivity of 4–7 nmol/l.

The methods based on statistical analysis of variance (SPSS 2006) were used for the evaluation received results.

RESULTS AND DISCUSSION

The obtained results concerning analyzed indicators are presented in the Table.

No significant differences between the heart rate and the number of respiration, depending on females temperament type were observed. The results also indicate the lack of relationship between the estimated parameters and the temperament (Bakken et al. 1994). The parameters are consistent with the physiological norms for that species (Pedersen 1996, Mononen et al. 1999).

It was found a statistically significant difference between the body temperature of aggressive and trustful foxes (Table). The temperature of animals classed as aggressive was higher than trustful of 0.66°C (these differences were statistically significant at $P < 0.05$). Similar relationships were described by Zoń et al. (1998). They also observed an increased body temperature of aggressive foxes relative to the trustful animals.

It was also observed a higher level of cortisol in the blood serum of females classified as aggressive, especially when compared with trustful foxes: this difference was statistically significant at level 0.01. It was also observed statis-

TABLE. The value of selected physiological measurements and cortisol level in blood serum in polar vixens with different temperament

Indicator	Group of vixens					
	aggressive		trustful		fearful	
	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
Heart rate per min	89.13	11.28	83.62	8.59	87.20	11.32
Number of respirations per min	39.20	5.00	39.62	4.08	39.00	5.68
Body temperature (°C)	40.57[a]	0.79	39.91[a]	0.56	40.22	0.78
Cortisol level (nmol/l)	119.53[A]	38.77	74.19[AB]	18.33	108.31[B]	26.74

a – statistical differences significant at level $P < 0.05$.
A, B – statistical differences significant at level $P < 0.01$.

tically significant difference between trustful and fearful females ($P < 0.01$). The observed results confirms thesis presented by Kowalski (1996), that the fearful animals have an increased levels of corticoids in blood serum. Aggressive and fearful animals are considered to be less adapted to the farm environment and adaptive processes do not run with them in an optimal way (Kowalski 1998).

The values obtained are within the norms considered to physiologically correct for the blue foxes, comprised between values of 49.9 and 140.9 nmol/l (Rekila 1997, Mononen et al. 1999). According to these authors, the difference in the level of cortisol may be related not with the temperament, but with seasonal changes in animal physiology.

CONCLUSION

To summarize it can be concluded that the obtained results indicate the existence of relationship between foxes temperament, specified by using behavioral tests, body temperature and the level of cortisol in the blood when comparing foxes aggressive and fearful with trustful.

The applied parameters may be useful as an additional method for assessing temperament foxes; however, full verification of the observed dependences requires confirmation from greater material.

Acknowledgement

PhD thesis carried out under the grant PO6E-045-15.

REFERENCES

BAKKEN M., HARRI M., JEPPESEN L., 1994: Production conditions, behaviour and welfare of farm foxes. Scientifur 18 (4): 233–248.

BRZOZOWSKI M., FRINDT A., SUCHODOLSKA A., KUDERSKA E., 1999: Próba określenia zależności między typami zachowań samic lisów polarnych a wynikami ich rozpłodu. Zesz. Nauk. Prz. Hod. PTZ 42, 303–309.

FILISTOWICZ A., PRZYSIECKI P., SYTA M., WIERZBICKI H., 2003: Wpływ bojaźliwego i ufnego zachowania samic na wyniki użytkowania rozpłodowego. Polski Hodowca Zwierząt Futerkowych 3 (4): 9–11.

GACEK L., 1999: The proposal of a new behavioral test for the Polar fox. Emphatic test. Scientifur 23 (3): 201–205.

HANSEN S., 1993: Stress and behavioural biology research in fur animals at the Natinal Ins. of Anim. Sci. Denmark. Annual report NIAS: 73–79.

KALETA T., 1982: Behawior agonistyczny samców lisów srebrzystych a ich użytkowość rozpłodowa. Rozprawa doktorska, SGGW [manuscript].

KOWALSKI A., 1996: Behawioralne i hormonalne wskaźniki adaptacyjne u szczurów i świń. Acta Acad. Agricult. Techn. Olsten. Vet. 22, Supl. C.

KOWALSKI A., 1998: Poziom hormonów stresowych a zmiany w behawiorze zwierząt podczas procesów adaptacyjnych. Nowa Wet. 1: 45–52.

MONONEN J., HARRI M., REKILA T., KASANEN S., NURMNIEN L., AHOLA L., 1999: Some correlations between behaviour, physiology and growth in juvenile male blue foxes. Zesz. Nauk. PTZ 42: 233–244.

PEDERSEN V., 1996: Combined behavioural and physiological measurements as a basis of the assessment of animal welfare. Acta. Agric. Scand. Sect. A, Anim. Sci., Suppl. 27: 69–75.

PRZYSIECKI P., NOWICKI S., NAWROCKI Z., FILISTOWICZ A., OTULAKOWSKI G., FILISTOWICZ A., 2010: Użytkowość reprodukcyjna samic lisa polanego (*Alopex lagopus*) o różnym typie zachowań. Aparatura Badawcza i Dydaktyczna 1: 39–44.

REKILA T., JALKANEN L., PYYKONEN T., HARRI M., 1997: Seasonal rhythm in serum cortisol in blue foxes. Scientifur 21 (4): 308.

ZOŃ A., SŁAWOŃ J., KALETA T., 1998: Próba określenia przydatności różnych testów behawioralnych w selekcji lisów niebieskich. „Nauka w Polskiej Zootechnice XXI w.". Sympozjum Naukowe. AR Lublin 10–11.09.1998: 315–316.

Streszczenie: *Wstępne badania nad zależnością między temperamentem samic lisów polarnych a wartościami wybranych wskaźników fizjologicznych i poziomem kortyzolu w surowicy krwi.* Celem badań była próba określenia, czy istnieje zależność między typem temperamentu lisic polarnych, określonym na podstawie testów behawioralnych, a poziomem wybranych wskaźników fizjologicznych i poziomem kortyzolu w surowicy krwi. W wyniku przeprowadzonych badań nie zaobserwowano różnic między liczbą oddechów i tętnem u samic o różnym typie temperamentu. Stwierdzono wyższą temperaturę ciała u samic o agresywnym typie temperamentu w porównaniu do samic ufnych (różnica istotna na poziomie P <0,05). Poziom kortyzolu w surowicy krwi u lisów agresywnych i bojaźliwych był znacznie wyższy w porównaniu do zwierząt ufnych (różnica istotna na poziomie P <0,01). Podsumowując badania, można stwierdzić, że określenie temperatury ciała oraz poziomu kortyzolu w surowicy krwi lisic może być uzupełniającą metodą oceny przydatności samic jako zwierząt hodowlanych. Pełna weryfikacja zaobserwowanych tendencji wymaga ich potwierdzenia w badaniach na liczniejszym materiale.

Słowa kluczowe: lisy polarne, samice, temperament, parametry fizjologiczne, kortyzol

MS received 28.08.2016
MS accepted 13.03.2017

Authors' address:
Marian Brzozowski
Katedra Szczegółowej Hodowli Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8
02-786 Warszawa
Poland
e-mail: marian_brzozowski@sggw.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 37–42
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.5

Interaction of hierarchical nanoporous carbons (HNCs) with chicken embryo red blood cells (RBC)

ŻANETA BUGAJSKA, SŁAWOMIR JAWORSKI, KAROLINA DANILUK, PAULINA BINIECKA, MALWINA SOSNOWSKA
Department of Animal Nutrition and Biotechnology, Warsaw University of Life Sciences – SGGW

Abstract: *Interaction of hierarchical nanoporous carbons (HNCs) with chicken embryo red blood cells (RBC).* The purpose of this study was to characterize toxicity of hierarchical nanoporous carbons (HNCs) on chicken embryo red blood cells (RBC), which are a perfect model to adapt the hemolysis assay in evaluation of the *in vitro* blood compatibility of nanoparticles. The samples of blood were treated with different concentration of HNCs (10, 50 and 100 μg/ml). Hemolysis assay showed that the hemolytic activity depends on the dose of HNCs. The microscope observations have shown a difference in morphology between treated and untreated RBC: changes in cell membrane shape and the occurrence of pathological erythrocytes forms.

Key words: hierarchical nanoporous carbon, nanoparticles, red blood cells, toxicity

INTRODUCTION

The nanoparticles of carbon allotropes have recently been widely investigated due to their *in vitro* and *in vivo* biological activity. The results of experiments on diverse carbon nanomaterials have shown that their different behavior in living organism may be useful in all biology and medicine fields, including areas such as drug/gene delivery (Feng et al. 2011, Wu et al. 2014), bioimaging, biosensing (Shen et al. 2012), antibacterial materials (Hu et al. 2010), and cancer therapy (Farokhzad et al. 2006, Cho et al. 2008). In one of the studies appeared that carbon nanotubes are needle-like potential carriers of bioactives including drug, genes and proteins. Moreover, functionalized nanotubes are more soluble, biocompatible, and have greater potential for attaching certain molecules and targeting them into cancer cells (Torchilin 2011, Mody et al. 2014). Another promising carbon nanoparticle is graphene. Flakes or a surface of graphene may be used as bioactive molecules (Sawosz et al. 2014). Furthermore, the nanodiamonds have a great biocompatibility but they are still a foreign non-degradable material for biological organisms (Zhu et al. 2012).

The new nanoparticles synthetized in the Military University of Technology in Warsaw called hierarchical nanoporous carbons (HNCs) are carbon allotropes, which are the first nanoparticles occurring in a cubic shape. The HNCs are closed-cage nanoparticles with really thick walls such as those of a single graphenepetal. The structure of HNCs is analogous to the structure of fullerenes – other carbon nanoparticles, which

were already tested for their anticancer properties. The difference in the structure revealed that the HNCs contain mixture of magnesium and oxalic acid powders (MgO) (Dyjak et al. 2016).

The main parameters for the biocompatibility of carbon nanoparticles are hemolytic properties and interactions with red blood cells (RBC) thus they have a great potential in drug delivery systems (Mocan 2013). The erythrocyte model is much closer to the physiological condition of living cells and their results are more accredited to be the foundation of the *in vivo* study. Therefore, we decided to choose chicken erythrocytes since on the contrary to the human ones, they contain a nuclear (Zhang et al. 2014). The proper assessment of potential toxic effects of carriers drug/gene delivery is important forasmuch almost every platform in such systems requires intravenous administration. In the use of nanoparticles in the drug delivery, it is necessary to determine the toxicity of the RBC. Most of the biomedical applications of nanoparticles require intravenous administration, which enables them to interact with RBC and other immune cells. Therefore, hemocompatibility assays are of utmost importance (Pan et al. 2016).

MATERIAL AND METHODS

Preparation and characterization of hierarchical nanoporous carbons (HNCs)

Hierarchical nanoporous carbons (HNCs) synthetized in the Military University of Technology (Warsaw, Poland). Shape and size of HNCs were evaluated using a JEM-2000EX transmission electron microscope (TEM) at 80 keV (JEOL Ltd, Tokyo, Japan). The samples for TEM were prepared by placing hydrocolloid droplets into formvar-coated copper grids (Agar Scientific, Stansted, UK). The test was performed in triplicate. Zeta potential was measured in milli-Q water by a ZEN3500 Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Prior to application, the carbon nanoparticles were dispersed in phosphate buffered saline (PBS) to prepare the following concentrations: 10, 50, and 100 μg/ml. The solutions were then sonicated for 30 min.

Embryo model

Fertilized eggs (*Gallus gallus*, n = 15) from Hubbard Flex Line hens were obtained from a commercial hatchery (Dembówka, Poland). After 19 days of eggs incubation (temperature 37°C, 70% humidity, turning once per hour), the embryos were immediately decapitated while blood samples were collected from the jugular vein. Blood samples were divided into the following groups: control untreated (0% hemolysis), positive control treated with 3% hydrogen peroxide (100% hemolysis), HNCs 10 μg/ml, HNCs 50 μg/ml, HNCs 100 μg/ml, hydrocolloids diluted in PBS. The samples were placed in Vacutainer tubes (BD Inc., Franklin Lakes, NJ, USA) containing ethylenediaminetetraacetic acid (EDTA), gently mixed on a rotary shaker, and incubated for 3 h at 37°C. The incubation time was based on Asharani et al. (2010). All measurements were performed eight times.

Blood cell morphology

Blood cell morphology was investigated using light microscopy and transmission

electron microscopy (TEM). Peripheral blood smears were prepared using 5 µl of whole blood, air-dried, stained peripherally with May–Grünwald–Giemsa, and examined at a magnification of ×1.000 (Leica DM750, Leica Microsystems, Nussloch, Germany). For the TEM examination, the blood samples were fixed in 2.5% glutaraldehyde and centrifuged at 1,200 rpm. The supernatant was discarded, RBCs were dispersed in deionized water. The samples for TEM were prepared by placing hydrocolloid droplets into formvar-coated copper grids. The test was performed in triplicate.

Hemolytic assay

The hemolysis assay was performed with embryo whole blood. After incubation, the tubes containing blood samples: control untreated (0% hemolysis), positive control treated with 3% hydrogen peroxide (100% hemolysis), HNCs 10 µg/ml, HNCs 50 µg/ml, HNCs 100 µg/ml were centrifuged for 10 min at 1,200 rpm. The absorbance of the supernatant, which includes plasma and lysed erythrocytes, was measured at 540 nm (Infinite M200, Tecan, Durham, NC, USA). Percent hemolysis was determined as compared to Shiny et al. (2014).

Statistical analysis

Statgraphics Centurion software (StatPoint Technologies, Warrenton, VA, USA) was used for the statistical analysis. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. Values of P below 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Red blood cells from a chicken embryo are a model that allows high precision in evaluating the toxicity. Moreover, they allow to prove the hemocompatibility (Zhang et al. 2014). With increasing concentration of HNCs (ζ = –28) a higher hemolytic activity was observed (Fig. 1). In contrast to the control group it increased at 10 µg/ml to 8%, at 50 µg/ml to 30% and the percentage of hemolysis was the highest (36%) at a concentration of 100 µg/ml. Both the lack of hemolysis

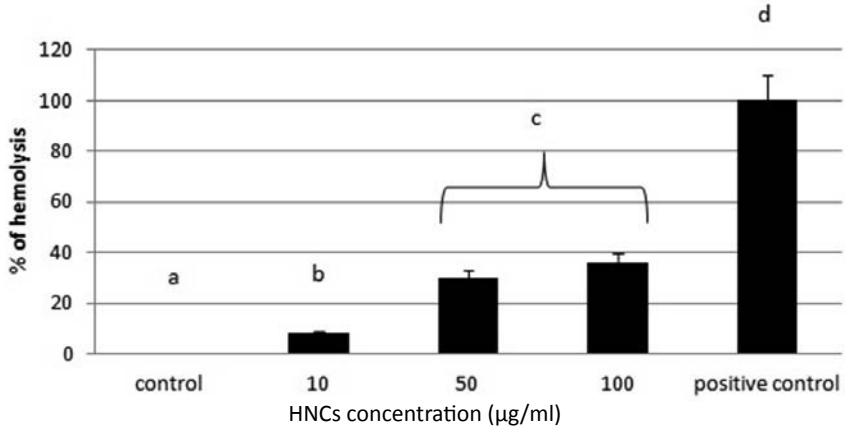

FIGURE 1. The hemolytic effect on chicken embryo blood exposed to HNCs in 10, 50 and 100 µg/ml concentration. The columns with different letters (a–d) indicate significant differences (P <0.001)

in the control group and an almost 100% hemolysis rate in the positive control group treated with 3% solution of the hydrogen peroxide, confirmed the accuracy. HNCs exhibited dose-dependent hemolytic activity towards RBC. Compared to other studies of silica nanoparticle, the size and the shape could be another factors causing induced hemolysis other than the concentration (Yu et al. 2011).

The microscope observations have shown a difference in morphology between control and treated samples with HNCs (Figs 2, 3). The membranes of RBC were disintegrated. The pictures of samples, which were treated with HNCs, are comparable to positive control. There were differentiations in the shape of cells, which were deformed. Cells also lost their biconcavity. The observation showed that the increasing level of swollen cells and other pathological forms of erythrocytes, such as echinocytes and knizocytes, depends on the degree of hemolysis (Lim et al. 2002). The most pathological forms occurred at 100 µg/ml concentration of HNCs. Moreover, all the HNCs treated groups showed the presence of ghost cells, which are the result of cell lysis. In the pictures of treated samples, the RBC had lost their typical discoid-shape and the degradation of the membranes was clearly visible. Therefore, further studies

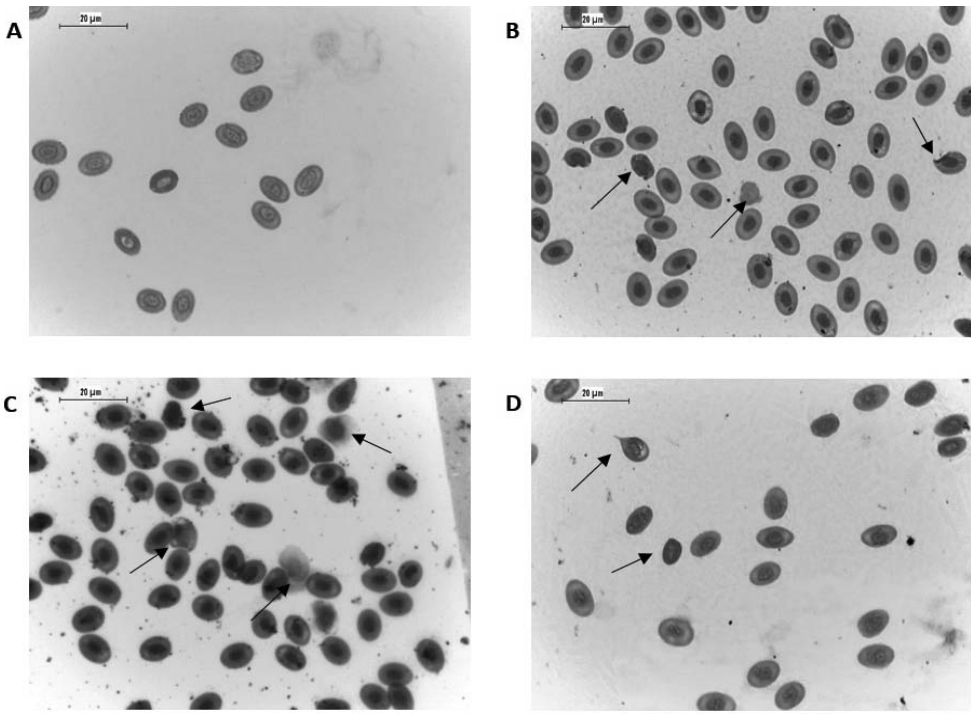

FIGURE 2. Results of blood smears stained peripherally with May–Grünwald–Giemsa from light microscope. A – control, B – sample treated with 50 µg/ml HNCs, C – sample treated with 100 µg/ml HNCs, D – sample treated with 10 µg/ml HNCs. Arrows indicate pathological forms of erythrocytes

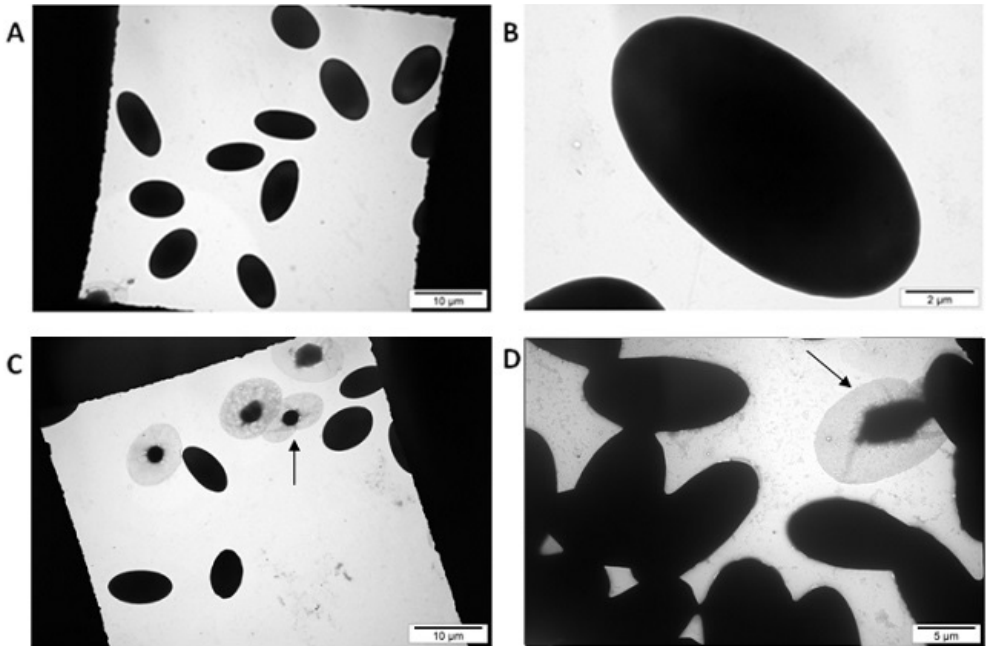

FIGURE 3. Pictures from TEM. A, B – control, C, D – samples treated with 50 µg/ml HNCs. Arrows indicate ghost cells

should concentrate on reducing the dose and finding other than intravenous ways of administration of the nanoparticles.

CONCLUSION

Treatment with HNCs damages the membrane in RBC differentiation: alters the shape and causes the occurrence of pathological forms such as echinocytes and knizocytes. Cell lysis results in the presence of multiple ghost cells. In the conducted study, HNCs exhibited dose--dependent hemolytic activity towards RBC.

Acknowledgement

Grant NCN PRELUDIUM 2013/09/N/ /N29/01898.

REFERENCES

ASHARANI P.V., SETHU S., VADUKUMPULLY S., ZHONG S., LIM C.T., HANDE M.P., VALIYAVEETTIL S., 2010: Investigations on the structural damage in human erythrocytes exposed to silver, gold, and platinum nanoparticles. Adv. Funct. Mater. 20: 1233–1242.

CHO K., WANG X., NIE S., CHEN ZG., SHIN DM., 2008: Therapeutic nanoparticles for drug delivery in cancer. Clin. Cancer Res. 14 (5): 1310–1316.

DYJAK S., KICIŃSKI W., NOREK M., HUCZKO A., ŁABĘDŹ O., BUDNER B., POLAŃSKI M., 2016: Hierarchical, nanoporous graphenic carbon materials through an instant, self-sustaining magnesiothermic reduction. Carbon 96: 937–946.

FAROKHZAD O.C., CHENG J., TEPLY BA., SHERIFI I., JON S., KANTOFF P.W., RICHIE J.P., LANGER R., 2006: Targeted nanoparticle-apatamer bioconjugates for cancer chemotherpay *in vivo*. Proc. Natl. Acad. Sci. USA 103 (16): 6315–6320.

FENG L., ZHANG S., LIU Z., 2011: Graphene based gene transfection. Nanoscale 3: 1252––1257.

HU W., PENG C., LUO W., LV M., LI X., LI D., HUANG Q., FAN C., 2010: Graphene-based antibacterial paper. ACS Nano 4 (7): 4317–4323.

LIM H.W.G., WORTIS M., MUKHOPADHYAY R., 2002: Stomatocyte–discocyte–echinocyte sequence of the human red blood cell: Evidence for the bilayer-couple hypothesis from membrane mechanics. Proc. Natl. Acad. Sci. USA 99 (26): 16766–16769.

MOCAN T., 2013: Hemolysis as expression of nanoparticles-induced cytotoxicity in red blood cells. BMBN 9: 7–12.

MODY N., TEKADE R.K., MEHRA N.K., CHOPDEY P., JAIN N.K., 2014: Dendrimer, Liposomes, Carbon Nanotubes and PLGA Nanoparticles: One Platform Assessment of Drug Delivery Potential. AAPS PharmSciTech 15 (2): 388–399.

PAN D., VARGAS-MORALES O., ZERN B., ANSELMO A.C., GUPTA V., ZAKREWSKY M., MITRAGOTRI S., MUZYKANTOV V., 2016: The Effect of Polymeric Nanoparticles on Biocompatibility of Carrier Red Blood Cells. PLOS ONE 11 (3): e0152074.

SAWOSZ E., JAWORSKI S., KUYWIN M., HOTOWY A., WIERZBICKI M., GRODZIK M., KURANTOWICZ N., STROJNY B., LIPIŃSKA L., CHWALIBOG A., 2014: Toxicity of pristine graphene in experiments in a chicken embryo model. Int. J. Nanomedicine 9: 3913–3922.

SHEN H., ZHANG L., LIU M., ZHANG Z., 2012: Biomedical applications of graphene. Theranostics 3: 283–294.

SHINY P.J., MUKHERJEE A., CHANDRASEKARAN N., 2014: Haemocompatibility assessment of synthesised platinum nanoparticles and its implication in biology. Bioprocess Biosyst. Eng. 9: 9911–9997.

TORCHILIN V., 2011: Tumor delivery of macromolecular drugs based on the EPR effect. Adv. Drug. Del. Rev. 63: 131–135.

WU S., ZHAO X., CUI Z., ZHAO C., WANG Y., DU L., LI Y., 2014: Cytotoxicity of graphene oxide and graphene oxide loaded with doxorubicin on human multiple myeloma cells. Int. J. Nanomedicine 9: 1413–1421.

YU T., MALUGIN A., GHANDEHARI H., 2011: Impact of silica nanoparticle design on cellular toxicity and hemolytic activity. ACS Nano 5 (7): 5717–5728.

ZHANG J., HOU X., AHMAD H., ZHANG H., ZHANG L., WANG T., 2014: Assessment of free radicals scavenging activity of seven natural pigments and protective effects in AAPH-challenged chicken erythrocytes. Food Chem. 145: 57–65.

ZHU Y., LI J., LI W., ZHANG Y., YANG X., CHEN N., SUN Y., ZHAO Y., FAN C., HUANG Q., 2012: The Biocompatibility of Nanodiamonds and Their Application in Drug Delivery Systems. Theranostics 2 (3): 302–312.

Streszczenie: *Interakcja hierarchicznych nanoporowatych nanocząstek węglowych (HNCs) z czerwonymi krwinkami zarodka kury.* Celem tego badania było określenie toksyczności hierarchicznych nanoporowatych nanocząstek węglowych (HNCs) wobec krwinek czerwonych, pozyskanych z zarodka kury (RBC). Są one idealnym modelem do oceny testu hemolizy w badaniach zgodności nanocząstek w warunkach *in vitro*. Do próbek krwi zarodka kurzego zostały dodane HNCs w różnych stężeniach (10, 50 i 100 µg/ml). Test hemolizy wykazał, że aktywność hemolityczna zależy od dawki HNCs. Podczas obserwacji mikroskopowych zaobserwowano różnice w morfologii między traktowanymi HNCs a nietraktowanymi RBC. Stwierdzono zmianę kształtu błony komórkowej krwinek i występowanie patologicznych form erytrocytów.

Słowa kluczowe: hierarchiczne nanoporowate nanocząstki węglowe, nanocząstki, czerwone krwinki, toksyczność

MS received 10.10.2016

MS accepted 27.03.2017

Authors' address:
Żaneta Bugajska
Zakład Nanobiotechnologii
Katedra Żywienia i Biotechnologii Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8
02-786 Warszawa
Poland
e-mail: zaneta.bugajska@gmail.com

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 43–51
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.6

Influence of melittin on viability and integrity of cell membrane on grade IV glioma

KAROLINA DANILUK, SŁAWOMIR JAWORSKI, PAULINA BINIECKA, ŻANETA BUGAJSKA, MALWINA SOSNOWSKA
Department of Animal Nutrition and Biotechnology, Warsaw University of Life Sciences – SGGW

Abstract: *Influence of melittin on viability and integrity of cell membrane on grade IV glioma.* The grade IV glioma is one of the malignant human tumours. Today there is no effective treatment for this type of cancer. Alternative methods are sought-after in glioma treatment, and lately melittin has been found to be useful in anticancer therapy. The aim of the study was to investigate the effect of melittin on the viability and the integrity of cell membranes of the grade IV glioma cells. The U87 glioma line cells were treated of melittin in increasing concentrations (5, 10, 15, 20 and 50 μg/mL) and incubated for 24 h. After incubation, the tests were performed in order to investigate the cell morphology, cell viability, membrane integrity and the way of cell death. The results have shown the devastating effect of melittin on the glioma cells. The melittin causes disintegration of cell membranes and induces cell death by apoptosis and less by necrosis.

Key words: glioma, melittin, toxicity, apoptosis

INTRODUCTION

Melittin is the main toxic element of the bee poison. It is composed of 26-amino acid and is an alfa-helical polypeptide with amphipathic character (C-end is hydrophilic and N-end is hydrophobic) (Raghuraman and Chattopadhyay 2007). The peptide has hemolytic proprieties and attacks every one of lipid membranes (Hoskin et al. 2008). Effects of the polypeptide consist of the destruction of lipid membrane on the physical and chemical level (Lee et al. 2004).

The interaction between melittin and lipid membranes is possible by the exchange of melittin conformation. Four alfa-helical monomers connect to each other and form tetramers. The form of tetramers inside cell membranes creates the ion channels which modify cell membranes permeability (Tosteson and Tosteson 1981). Initially, melittin monomers stick parallel to the lipid membrane then, as tetramers, change the location on the perpendicular and made pores inside the cell membranes, leading to the cell death.

Melittin also activates phospholipase and calmodulin by facilitating phospholipase A_2 (Mollay et al. 1974) and bonds degradation inside lipid membranes (Keith et al. 2010). Traditional medicine used the melittin against many diseases like rheumatism, chronic infections, even cancer. Some research has shown that major element of the bee venom, melittin, has also anti-

microbial activities. Many studies have demonstrated that melittin has strong anticancer activity (Gajski and Garaj-Vrhovac 2013). The study conducted on the colon, gastric, lung, prostate and ovarian cancer cells gave satisfactory results (Huh et al. 2012, Jo et al. 2012, Park et al. 2012, Zheng et al. 2015, Kong et al. 2016).

Grade IV glioma is a malignant brain tumour which comes from glial cells (www.abta.org). According to World Health Organization (WHO), it has the highest grade of malice. It is the most common malignant brain tumour and also one of the most mortal human tumours. Most-ly, treatment of glioma begins with surgery, but this is a difficult and risky procedure. This tumour can revive from the single cells, so innovative therapies are needed to treat glioma. In 2013 published research suggested that melittin may be useful in the anticancer treatment (Shin et al. 2013). Melittin as a factor perforating the cell membrane may cause damage to the cell membranes of grade IV glioma. The aim of the study was to investigate the effect of melittin on the viability and the integrity of the outer cell membranes and the mitochondrial membranes using the cell line U87 as a model.

MATERIAL AND METHODS

Cell cultures

Human glioma cell line U87 and was obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's culture medium containing 10% fetal bovine serum (Life Technologies, Houston, TX, USA), 1% penicillin and streptomycin (Life Technologies) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in a NuAire DH Auto Flow CO_2 Air-Jacketed Incubator (Plymouth, MN, USA).

Preparation of melittin

The melittin was obtained from Sigma Aldrich (Munich, Germany). The melittin powder was solubilising in ultrapure water to prepare stock 1.0 mg/mL solution. The solution was diluted to different concentrations (5, 10, 15, 20, 50 mg/L) with 1× Dulbecco's modified Eagle's culture medium (Life Technologies, Houston, TX, USA) immediately prior to exposure to the cells.

Cell morphology

U87 cells were plated in six-well plates (1×10^5 cells per well) and incubated for 24 h. Cells cultured in medium without the addition of melittin were used as the control. Melittin was introduced to the cells at increasing concentrations (5, 10, 15, 20 and 50 μg/mL). The same procedure was repeated during following tests. Cell morphology was recorded using an optical microscope (Olympus CKX4, Poland) at 24-hour post-exposure.

Cell viability

Cell viability was evaluated using PrestoBlue® Cell Viability Assay (Life Technologies, Taastrup, Denmark). The reagent added to cells, is reduced by metabolically active cells and turns red in colour, allowing a quick measure of viability and cytotoxicity. U87 cells were incubated on 96-well plates (5×10^3 cells per well) for 24 h. After incubation, the medium was removed and melittin

solutions (5, 10, 15, 20 and 50 μg/mL) were added to the wells at increasing concentration (90 μl per well) and the plates were incubated for an additional 24 h. Cells incubated without melittin were used as a control group. In the next step, 10 μl of PrestoBlue™ Reagent was added directly to cells in culture medium and incubated for 2 h at 37°C. After incubation the optical density of each well was recorded at 570 nm on an enzyme-linked immunosorbent assay reader (Infinite M200, Tecan, Durham, NC, USA). Cell viability was expressed as the percentage (OD-test × 100%) / / (OD-control, where "OD-test" is the optical density of cells exposed to melittin and "OD-control" is the optical density of the control sample.

Membrane integrity

A lactic dehydrogenase (LDH) test (LDH-based in vitro toxicology assay kit, Sigma-Aldrich) was used to evaluate cell membrane integrity. This test is based on enzymatic reactions resulting in a coloured product determined spectrophotometrically. U87 cells were plated in 96-well plates (5×10^3 cells per well) and incubated for 24 h. In the next step, the medium was removed and melittin solutions were added to the cells at increasing concentration. After 24 h of incubation, half of the volume of the culture medium was removed and added LDH assay mixture. A final volume for LDH assay was a 100 μl per well. The plate was incubated for 20 min without light at room temperature. The OD was detected as outlined and the LDH leakage was expressed as the percentage of OD using a formula: 100 – (OD-control / / OD-test) × 100%.

Mitochondrial transmembrane potential

Mitochondrial Transmembrane Potential Apoptosis Detection Kit (Abcam, Cambridge, UK) was used to test mitochondrial transmembrane potential as an indicator of cell death. In this test U87 glioma cells were plated in six-well plates (1×10^5 cells per well) and incubated for 24 h. In the next step, the medium was removed and melittin solutions were added to the cells at increasing concentration and were incubated for 24 h. Cells cultured in medium without the addition of melittin were used as the control. The cells were harvested and suspended in 1 ml of the diluted MitoCapture solution. After 20 min of incubation with the reagent in 37°C and 5% CO_2 mitochondrial permeability was analysed using fluorescence microscope (Olympus CKX41, Poland) 24 h after exposure. MitoCapture that has aggregated in the mitochondria of healthy cells fluoresces red, MitoCapture cannot accumulate in mitochondria, it remains as a monomer in the cytoplasm, and fluoresces green.

Apoptosis/necrosis assay

An annexin V/PI assay (Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit with AlexaFluor 488 Annexin V and propidium iodide (PI) for flow cytometry, Life Technologies) was performed to test way of cells death. After 24 h incubation of U87 glioma cells in 75 ml flasks (1×10^6 cells per flask), the medium was removed, and melittin solutions were added at 20 μg/mL. After further 24 h incubation, the medium was removed and the cells were washed in

ice-cold PBS. Harvested cells were suspended in 100 µl annexin-binding buffer (Invitrogen, Carlsbad, CA, USA) and subsequently, 5 µl of annexin V linked with Alexa Fluor 488 and 1 µl of PI were added (Invitrogen, Carlsbad, CA, USA). Cells were analysed using FACStrak (Becton-Dickinson, Germany; software – SimulSet), measuring the fluorescence emission at 530 nm and 575 using excitation at 488 nm.

Statistical analysis

Data were analysed using multifactorial analysis of variance with Statgraphics® Plus 4.1 (StatPoint Technologies, Warrenton, VA, USA). The differences between groups were tested using Tukey's multiple range tests. All mean values are presented with the standard deviation.

RESULTS AND DISCUSSION

In this study, we evaluated the influence of melittin on the morphology, viability, and cell membrane integrity of glioblastoma cells. Additionally, apoptosis and necrosis were evaluated. Glioma cells were treated with melittin in increasing concentration (5, 10, 15, 20 and 50 µg/mL). Figure 1 shows representative images from the optical microscope. We observed a significant difference between control group and cells treated with 15 µg/mL. After incubation with melittin U87 glioma cells changed their morphology. Their protrusions were thinner in comparison with the control cells. Moreover, they collapsed similarly to ovarian cancer cells (Bei et al. 2015). However, they used lower concentration (3 µM ~ 8.5 µg/mL) of melittin, thus glioma cells are probably more resistant to bee venom influence.

Zhang et al. (2014) suggested that melittin has a toxic influence in higher concentration on cancer cells and toxicity is dose-dependent. Increased concentrations of melittin resulted in decreased vitality on glioma cells. The viability was reduced to 51.6% followed by 15 µg/mL of melittin and the lowest viability was observed at the concentration of 50 µg/mL, i.e. 19.5% (Fig. 2). However, the difference between control and treated cells was significant at 10 µg/mL. Melittin due to the ability to form pores in lipid membranes can perforate a cell membrane of grade IV glioma. Cells whose integrity of outer membrane was interrupted may be directed to the death or have reduced metabolic activity.

Previous studies proved that melittin inhibits proliferation of human glioma cells and causes cell death in high concentration (Yang et al. 2007). In this study, membrane integrity was monitored by LDH assay. Melittin destabilizes cell membrane functionality and integrity, and there were significant differences between melittin-treated groups (Fig. 3). Melittin had the highest toxicity at concentrations of 20 and 50 µg/mL, with membrane disintegration of 72 and 67% respectively. Several studies show that melittin has the ability to perforation of lipid membranes and our results are consistent with this. Melittin also destroys the inner cell membranes, especially mitochondrial (Dombrowski et al. 2012).

The pictures from the fluorescence microscope (Fig. 4) show that control group has a lot of active mitochondria what is seen as lighter areas, marked letter R. It corresponds to the red colour on the fluorescence microscope images.

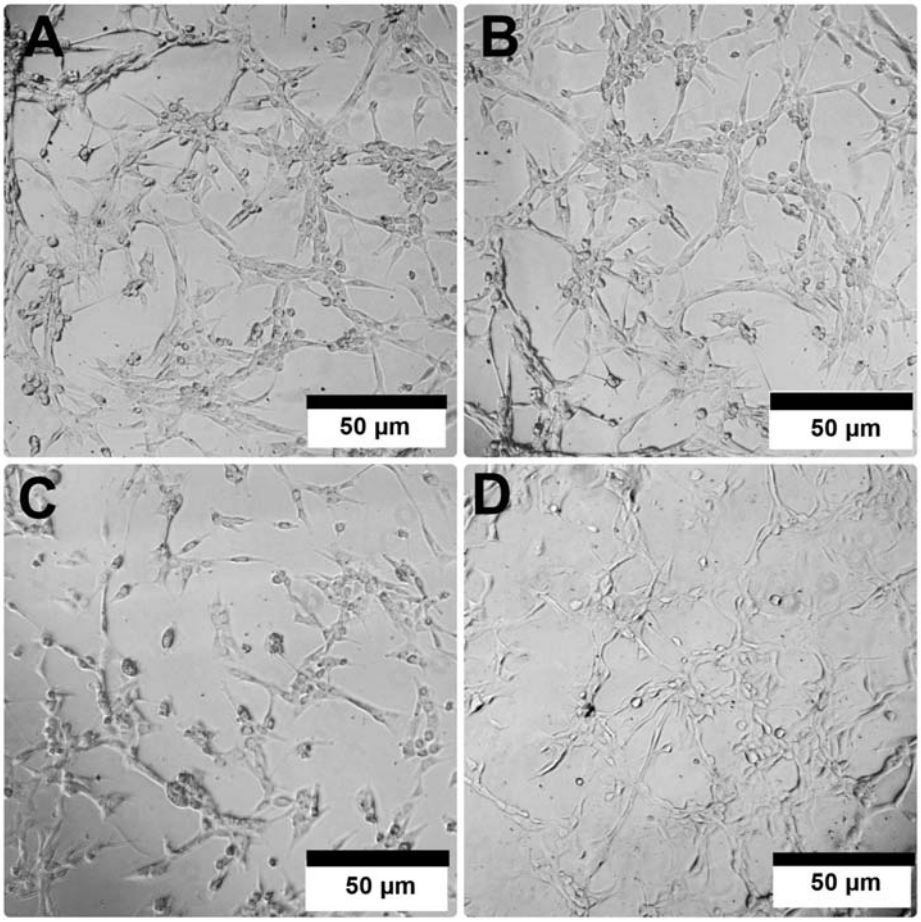

FIGURE 1. Optical microscopy images of melittin-treated and untreated U87 glioma cells. A – control; B – cells treated 5 µg/mL of melittin; C – cells treated 15 µg/mL of melittin; D – cells treated 50 µg/mL of melittin

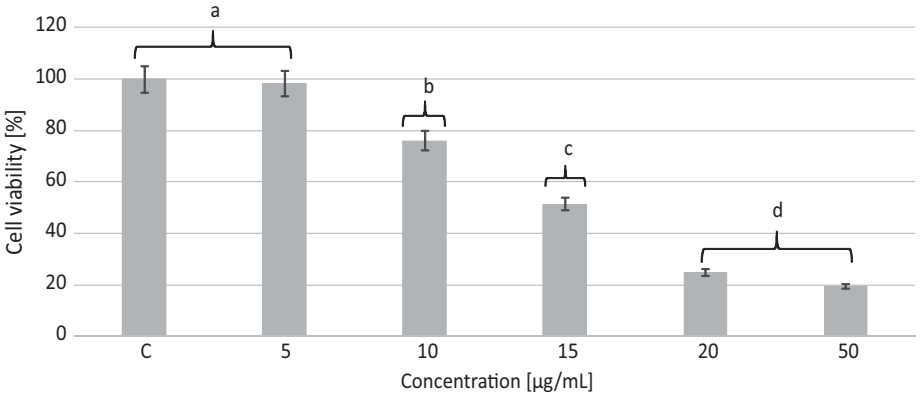

FIGURE 2. Effect of melittin, on the viability of U87 glioma cells. C – untreated cells, control group. The columns with different letters (a–d) indicate significant differences between groups (P <0.001)

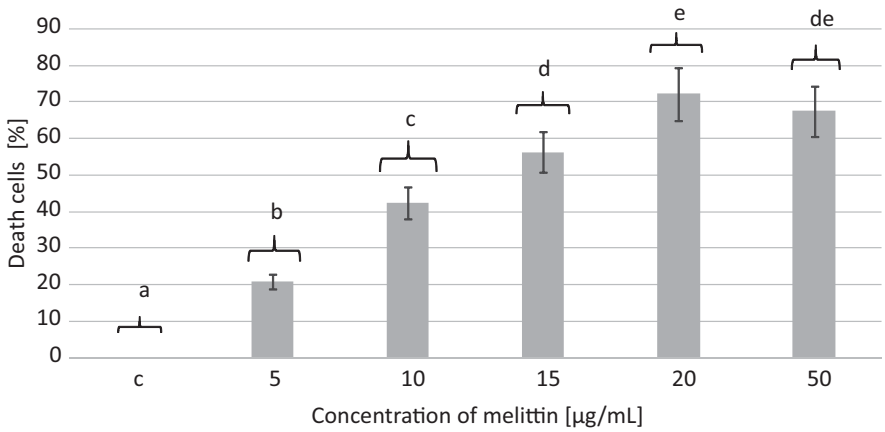

FIGURE 3. Effect of melittin on the mortality of U87 glioma cells. C – untreated cells, control group. The columns with different letters (a–e) indicate significant differences between groups (P <0.001)

The melittin-treated cells have much less active mitochondria. On the Figure 4b are noticeable mainly darker areas, marked letter G, corresponding to the green colour on the fluorescence microscopy. As melittin has the ability to perforation of mitochondrial membranes, it may induce the intrinsic apoptosis pathway, however, the cancer cells are the cell type in which very rarely death occurs by apoptosis (Wong 2011). This constitutes one of the main problems in the design of anticancer therapy. Kong et al. (2016) reported that melittin induces apoptosis in human gastric cancer cell. Jo et al. (2012) proved that melittin-induced death by apoptosis in ovarian cancer cells. In this study, the way of glioma cell death was examined by melittin. The previous studies showed that melittin induces apoptosis by intrinsic pathway (Kong et al. 2016). We received the similar results using a flow cytometer. Figure 5 shows results from the apoptosis assay. Melittin induced apoptosis to a substantial degree in U87 glioma cells (60%). The degree of necrosis was 22% only. This is a desirable effect because necrosis induces inflammation, which is not present during apoptosis (Stępień 2007). Apoptosis has two well-known pathways: intrinsic and extrinsic. The mechanism of interactions of melittin with cell membrane may induce the outer pathway of apoptosis (LDH assay). However, our research shows that mitochondrial pathway is passible too (Fig. 4). On the picture from fluorescence microscope, the control group has a lot of active mitochondria what is seen as lighter areas, marked letter R. It corresponds to the red colour on the fluorescence microscope images. The melittin-treated cells have much less active mitochondria. On the Figure 4b are noticeable mainly darker areas, marked letter G, corresponding to the green colour on the fluorescence microscopy. The mitochondrial transmembrane potential assay showed that melittin disintegrates mitochondrial membranes, which may indicate that melittin induces apoptosis by a mito-

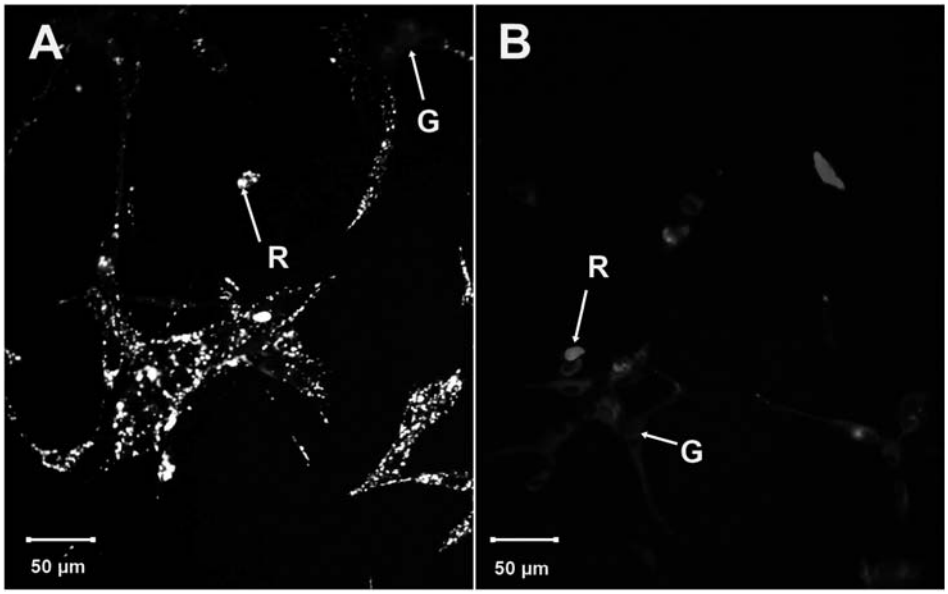

FIGURE 4. Effect of melittin on the mitochondrial membranes integrity. A – control group, untreated cells; B – cells treated 20 µg/mL of melittin (explanations in the text)

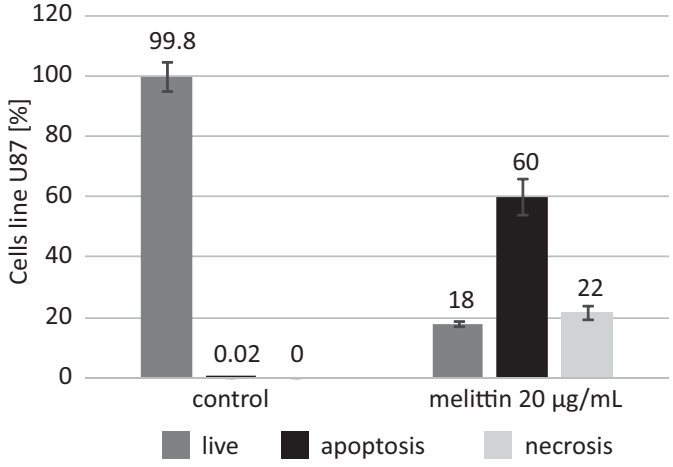

FIGURE 5. Annexin V-Alexa Fluor® 488 and PI assay analysis. Diagrams of cells exposed to 20 µL/mL of melittin

chondrial pathway in glioma cells, but to know detailed way of glioma cell death by melittin more tests are needed.

To sum up, melittin has a harmful influence on human glioma cells. By the ability to cell membrane perforation kills cancer cells by apoptosis mostly. This fact is important because the risk of inflammation is reduced. Melittin interaction with cell membranes could be exploited

for anticancer therapy. However, we need more information about the effects of this compound on the level of proteins, genes and enzymes. The present results are preliminary and follow-up research to elucidate the molecular mechanisms involved in the interactions between melittin and cells is necessary. At this stage, the results indicate that the contact between melittin and U87 cell membranes may be the key cause of melittin toxicity. Consequently, the morphology of glioma cells, the molecular status of cell lines, and the melittin concentration may act together to regulate the biological response of glioma cells to melittin.

CONCLUSION

The results demonstrate that cytotoxicity of melittin on glioma cells increases with increasing melittin concentrations from 5 to 50 μg/mL. Melittin causes perforation of cell membranes and mitochondrial membranes. However, melittin induces apoptosis and to less extent necrosis in the U87 cell line, indicating that melittin is a promising candidate for anticancer therapy.

Acknowledgement

Grant NCN PRELUDIUM 2013/09/N/ /N29/01898.

REFERENCES

BEI C., BINDU T., REMANT B.C., PEISHENA X., 2015: Dual secured nano-melittin for the safe and effective eradication of cancer cells. J. Mater. Chem. B. Mater. Biol. Med. 3 (1): 25–29.

DOMBROWSKI Y., PERIC M., KOGLIN S., KAYAMAKANOV N., SCHMEZER V., REINHOLZ M., RUZICKA T., SCHAUBER J., 2012: Honey bee (*Apis mellifera*) venom induces AIM2 inflam-masome activation in human keratinocytes. Allegry 67 (11): 1400––1407.

GAJSKI G., GARAJ-VRHOVAC V., 2013: Melittin: a lytic peptide with anticancer properties. Environ. Toxicol. Pharmacol. 36 (2): 697–705.

HOSKIN D.W., RAMAMOORTHY A., 2008: Studies on anticancer activities of antimicrobial Peptides. Biochim. Biophywhuchs. Acta 1778 (2): 357–375.

HUH J.E., KANG J.W., NAM D., BAEK Y.H., CHOI D.Y., PARK D.S., LEE J.D., 2012: Melittin suppresses VEGF-A-induced tumour growth by blocking VEGFR-2 and the COX--2-mediated MAPK signalling pathway. J. Nat. Prod. 75: 1922–1929.

JO M., PARK M.H., KOLLIPARA P.S., AN B.J., SONG H.S., HAN S.B., KIM J.H., SONG M.J., HONG J.T., 2012: Anti-cancer effect of bee venom toxin and melittin in ovarian cancer cells through induction of death receptors and inhibition of JAK2/STAT3 pathway. Toxicol. Appl. Pharmacol. 258: 72–81.

KEITH D., ESHLEMAN A., JANKOWSKY A., 2011: Melittin stimulates fatty acid release through non-phospholipase-mediated mechanisms and interacts with the dopamine transporter and other membrane spanning proteins. Eur. J. Pharmacol. 650 (2–3): 501–510.

KONG G.M., TAO W.H., DIAO Y.L., FANG P.H., WANG J.J., BO P., QIAN F., 2016: Melittin induces human gastric cancer cell apoptosis via activation of mitochondrial pathway. World J. Gastroenterol. 22 (11): 3186–3195.

LEE M.T., CHEN F.Y., HUANG H.W., 2004: Energetics of pore formation induced by membrane active peptides. Biochemistry 43: 3590––3599.

MOLLAY C., KREIL G., 1974: Enhancement of bee venom phospholipase A2 activity by melittin, direct lytic factor from cobra venom and polymyxin. B. FEBS 46: 141–144.

PARK M.H., CHOI M.S., KWAK D.H., OH K.W., YOON D.Y., HAN S.B., SONG H.S., SONG M.J., HONG J.T., 2011: Anti-cancer effect of bee venom in prostate cancer cells through activation of caspase pathway via inactivation of NF-κB. Prostate 71 (8): 801–812.

RAGHURAMAN H., CHATTOPADHYAY A., 2007: Melittin: a membrane-active peptide with diverse functions. Biosci. Rep. 27 (4–5): 189–223.

SHIN J.M., JEONG Y.J., CHO H.J., PARK K.K., CHUNG I.K., LEE I.K., KWAK J.Y., CHANG H.W., KIM C.H., MOON S.K., KIM W.J., CHOI Y.H., CHANG Y.C., 2013: Melittin suppresses HIF-1α/VEGF expression through inhibition of ERK and mTOR/p70S6K pathway in human cervical carcinoma cells. PLOS ONE. 8 (7): e69380.

STĘPIEŃ A., IZDEBSKA M., GRZANKA A., 2007: The types of cell death. Postepy Hig. Med. Dosw. 61: 420–428.

TOSTESON M.T., TOSTESON D.C., 1981: The sting. Melittin forms channels in lipid bilayers. Biophys. J. 36 (1): 109–116.

WONG R.S.Y., 2011: Apoptosis in cancer: from pathogenesis to treatment. J. Exp. Clin. Canc. Res. 30: 87.

YANG Z.L., KE Y.Q., XU R.X., PENG P., 2007: Melittin inhibits proliferation and induces apoptosis of malignant human glioma cells. Nan Fang Yi Ke Da XueXueBao. 27 (11): 1775––1777.

ZHANG H., ZHAO B., HUANG C., MENG X.M., BIAN E.B., LI J., 2014: Melittin restores PTEN expression by down-regulating HDAC2 in human hepatocelluar carcinoma HepG2 cells. PLOS ONE 9: e95520.

ZHENG J., LEE H.L., HAM Y.W., SONG H.S., SONG M.J., HONG J.T., 2016: Anti-cancer effect of bee venom on colon cancer cell growth by activation of death receptors and inhibition of nuclear factor kappa B. Oncotarget. 6 (42): 44437–44451.

Streszczenie: *Wpływ melityny na żywotność i integralność błon komórkowych glejaka IV stopnia.* Glejak IV stopnia jest jednym ze złośliwych nowotworów ludzkich. Do dziś nie wynaleziono skutecznego leku do walki z tym nowotworem. Melityna to jeden ze składników jadu pszczelego, który wykazuje działania antyrakowe. Celem badań było zbadanie wpływu melityny na żywotność i integralność błon komórkowych glejaka IV stopnia linii U87. Komórki glejaka IV stopnia linii U87 były traktowane roztworami melityny we wzrastającym stężeniu (5, 10, 15, 20 i 50 µg/mL) i inkubowane przed 24 h. Po inkubacji przeprowadzono badania w celu sprawdzenia morfologii komórek, ich żywotności, integralności błon komórkowych oraz drogę śmierci komórkowej. Wyniki wskazują na niszczący wpływ melityny na komórki glejaka. Melityna powoduje dezintegrację błon komórkowych oraz indukuje śmierć komórkową na drodze apoptozy oraz, w mniejszym stopniu, nekrozy.

Słowa kluczowe: glejak, melityna, toksyczność, apoptoza

MS received 10.10.2016
MS accepted 23.03.2017

Authors' address:
Karolina Daniluk
Zakład Nanobiotechnologii
Katedra Żywienia Zwierząt i Biotechnologii
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8, 02-786 Warszawa
Poland
e-mail: kdaniluk1@gmail.com

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 53–63
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.7

Assessment of slaughter value of sheep on the basis of linear measurements made on carcass digital images

ALEKSANDRA DASIEWICZ[1], WITOLD RANT[2], MARCIN ŚWIĄTEK[2], AURELIA RADZIK-RANT[2]

[1] Department of Animal Nutrition, The Kielanowski Institute of Animal Physiology and Nutrition – Polish Academy of Sciences

[2] Department of Animal Breeding, Warsaw University of Life Sciences – SGGW

Abstract: *Assessment of slaughter value of sheep on the basis of linear measurements made on carcass digital images.* The aim of this study was to develop a method of assessing slaughter value of sheep using digital carcass images. Research material consisted of 38 ovine carcasses. Carcasses were classified in the EUROP grading system in terms of muscle and fat and photographed from three sides and then computer image analysis were used. Analyzed data of linear and area measurements of sheep carcasses using computer image analysis and compare them with the results of slaughter analysis conducted by the conventional method. To compare obtained results statistical analysis was performed. The correlation between computer linear measurements and meat cuts weight and EUROP classification were examined. The preliminary analysis showed the possibility of using computer image analysis to evaluate the meat yield of sheep, and the resulting measurements are correlated to the proportion of meat cuts and tissues, but in order to obtain high accuracy results further studies are needed.

Key words: meat yield, linear measurements, digital images, sheep carcasses

INTRODUCTION

The increasing consumer demands as well as increasingly stringent regulations exposes the producers to problem of providing high and repeatable quality (Brosnan and Sun 2004). A study by Luong (1997) pointed out that food industry destined 1.5–2% of annual income from total production for monitoring the quality. In the connection to the above-mentioned trend more and more studies in field of engineering and manufacturing technology focuses on the development of a fully automated and objective method of quality control and product assessment. Over the past few years scientist have tried to develop method that would meet the above requirements, but also minimize time-consuming and cost effectiveness of product evaluation. The result of this study was, i.a., application in the food industry computer tomography and ultrasound (Abdullah et al. 2004). However, none of mentioned methods was fully effective. After application of the computer image analysis expected result were obtained. Computer image analysis as a non-destructive method, allowing to obtain fast, repeatable and objective evaluation of the quality, more and more often is used to measure and

predict the quality of raw materials in almost every industry sector. Currently, image analysis is widely used in chemical engineering, medicine, textiles and even architecture (Iqbal et al. 2010). In agriculture computer image analysis is used, among others, in the studies of plant protection products (identification of pests and diseases) to evaluate the efficacy of active substances. It might be also used to evaluate the quality of agricultural products, as well as may be used in agricultural technology, were it becomes an integral part of the technological processes. Assessment based on image analysis enable determine the colour, damage and defects in the surface of vegetables, fruits and meat. It is also possible to estimate the quality level, size and texture. Moreover, products such as bread, cereals and some type of chees could also be tested (Frączek 2005). It should be noted that computer image analysis was widely used also in classification of pig and beef carcasses. Measurements performed by this method are characterised by high accuracy and repeatability, moreover, it is not vitiated by an error resulting from a subjective approach of classifier (Olivier et al. 2010). Those features enabled Meat and Livestock Commission approved computer image analysis as the official method for grading pig carcasses. Different countries have already begun taking significant steps to put method based on video image analysis into effect on slaughter lines to enhance precision of the official EUROP carcass classification system for beef, pigs and lambs (Rius-Vilarrasa et al. 2009).

The aim of this study was to analyse the data of linear and surface measurements of ram lambs carcasses of two Polish breeds using computer images and compare them with the results of slaughter analysis conducted by the conventional method.

MATERIAL AND METHOD

The study was conducted at the Department of Breeding Sheep and Goats SGGW in Warsaw. The material consisted of 38 ram lambs carcasses derived from the Experimental Farms Sheep and Goats SGGW in Żelazna, Poland. Rams two breeds – Polish lowland sheep (28 animals) and Wrzosówka sheep (10 animals), were slaughtered after reaching weight 35–40 kg of live weight. The animals were slaughtered at the abattoir according to standard commercial procedures. The hot carcass weight (CW) was determined after dressing. The carcasses were then chilled at 4°C for 24 h and graded using the six EUROP conformation class notation system (from 6 for S to 1 for P) and the three fat cover class notation system (Council Regulation 1183/2006).

During the evaluation carcasses were hanging in a specially prepared for this purpose rack, and then using an Konica Minolta Z2 digital camera three pictures were taken – from the dorsal, ventral and left lateral side. Carcasses were classified in the EUROP system in terms of muscle and fat, and then weighed. Also the pH_{24} was studied, measured depth, perimeter and length of the leg, the surface and the width and depth of the loin eye. Besides it was defined the thickness of the fat layer of subcutaneous fat over the *musculus longissimus dorsi* and the mass and percentage of kidney with the fat in carcass

weight. Carcases were divided into meat cuts: front and rear shank, ham, lamb neck, shoulder, ribeye, flank, saddle, tenderloin and haunch. Received meat cuts were weighed and their percentage in the carcass was calculated. There were also conducted leg dissection, which enabled the determination of the percentage and muscle, fat and bones weight in this element.

The ten linear measurements of carcasses, using the computer images has been elaborated. Carcass measurements on the pictures were carried out using MultiScan (MultiScanBase v. 18.03, Computer Scanning Systems Ltd, Poland), which is a measurement system operating in a Windows environment. On the picture showing the dorsal side of carcass (Fig. 1) seven measurements were performed: haunch length measured as distance from pastern to the tail point (L_1), the widest position below the groin point (W_1), the rump width at the base of the tail (W2), width at the rump narrowest point (W_3), the widest point of the carcase (W4), the thinnest width below W4 and above shoulder (W_5) and area of the rump (A_1). Then pictures were analysed on the left side of the carcass, which set three values (Fig. 2): the widest position of the haunch (D_1), the widest position of the chest (D_2) and the length of the carcass from tail point to base of the neck (L_2).

Statistical processing of the data was carried out using SPSS version 23.0 (SPSS for Windows). The data obtained were analyzed statistically by the analysis of variance where the effect of breed, EUROP conformation and EUROP fatness grade, and body weight at slaughter as a covariate were included in the

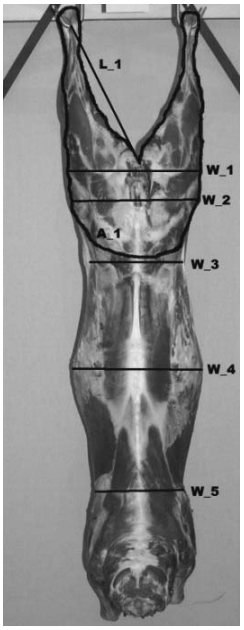

FIGURE 1. The measurements performed on the dorsal side of carcass

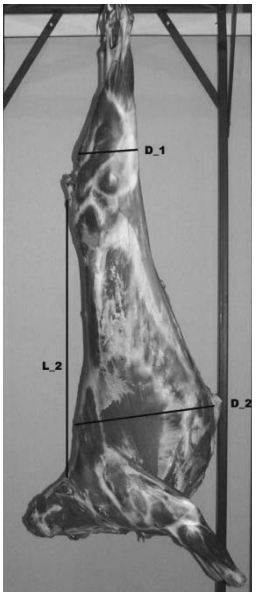

FIGURE 2. The measurements performed on the left side of carcass

model. Furthermore, the correlation between traits has been analysed. The data was summarized in the tables in the form of least squares means marked as (*LSM*) and standard error (*SE*).

RESULTS AND DISCUSSION

The results of analysis of selected features of slaughter value are presented in Table 1. Obtained by linear measurements made on digital carcass images results allow to conclude that there are differences between carcasses of Polish lowland sheep and Wrzosówka sheep. Both in terms the linear traits measured using a computer method and the results of the dissection higher values can be assigned to the lowland sheep. A significant difference was observed in the mass of valuable parts and percentage of haunch in the carcase. Suchlike results were reported by Rant (2013) where slaughter value of Wrzosówka and Polish lowland ram lambs have been considered. Results of haunch dissection showed that a higher percentage of meat and the lower fat content were observed in Wrzosówka sheep. However this trend is not reflected in the relation to the value specified in kilograms. Similar results have been obtained by Kiyanzad (2004), comparing the two Iranian sheep breeds Moghani and Makui. According to Ramsey studies (1991), this discrepancy may be due to differences in nutrition and date of slaughter, and thus the degree fatness of animals. Values of computer measurement such as the length and width of the five areas of the carcase Polish lowland sheep were slightly higher than in Wrzosówka. This translates to the larger surface of rump. The results relating to the value of selected features of the slaughter value and computer linear measurement depending on the classification scale EUROP shown in Table 2. It was found that the carcasses classified as U were characterized by a highly significantly higher value of traits measured directly on the carcass. These differences concern: the depth and weight of the haunch the weight of valuable meat cuts and fat weight in the round compared to the class O and P. Likewise the linear measurements obtained by the computer measurements (W_1, W_2, W_3, W_4, W_5, D_1, L_2) were significantly higher. Also in the carcasses qualified for the R class was demonstrated a highly significantly higher value of the studied traits than in the carcasses classified into lower classes of conformation. Similar dependences were presented in study of Oliver et al. (2010), where the relationship between body weight, affiliation to the EUROP class and computer analysis of linear measurements of carcasses of regional breeds Spanish bulls have been studied. Another experiment was conducted by Rius-Vilarrasa (2008). In his work has studied the possibility of the application of computer image analysis to the EUROP classification system for sheep carcasses. Results of his studies indicated that without considering the carcass weight and degree of fatness, computer analysis is a poor predictor of slaughter value. Thereby, this confirmed results obtained in previous studies of Kempster (1981) and Horgan et al. (1995). Analogous results were obtained by Craigie et al. (2013) in the study focused on relationship between image analysis (VIA system), visual classification and meat yield of cattle of different breed and

TABLE 1. Selected features of the slaughter and computer linear measurements depending on the breed

Trait	PLS		WRS	
	LSM	*SE*	*LSM*	*SE*
Circuit of haunch	37.18[a]	0.483	34.95[b]	0.794
Depth of haunch	19.78	0.296	19.35	0.486
Lenght of haunch	24.94	0.190	24.65	0.313
Width of loin eye	5.93	0.076	5.73	0.124
Depth of loin eye	2.74	0.069	2.50	0.114
Thickness of fat	0.92	0.113	0.54	0.186
Rib eye weight (kg)	0.51[a]	0.019	0.43[b]	0.031
Rib eye content (%)	6.43	0.103	6.14	0.169
Saddle weight (kg)	0.52[a]	0.018	0.43[b]	0.030
Saddle content (%)	6.57	0.129	6.18	0.212
Haunch weight (kg)	2.24[A]	0.076	1.86[B]	0.125
Haunch content (%)	28.06[a]	0.252	26.67[b]	0.414
Valuable meat cuts (kg)	3.27[A]	0.109	2.72[B]	0.180
Meat in haunch (kg)	1.58[a]	0.047	1.36[b]	0.077
Fat in haunch (kg)	0.23[a]	0.018	0.15[b]	0.029
Bone in haunch (kg)	0.39	0.016	0.34	0.026
L_1	23.21	0.188	22.69	0.309
W_1	20.99	0.247	20.32	0.405
W_2	21.28	0.300	20.20	0.493
W_3	15.21	0.273	14.51	0.448
W_4	21.79	0.371	20.95	0.610
W_5	13.93[a]	0.227	13.04[b]	0.374
A_1	572.50[a]	13.084	511.71[b]	21.499
D_1	10.50	0.194	10.19	0.320
D_2	23.64	0.257	23.64	0.423
L_2	50.65	0.676	50.09	1.111

Values in rows with different letters differ significantly: a, b ($P \leq 0.05$), A, B ($P \leq 0.01$); PLS – Polish lowland sheep; WRS – Wrzosówka sheep; L_1 – haunch length; W_1 – widest position below the groin point; W_2 – the rump width at the base of the tail; W_3 – width at the rump narrowest point; W_4 – widest point of the carcase; W_5 – thinnest width below; W4 and above shoulder; A_1 – area of the rump; D_1 – widest position of the haunch; D_2 – widest position of the chest; L_2 – length of the carcass from tail point to base of the neck

TABLE 2. Selected features of the slaughter value and computer linear measurements depending on the EUROP classification

Trait	U		R		O		P	
	n = 9		*n* = 12		*n* = 10		*n* = 7	
	LSM	*SE*	*LSM*	*SE*	*LSM*	*SE*	*LSM*	*SE*
Circuit of haunch (cm)	37.67^{B}	0.704	38.38^{B}	0.737	34.50^{A}	0.707	34.25^{A}	1.581
Depth of haunch (cm)	20.74^{B}	0.406	19.76^{A}	0.424	19.00^{A}	0.407	18.25^{A}	0.911
Lenght of haunch (cm)	24.65	0.323	24.50	0.722	25.08	0.336	24.81	0.322
Width of loin eye (cm)	6.11	0.116	5.90	0.121	5.72	0.116	5.55	0.260
Depth of loin eye (cm)	2.73	0.114	2.86	0.119	2.50	0.115	2.62	0.256
Thickness of fat (mm)	0.86^{B}	0.104	1.51^{C}	0.109	0.49^{A}	0.104	0.38^{A}	0.233
Rib eye (kg)	0.55^{B}	0.020	0.55^{B}	0.027	0.39^{AB}	0.026	0.42^{A}	0.058
Saddle (kg)	0.55^{B}	0.024	0.57^{B}	0.025	0.40^{AB}	0.024	0.46^{A}	0.055
Haunch (kg)	2.37^{B}	0.104	2.41^{B}	0.109	1.76^{AB}	0.105	1.72^{A}	0.234
VMC (kg)	3.47^{B}	0.146	3.54^{B}	0.153	2.55^{AB}	0.107	2.60^{A}	0.328
L_1	23.20	0.326	23.270	0.341	22.749	0.327	22.40	0.731
W_1	21.45^{B}	0.293	21.57^{B}	0.307	19.84^{A}	0.295	18.89^{A}	0.659
W_2	21.79^{B}	0.339	22.08^{B}	0.354	18.52^{A}	0.760	19.55^{A}	0.340
W_3	16.00^{B}	0.338	15.72^{B}	0.353	13.90^{A}	0.339	13.01^{A}	0.759
W_4	22.20^{B}	0.477	23.08^{B}	0.499	20.13^{A}	0.479	19.09^{A}	1.071
W_5	14.46^{B}	0.242	14.49^{B}	0.253	12.27^{A}	0.243	12.36^{A}	0.543
D_1	10.78^{B}	0.267	10.89^{B}	0.279	9.64^{A}	0.268	9.75^{A}	0.599
D_2	24.08	0.375	24.33	0.392	22.62	0.377	22.72	0.843
L_2	51.59^{B}	0.993	52.18^{B}	1.038	47.62^{A}	0.997	48.63^{A}	2.229

A, B, C – values in rows with different letters differ significantly ($P \leq 0.01$); VMC – valuable meat cuts; L_1 – haunch length; W_1 – widest position below the groin point; W_2 – the rump width at the base of the tail; W_3 – width at the rump narrowest point; W_4 – widest point of the carcase; W_5 – thinnest width below; W4 and above shoulder; D_1 – widest position of the haunch; D_2 – widest position of the chest; L_2 – length of the carcass from tail point to base of the neck

gender. Table 3 shows the parameters of selected features of the slaughter value measured directly on carcasses and computer measurements according to class of fatness in the EUROP system. As in the Hinz's studies (2007) shown that almost all of the analysed features demonstrated significantly higher values in carcasses selected for a third, the highest fatness class compared to the carcasses belonging to the first and the second class. Furthermore, highly significant differences were also observed between the second and first class. Correlations between the computer measurements and the weight of valuable meat cuts shown in Table 4.

TABLE 3. Selected features of the slaughter value and computer linear measurements depending on the degree of fatness in EUROP classification

Trait	1		2		3	
	n = 19		*n* = 11		*n* = 8	
	LSM	*SE*	*LSM*	*SE*	*LSM*	*SE*
Circuit of haunch (cm)	35.91^{A}	0.623	37.64^{A}	0.772	39.01^{B}	0.968
Depth of haunch (cm)	19.88	0.359	19.43	0.444	20.19	0.558
Lenght of haunch (cm)	24.66	0.285	24.89	0.352	25.19	0.442
Width of loin eye (cm)	5.83	0.102	5.87	0.127	6.12	0.159
Depth of loin eye (cm)	2.71	0.101	2.72	0.125	2.81	0.157
Kidney weight (kg)	0.12^{A}	0.012	0.19^{B}	0.015	0.17^{A}	0.019
Thickness of fat (mm)	0.61^{A}	0.092	0.88^{B}	0.114	1.90^{C}	0.143
Rib eye (kg)	0.47^{A}	0.023	0.53^{B}	0.028	0.59^{C}	0.036
Saddle (kg)	0.49^{A}	0.022	0.53^{B}	0.027	0.61^{C}	0.033
Haunch (kg)	2.01^{A}	0.092	2.29^{B}	0.114	2.60^{C}	0.143
VMC (kg)	2.97^{A}	0.129	3.36^{B}	0.160	3.80^{C}	0.201
L_1	22.75	0.288	23.29	0.357	23.48	0.448
W_1	20.19^{A}	0.260	21.39^{B}	0.321	22.12^{C}	0.403
W_2	20.18^{A}	0.300	21.74^{B}	0.371	22.74^{B}	0.466
W_3	14.58^{A}	0.299	15.42^{A}	0.370	16.46^{B}	0.465
W_4	20.76^{A}	0.422	22.17^{A}	0.523	23.83^{B}	0.656
W_5	13.31^{A}	0.214	14.26^{B}	0.265	14.87^{B}	0.333
A_1	522.93^{A}	13.511	601.29^{B}	16.727	627.45^{C}	20.992
D_1	10.55	0.236	10.31	0.292	10.83	0.367
D_2	23.41	0.332	24.24	0.411	24.22	0.516
L_2	50.22	0.878	51.57	1.087	51.77	1.365

A, B, C – values in rows with different letters differ significantly ($P \leq 0.01$); VMC – valuable meat cuts; L_1 – haunch length; W_1 – widest position below the groin point; W_2 – the rump width at the base of the tail; W_3 – width at the rump narrowest point; W_4 – widest point of the carcase; W_5 – thinnest width below; W4 and above shoulder; A_1 – area of the rump; D_1 – widest position of the haunch; D_2 – widest position of the chest; L_2 – length of the carcass from tail point to base of the neck

It should be emphasized that all the correlations were statistically significant and highly significant. The strongest linear relationship reaching values in the range of 0.76–0.88 exists between the weight of meat cuts and surface of rump. Also highly statistically significant correlations were found between measurements determining the width of the rump (W_1, W_2) with weight of haunch. Similar

TABLE 4. Correlations of computer linear measurements with weight of selected meat cuts

Trait	Rib eye (kg)	Saddle (kg)	Haunch (kg)	Valuable meat cuts (kg)	Haunch dissection		
					meat (kg)	fat (kg)	bone (kg)
L_1	0.33*	0.33*	0.42**	0.41*	0.44**	0.37*	0.50**
W_1	0.73**	0.68**	0.83**	0.81**	0.82**	0.73**	0.76**
W_2	0.78**	0.75**	0.87**	0.86**	0.87**	0.75**	0.79**
W_3	0.57**	0.62**	0.69**	0.68**	0.67**	0.58**	0.60**
W_4	0.64**	0.64**	0.72**	0.72**	0.66**	0.70**	0.60**
W_5	0.72**	0.73**	0.75**	0.77**	0.70**	0.73**	0.61**
A_1	0.80**	0.80**	0.89**	0.89**	0.86**	0.79**	0.76**
D_1	0.49**	0.55**	0.58**	0.58**	0.56**	0.35*	0.52**
D_2	0.41*	0.33*	0.43**	0.42*	0.45**	0.36*	0.30*
L_2	0.59**	0.62**	0.61**	0.63**	0.51**	0.60**	0.53**

*correlation significant at $P \leq 0.05$; **correlation significant at $P \leq 0.01$.

L_1 – haunch length; W_1 – widest position below the groin point; W_2 – the rump width at the base of the tail; W_3 – width at the rump narrowest point; W_4 – widest point of the carcase; W_5 – thinnest width below W4 and above shoulder; A_1 – area of the rump; D_1 – widest position of the haunch; D_2 – widest position of the chest; L_2 – length of the carcass from tail point to base of the neck

results were obtained also by Oliver et al. (2010). They allow to state that with increasing width of the rear quarter of the carcass increases its content of valuable meat cuts. Correlation analysis of computer linear measurement and classification in EUROP grading system (Table 5) showed highly significant linear relationship. The highest value of the correlation coefficient (r = 0.73) were obtained for the EUROP scale and width in the throat behind the shoulders. The estimated value of the correlation was similar to that defined by La Ville (1996) (r = 0.69) in the study of young Charolais bulls. In the present study also found a strong relationship between the surface area of rump and conformation classification. Correlations between the computer measurement has shown that the strongest relationship occurs between hindquarter surface area and width. For the measurement at the W_1 point correlation coefficient was r = 0.91, and for the point W_2 r = 0.95. The lowest linear relationship was demonstrated for the length of the haunch and EUROP classification and between the length and depth of the haunch. This may be due to the fact that in ovine the haunch is longer the flatter it is and visual degree of "occupancy rate" of muscularity is lower (Hopkins 1996). The results indicate that computer measurements of ovine carcases can be used to determine the amount of valuable meat cuts, as well as to estimation of other parameters representing the slaughter value. Due to

TABLE 5 . Correlations of computer linear measurement and EUROP classification

Traits	EUROP	L_1	W_1	W_2	W_3	W_4	W_5	A_1	D_1	D_2	L_2
EUROP		0.22	0.62**	0.67**	0.64**	0.52**	0.73**	0.70**	0.38*	0.46**	0.47**
L_1			0.54**	0.55**	0.44**	0.52**	0.44**	0.55**	0.18	0.32	0.42**
W_1				0.96**	0.81**	0.81**	0.78**	0.91**	0.44**	0.61**	0.68**
W_2					0.83**	0.86**	0.85**	0.95**	0.51**	0.58**	0.71**
W_3						0.82**	0.79**	0.84**	0.45**	0.41**	0.57**
W_4							0.85**	0.82**	0.45**	0.53**	0.68**
W_5								0.89**	0.55**	0.59**	0.70**
A_1									0.51**	0.57**	0.72**
D_1										0.44**	0.48**
D_2											0.54**
L_2											

*correlation significant at $P \leq 0.05$; **correlation significant at $P \leq 0.01$.

L_1 – haunch length; W_1 – widest position below the groin point; W_2 – the rump width at the base of the tail; W_3 – width at the rump narrowest point; W_4 – widest point of the carcase; W_5 – thinnest width below W4 and above shoulder; A_1 – area of the rump; D_1 – widest position of the haunch; D_2 – widest position of the chest; L_2 – length of the carcass from tail point to base of the neck

the small number of observations in this study, the work on this issue requires further continuation.

CONCLUSION

The method of linear measurements made on digital carcass images can be considered as a supplement to the assessment of sheep carcasses in EUROP grading system. It enables objectively estimate the meat yield and valuable meat cuts content, and thus the profitability of the production of lamb. It was found that preliminary analysis shows the possibility of using this method to assess the slaughter value of sheep, and the resulting measurements are correlated to the proportion of meat cuts and tissues, however, to obtain high accuracy results further studies are needed on populations of large numbers.

Acknowledgement

This study was carried out as part of research project N N311081240, supported by the National Science Center of Poland.

REFERENCES

ABDULLAH M.Z., GUAN L.C., LIM K.C., KARIM A.A., 2004: The applications of computer vision system and tomographic radar imaging for assessing physical properties of food. J. Food Eng. 6: 125–135.

BROSNAN T., SUN D.W., 2004: Improving quality inspection of food products by computer vision – a review. J. Food Eng. 61: 3–16.

CRAIGIE C.R., NAVAJAS E.A., PURCHAS R.W., 2013: A review of the development and use of video image analysis (VIA) for beef carcass evaluation as an alternative to the current EUROP system and other subjective systems. Meat Sci. 92: 307–318.

Council Regulation 1183/2006 of 24 July 2006 concerning the Community scale for the classification of carcases of adult bovine animals (codified version). Official Journal of the European Communities 214.

FRĄCZEK J., 2005: Digital image analysis in agricultural technology. Inżynieria Rolnicza 6 (66): 149–158.

HINTZ A., 2007: Video image analysis (VIA). A summary report for sheep carcases. Available from: www.hccmpw.org.uk/medialibrary/pdf/416.pdf [accessed: 14.11.2016].

HOPKINS D.L., 1996: The relationship between muscularity, muscle – bone ratio and cut dimensions in male and female lamb carcasses and the measurement of muscularity using image analysis. Meat Sci. 44: 307–317.

HORGAN G.W., MURPHY S.V., SIMM G., 1995: Automatic assessment of sheep carcasses by image analysis. Anim. Sci. 60: 197–202.

IQBAL A., VALOUS N. A., MENDOZA F., SUN D.-W., ALLEN P., 2010: Classification of pre-sliced pork and Turkey ham qualities based on image colour and textural features and their relationships with consumer responses. Meat Sci. 84: 455–465.

KEMPSTER A.J., ARNALL D., ALLISTON J.C., BARKER J.D., 1981: An evaluation of two ultrasonic machines (Scanogram and dans-canner) for predicting the body composition of live sheep. Anim. Prod. 34: 249–255.

KIYANZAD M., 2004: Using Linear Body Measurements of Live Sheep to Predict Carcass Characteristics for Two Iranian Fat-tailed Sheep Breeds. Asian-Australian J. Anim. Sci. 17 (5): 693–699.

La VILLE E., MARTIN V., BASTIEN O., 1996: Prediction of composition traits of young Charolais bull carcasses using a morphometric method. Meat Sci. 44: 93–104.

LUONG J.H.T., BOUVRETTE P., MALE K.B., 1997: Developments and application of biosensors in food analysis. Trends Biotechn. 15: 369–377.

OLIVER A., MENDIZABAL J.A., RIPOLL G., ALBERTÍ P., PURROY A., 2010: Predicting meat yields and commercial meat cuts from carcasses of young bulls of Spanish breeds by the SEUROP method and an image analysis system. Meat Sci. 84: 628–633.

RAMSEY C.B., KIRTON A.H., HOGG B., DOBBIE J.L., 1991: Ultrasonic, needle, and carcass measurements for predicting chemical composition of lamb carcasses. J. Anim. Sci. 69: 3655–3664.

RANT W., 2013: Wpływ rasy i standardu wagowego na wartość rzeźna tusz i cechy jakościowe mięsa tryczków utrzymywanych w warunkach hodowli zachowawcze. Rozprawy Naukowe i Monografie. Wydawnictwo SGGW, Warszawa.

RIUS-VILARRASA E., BÜNGER L., MALTIN C.A. MATTHEWS K., ROEHE R., 2008: Evaluation of video image analysis (VIA) technology to predict lean meat yield of sheep carcasses online under abattoir conditions. Meat Sci. 80 (4): 1138–1149.

RIUS-VILARRASA E., BÜNGER L., MALTIN C.A., MATTHEWS K., ROEHE R., 2009: Evaluation of Video Image Analysis (VIA) technology to predict meat yield of sheep carcasses on-line under UK abattoir conditions. Meat Sci. 82: 94–100.

Streszczenie: *Ocena wartości rzeźnej tusz owczych z wykorzystaniem komputerowych pomiarów tusz.* Materiał badawczy stanowiło 38 tusz. Tusze fotografowano z trzech stron, a zdjęcia poddano komputerowym pomiarom. Tusze klasyfikowane były w systemie EUROP pod względem umięśnienia oraz otłuszczenia. Następnie przeprowadzono analizę wartości rzeźnej tusz. W celu porównania otrzymanych wyników przeprowadzono analizę statystyczną. Zbadano korelacje między poszczególnymi komputerowymi pomiarami liniowymi z masą wybranych wyrębów oraz klasyfikacją EUROP. Obliczono średnie wartości oraz błąd standardowy wszystkich badanych cech. Stwierdzono, iż wstępna analiza wykazuje możliwości zastosowania komputerowej analizy obrazu do oceny wartości rzeźnej owiec, a otrzymane pomiary są skorelowane z proporcjami wyrębów i tkanek, jednak dla uzyskania dużej dokładności wyników niezbędne są dalsze badania na populacji o dużej liczebności.

Słowa kluczowe: wartość rzeźna, komputerowe pomiary liniowe, tusze owcze

MS received 12.12.2016
MS accepted 03.04.2017

Authors' address:
Aleksandra Dasiewicz
Zakład Żywienia Zwierząt
Instytut Fizjologii i Żywienia Zwierząt im. Jana Kielanowskiego PAN w Jabłonnie
Instytucka 3, 05-110 Jabłonna
Poland
e-mail: a.dasiewicz@ifzz.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 65–74
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.8

Gender and age structure as well as body weight of partridge (*Perdix perdix* L.) during periods of high and low population density in the Lublin Upland

MARIAN FLIS[1], MAREK PANEK[2]
[1] Department of Zoology, Animal Ecology and Wildlife Management, University of Life Sciences in Lublin
[2] Polish Hunting Association, Research Station in Czempiń

Abstract: *Gender and age structure as well as body weight of partridge (*Perdix perdix *L.) during periods of high and low population density in the Lublin Upland.* Studies upon structure of gender and age as well as body weight of partridges were carried out in the Lublin Upland in 1988 and 2015, different in relation to the levels of these animals population density. These features were evaluated in 104 individuals culled in early October. Nearly four-fold decrease in the population density of this species was recorded between the compared periods. Over 80-fold decrease in the hunting acquisition of partridge occurred the same period. A decrease in the share of females in the population after the breeding period was also found, which probably resulted from an increase in this gender mortality during the breeding season. Proportions of the number of juveniles to adult animals (2.4 and 1.7), and to adult males (4.0 and 1.7) in two subsequent study years, i.e. indicators of the young partridges production, did not differ significantly, but it could be mainly due to a small sample in the latter year. The gender structure of juvenile partridges was 0.94 male per female in 1988 and 0.71 male per female in 2015, which did not significantly differ between periods (χ^2 = 0.178). Among adult individuals, 1.5 male per female were recorded in 1988, while only males were reported in 2015. Body weight of young partridges decreased between the years of research by 22.5 g, i.e. 6%, while adult animals by 12.5 g, which indicates the decrease in bird size, and thus the individual condition, presumably due to limited food resources. Differences in body weight between periods were statistically significant only at young animals. Such situation can cause enhanced mortality of young partridges in autumn and winter, therefore worsen the existing regress in this species population. The results confirmed that the previous assessment of the causes of the decline in partridges population in Poland, showing an increase in losses during the breeding season, as the main demographic mechanism, were valid also for the second decade of the 21st century. It follows that improving the environmental conditions during the breeding season, besides reducing the number of predators, should be an essential part of the partridge active protection programs.

Key words: grey partridge, body mass, age, sex, population density, Lublin Upland

INTRODUCTION

In recent decades, there has been a sharp decrease in the number of small animals in the most of hunting grounds in Poland, as well as in other European countries. The most drastic decrease concerned hares and partridges (Dziedzic et al. 2002, Smith et al. 2005, Kamieniarz and

Panek 2008, Flis 2009a, Kuijper et al. 2009). The increase in predators' pressure and progressive transformation of the agricultural landscapes are counted to the main reasons for the decreasing number of these species in Poland in previous decades. Increased mortality of hares and partridges as an effect of predation resulted mainly from a significant increase in fox population (Panek 2005, Panek et al. 2006, Wasilewski 2007), which in turn was conditioned by the introduction of annual preventive vaccination of this species against rabies (Flis 2010, 2013). Transformation of the agricultural landscape manifested mainly as simplifying its structure (increase in the field sizes, elimination of places excluded from crops and overgrown with wild vegetation), changes in the share of individual plant species in the structure of crops, and intensification of agricultural production (Panek 1997, Panek and Kamieniarz 1998, 1999, Wübbenhorst and Leuschener 2006, Jezierski 2007, Motyl 2007, Flis 2009a).

Previous studies conducted in Poland and other European countries indicate that the combination of these adverse changes in the environment caused a decline in the number of partridges affecting mainly through a negative impact on the course and results of these birds breeding (Potts 1986, Panek 2005, Kuijper et al. 2009). In Poland, this concerned mainly the breeding success deteriorating as a result of limiting the availability of nesting sites and increased mortality of adult individuals during breeding, especially hatching females (Panek and Kamieniarz 2000, Panek 2002a, 2005). Additionally, there was some decrease in the survival of chick partridges (Panek 2005) in connection with the reduction of their food resources used during the first weeks of life, i.e. decrease in the abundance of some groups of insects, which is negatively affected by an intensive use of chemical plant protection means and simplified structure of the agricultural landscape (Rands 1985, Potts 1986, Panek 1997).

In order to reverse the negative trend of the partridge population situation in Poland, various activities of active conservation of this species are undertaken. One of such measures is to reduce or suspend the hunting gaining in areas, where the decline was deep and density of partridges reached a low level, which in the first decade of the 21st century concerned the majority of hunting grounds, especially in the west and north of the country (Kamieniarz and Panek 2008, Budny et al. 2011). Since the late 1990s of the 20th century, the settlement using birds coming from captive breeding has been also applied in many areas (Kamieniarz and Panek 2008). The research upon the survival and reproduction of discharged partridges has been conducted as well, i.e. an assessment of the reintroduction effectiveness, as well as measurements of potential resources availability for these birds in their reintroduction sites (Kamieniarz and Panek 2011, Nasiadka and Świtalska 2014). The attempt to modify habitats in a direction of their optimization taking into account the ecology of this species should be an important element of programs for the reconstruction of partridge population (Kamieniarz and Panek 2008, Flis 2009a). However, to carry out the activities to improve the living conditions of partridges, a thorough knowledge about the causes of their

decline is needed. While the phenomena leading to a significant reduction in these birds population in the 1990s have been the subject of detailed analysis (Panek 2005), conditions of their situation during the first 10 years of the 21st century remain poorly known. Nevertheless, results of the national monitoring of partridges show that after a period of relative stabilization of their population in the first decade of the 21st century, further decrease in the average density of these birds in Poland occurred at the turn of the first and second decade (Budny et al. 2011). One of the reasons for poor recognition of conditions of current partridge situation is undoubtedly the limited availability of the research material because of their low densities and the widespread suspension of hunting.

From the above review of the causes of the partridge population decline in Poland in previous decades follows that it should be accompanied by changes in the structure of gender and age in the populations of these birds after the breeding period, resulting from deterioration of the results of breeding and chicks survival. In addition, since limiting the food resources was pointed as the main cause of the increase in chicks losses, it can also be expected to reduce the weight of young partridges that survived a critical period of dependence on insects as a food.

The aim of the study was to test of the above predictions about the causes and consequences of the partridge number decline in relation to the current situation of these birds. Therefore, the structure of gender and age and body weight of partridges during the period with relatively high density before falling of their numbers in 1990s, as well as in the second decade of the 21st century, i.e. a period with significantly lower their density, was compared. The evaluation was carried out on the basis of material collected in the game shooting district located in the Lublin Upland.

THE STUDY AREA

Lublin Upland is characterized by chernozem soil type, and thus it is one of the most fertile areas in the country (Witek 1991). It determines the dominance in the field cultivation of plants with high soil requirements. At the same time, this area is characterized by a rather low intensification of agricultural production. In the areas of research, the farmlands were almost entirely owned by individual farms, which was associated with high fragmentation of fields (the average size of individual field area amounted to 1.1 ha) and mosaicism of crops, i.e. considerable diversity of agricultural landscape. The crop structure was dominated by cereals, root crops, and in recent years an increasing share accounted for rape cultivation. In addition, field cultures of soft fruits increasingly appeared. The differentiation of the field environment occurring in the area of research seemed to be beneficial for partridges, the species preferring the heterogeneous agrocoenoses (Popławski 1962).

Material about gender, age and weight of partridges was collected in 1988 and 2015 in two game shooting district located in the Lublin Upland. In 1988, it was the district formerly referred to as No 59, which was the Centre for Animal Breeding, Polish Hunting Association in Wierzchowiska, located in the

present counties of Lublin and Świdnik. The area of this district was 9,700 ha, of which the forest land accounted for 17%. In 2015, the research was conducted in two adjacent game shooting districts: No 202 and No 219, leased by the hunting association and located in the district of Opole Lubelskie. The total area of these districts was 13,600 ha, and the share of forest land amounted to 25%. These were ones of the last districts in Lublin region, where the hunting acquisition of partridges was still conducted at that time. Similar agricultural landscapes, typical for this region dominated in both areas of the research. Mean maximum air temperature in the vicinity of Lublin during the main period of partridge chicks conduction (the second half of June and the first half of July) (Panek 2002b) was similar in the study years (21.7 in 1988 and 21.9 in 2015, data from the Institute of Meteorology and Water Management), thus the weather conditions, known as a factor affecting the living conditions and survival of chick partridges (Potts 1986, Panek 1992) should not have resulted in differences in their situation between the years of the research.

MATERIAL AND METHODS

Data on the number and acquisition of partridges in the area of research in five-year periods preceding the years of collecting the materials about sex, age and weight of these birds, i.e. 1984–1988 and 2010–2014, came from the hunting reports (sheets ŁOW-2, annual hunting plans) and referred to the former Lublin province or present Lublin PZŁ district (areas of these units overlap to a large extent). In the case of population number, there were estimates made each spring (March) by the tenants and managers of hunting grounds. Based on these data, density of partridges and their acquisition per field unit area in each year, was calculated.

Data on gender, age and body weight were collected for a sample of 85 partridges obtained through the so-called foreign exchange hunting on 11–12 October 1988 and 19 partridges acquired while hunting conducted on 4 October 2015. Age and gender was determined for each acquired partridge. The age was assessed on the basis of appearance and shape of the primary quills. An additional element helpful for the evaluation was the color of legs (Popławski 1962). Based on this, birds were qualified as this year's young and adult ones (over one--year old). Gender of partridges was rated on a basis of the look and shape of the outer shoulder feathers and wing covers, as well as the presence or absence of characteristic thoraco-abdominal feathers pigmentation, known as horseshoe (Popławski 1962). Body weight of birds was determined by weighing directly in the field, on the laboratory scale to the nearest 1 g accuracy.

All the material, i.e. total of 104 partridges acquired in two periods of study, was divided into gender-age groups, which allowed for determining the rate of gender (male per female) and age structure (young per adult) of obtained birds. To determine the effect of time, age, and gender on the weight of animals, a multi-factor and single--factor analysis of variance was applied. The proportions were compared using χ^2 test. Analyzes were performed using Statistica software.

RESULTS

During the five years preceding the first period of study, the spring density of partridges in game shooting districts of Lublin region decreased from 14.4 to 6.8 animal/100 ha, while hunting acquisition of the species was 0.8–2.6 animal/100 ha (Table 1). During the five years preceding the second period of study, density of partridge population in Lublin region showed subsequent decreasing trend from 3.4 to 2.5 animal/100 ha. The hunting acquisition amounted then 0.04–0.01 animal/100 ha. Average density of partridges decreased 3.8 times between the two periods (from 10.5 to 2.8 animal/100 ha), while the average acquisition 80 times (from 1.6 to 0.02 animal/100 ha). In the five-year period preceding the study, in the mid-1980s, the indicator of the hunting population exploitation averaged to 14.8%. On the other hand, in the years preceding the second period of study, this indicator was over 21 times lower and was at an average level of 0.7%.

TABLE 1. Indicators of population density and obtaining of partridges in the Lublin Upland

Years of assessment		Density (*n*/100 ha)	Hunting acquisition (*n*/100 ha)	Indicator of hunting exploitation of the population (%)
Period I	1984	14.4	2.6	18.1
	1985	13.2	1.6	12.1
	1986	9.7	1.6	16.5
	1987	8.2	0.8	9.8
	1988	6.8	1.2	17.6
Period II	2010	3.4	0.04	1.2
	2011	3.0	0.02	0.7
	2012	2.6	0.01	0.4
	2013	2.6	0.01	0.4
	2014	2.5	0.02	0.8

TABLE 2. Characteristics of grey partridges shot during two years in Lublin Upland (1988, 2015) the number of individuals, body weight (g), standard device

Specification		Age			
		young		adult	
		1988	2015	1988	2015
Male (♂)	*n*	29	5	15	7
	$\bar{x}$	376.4	355.0	394.0	377.1
	SD	32.1	12.3	22.1	10.8
Female (♀)	*n*	31	7	10	–
	$\bar{x}$	369.7	347.1	383.0	–
	SD	20.6	18.0	17.8	–
Total	*n*	60	12	25	7
	$\bar{x}$	372.9	350.4	389.6	377.1
	SD	26.8	15.7	20.8	10.8

The number of partridges qualified for each gender-age category and data of average body weight for each category in both study periods are given in Table 2. Structure of young partridges gender was 0.94 male per female in 1988, and 0.71 male per female, which did not significantly differ between periods ($\chi^2 = 0.178$, $P = 0.7$). Among adults, 1.5 male per female was found in 1988, while in 2015 only males were recorded and the difference between male and female proportions in two periods proved to be significant ($\chi^2 = 4.073$, $P = 0.04$), thus the share of females decreased the in last period.

The age structure (young per adult) was 2.4 in 1988 and 1.7 in 2015, not differing statistically between periods (χ^2 = = 0.402, P = 0.5). Proportion of juvenile to adult males number was 4.0 in 1988 and 1.7 in 2015 (which was equal to the previous indicator, due to the absence of females in a sample from that year), and neither did not differ significantly between periods (χ^2 = 2.399, P = 0.1).

The variance analysis of partridge body weight (Table 1) according to period, gender, and age revealed differences between study period (F = 10.98, P = 0.001) and bird's age (F = 11.14, P = 0.001), whereas there were no statistically significant differences between genders (F = 3.14, P = 0.08). Therefore, weight of adults and juveniles (both genders altogether) within two periods was compared separately using a single-factor variance analysis, that showed no significant differences in the case of adult animals (F = 2.296, P = 0.1), yet it confirmed differences in body weights of young birds between study periods (F = 7.877, P = = 0.006). Average body weight of young individuals between 1988 and 2015 decreased by 22.5 g, i.e. by 6% (Table 1).

DISCUSSION

Almost four-fold decrease in density of partridges occurred within nearly 30 years in the area of research. The indicator of partridge acquisition per unit area was higher in the 1970s in the region of Lublin Upland than the current spring density of these birds (Pielowski et al. 1993).

Comparison of partridge gender and age structure during former high densities of birds and in the last period, to low states of their populations, at least partially confirmed the predictions arising from the results of earlier analyzes of the causes of the decline in this species population in Poland. First of all, a significant change in the gender structure of adult partridges after the breeding period, i.e. decrease in females share, has been shown. The research material consisted of birds acquired in the course of hunting, meanwhile in Poland, it was found a higher susceptibility of adult males rather than other gender-age groups of partridges to hunting, thus their greater participation among culled individuals than in population present in the area during the hunting season (Olech 1971). Nevertheless, in both years of study, the material was collected by means of the same method. Therefore, reducing of the females share among acquired adult birds undoubtedly resulted from a decrease in the proportion of this gender in the population at the end of summer. This should be associated with increased mortality of females during reproduction, identified for this species in previous years in Poland, and also in some other European countries (Potts 1986, Bro et al. 2001, Panek 2002a). For example, up to 48% of female partridges acceding to breed were dying in the late 1990s in western Poland within three main months of the breeding season (in large part during hatching, mainly caught by carnivores, mainly foxes), while only 11% of males were lost in the same period (Panek 2002a). Any decrease in the ratio of the number of juveniles to adults after a period of reproduction between two periods of study, i.e. reduction in the rate of production of young in partridge populations, was not observed in Lublin Upland. The observed decrease in the share of females among adults meant, however, that recently the number of juveniles reared

within a given year was shared by a relatively smaller number of adults than in the case of samples from the past. Hence the potential reduction in the production of young between two periods of tests can be masked by changes in the survival of adult individuals. However, calculation of the percentage of young birds to the number of adult males (which can be regarded as an indicator of the number of breeding pairs accessing to hatching) (Potts 1986), although resulted in over two-fold difference of this parameter between periods, did not allow for rejection of statistical hypothesis on equality of that proportion for two study periods. Undoubtedly, the results of comparisons were significantly affected by small sample for the last period, which was a significant factor limiting the possibility of concluding on the basis of collected material. In the second half of the 1960s, within a large sample of partridges culled in different parts of Poland, the proportion of young to adult males ranged in each year from 1.9 to 3.5, with an average of 2.85 (Olech 1971). For Lublin Upland, the indicator was even higher in 1988 yet clearly lower than in 2015. Achieved data suggest therefore that the production of young in partridge populations in Lublin Upland in recent years was lower than in the past. Thus, the conclusions from previous studies indicating some increase in losses during the breeding season, and consequently reducing the production of young, as an important cause of the decline and the persistence of low states of partridges in Poland (Panek 2002a, 2005) were likely to be relevant in relation to the situation of these birds in Lublin Upland in the second decade of the 21st century, as well. It should be noted that the material of 2015 came from one of the last areas in the region, where hunting of partridge population was continued. This means that density of these birds were probably higher, thus processes leading to its reduction possibly weaker than in other regions of the country.

Average body weight of different gender-age partridges groups during the autumn hunting, according to previous studies in Poland and neighboring countries, included within 300–440 g (Pulliainen 1965, 1968, Szwykowska 1969, Olech 1971, Chlewski 1980). The values obtained in Lublin Upland in both periods were within this range, even in its central part, and thus they were typical for the species.

The observed differences in body weight of young partridges between two periods confirmed the predictions arising from prior knowledge about the causes of the decline in this species population in Poland. Material was acquired in similar terms, and significant changes in phenology of partridge breeding seem unlikely, so in both cases, young birds were rather at the same stage of ontogenetic development. Therefore, decrease in the average weight of young indicates a reduction in their size or individual condition. The most likely cause appears to be the same factor that led to the decline in survival of chick partridges in Europe in recent decades, i.e. reducing their food resources, decreased abundance of insects in fields mainly due to the intensive use of pesticides (Rands 1985, Potts 1986, Kuijper et al. 2009). Relatively small decrease in the survival rate of chick partridges was observed in Poland, in the 1990s and this phenomenon was a secondary reason for that decrease in the number of these birds (Panek 2005). However, at the beginning of the 21st century, the negative trend of chick partridges

survival had apparently continued as the results of national monitoring showed a progressive decrease in the average number of young birds in family flocks of birds observed after reproduction period. In the early 1990s, it ranged within 8–10, while at the end of the first decade of 21st century, only 6–8 young birds per flock were recorded (Budny et al. 2011). At the same time in recent decades, the increase in the use of chemical pesticides and fertilizers in agriculture occurred in Poland (Flis 2009b, GUS 2014). Results achieved during this study suggest that depletion of food resources for partridges during breeding not only leads to a decrease in chick survival and production of young, but also to reduce the weight of juveniles, which may adversely affect their survival in the subsequent months.

CONCLUSION

The obtained data allow to formulate the following statements and conclusions:

1. In less than a 30-year period, the density of partridges in Lublin Upland decreased almost 4-fold, and values found in the second decade of the 21st century should be assessed as very low. This resulted in a drastic reduction in hunting partridges acquisition in this region.
2. Comparison of gender structure of adult partridges between two periods of research indicates a reduction in the share of females in population after the breeding period, which probably was associated with an increase in mortality of this gender during the breeding season. Proportion of young to adults number did not differ statistically between periods, but this apparently was due to the small sample for the last period. Thus, the findings suggest that the decrease in density of partridges in the area of study was accompanied by an increase in losses during breeding period and possibly lowering the production of young.
3. Body weight of young partridges decreased between the periods of study. Such results of the research may suggest a reduction in their individual condition, probably due to limited food resources for chicks, i.e. abundance of insects in the fields (and therefore factor hitherto known from mainly negative effects on the survival of chick). This condition may result in increased mortality of young birds in autumn and winter, and thus deepen the regression of the species population. The subject of the study requires further investigation.
4. Achieved results give rise to a statement of the need to undertake action of active conservation of this species, primarily leading to the improvement in the conditions during breeding season, by optimizing the living environments, control and reduction of predators. Such activities should be accompanied by partridges settlement, often run by hunters to supply the local populations of this species.

REFERENCES

BUDNY M., BRESIŃSKI W., KAMIENIARZ R., KOLANOŚ B., MĄKA H., PANEK M., 2011: Sytuacja zwierząt łownych w Polsce w roku łowieckim 2010/2011 (wyniki monitoringu). Biul. Stacji Bad. PZŁ w Czempiniu: 8.

BRO E., REITZ F., CLOBERT J., MIGOT P., MASSOT M., 2001: Diagnosing the environmental causes of the decline in grey partridge *Perdix perdix* survival in France. Ibis 143: 120–132.

CHLEWSKI A., 1980: Kuropatwa (*Perdix perdix* L.) w ekosystemie polnym. Praca doktorska, Akademia Rolnicza w Poznaniu, Poznań [manuscript].

DZIEDZIC R., KAMIENIARZ R., MAJER-DZIEDZIC B., WÓJCIK M., BEEGER S., FLIS M., OLSZAK K., ŻONTAŁA M., 2002: Przyczyny spadku populacji zająca szaraka w Polsce. Wydaw. Ministerstwo Środowiska. Fundacja Ekonomistów Środowiska i Zasobów Naturalnych, Warszawa: 17–34.

FLIS M., 2009a: Zagęszczenie i siedliska wykorzystane przez kuropatwy *Perdix perdix* na Wyżynie Lubelskiej w okresie jesień-wiosna 2006/2007. Not. Ornitol. 50 (2): 143–146.

FLIS M., 2009b: Szkody w uprawach rolniczych w świetle szkodliwego oddziaływania rolnictwa na ekosystemy. In: Biotop. Zagrożenia biotopów leśnych. Uniwersytet Opolski, Opole: 123–132.

FLIS M., 2010: Wścieklizna w województwie lubelskim w latach 2002–2009 na tle dynamiki liczebności lisów wolno żyjących. Med. Wet. 66 (8): 562–565.

FLIS M., 2013: Sytuacja epizootyczna i epidemiologiczna wścieklizny w Polsce w latach 2002–2011 na tle dynamiki liczebności lisów wolno żyjących. Życie Wet. 88 (8): 657–660.

GUS, 2014: Rocznik statystyczny rolnictwa. Warszawa.

JEZIERSKI W., 2007: Możliwości życiowe niektórych gatunków zwierząt łownych. Syl. 151 (5): 3–16.

KAMIENIARZ R., PANEK M., 2008: Zwierzęta łowne w Polsce na przełomie XX i XXI wieku. Stacja Badawcza – OHZ PZŁ w Czempiniu, Czempiń: 78–83.

KAMIENIARZ R., PANEK M., 2011: Przebieg lęgów wsiedlonych kuropatw pochodzących z hodowli – badania radiotelemetryczne. Syl. 155 (11): 778–783.

KUIJPER D.P.J., OOSERVELD E., WYMENGA E., 2009: Decline and potential recovery of the European grey partridge (*Perdix perdix*) population – a review. Eur. J. Wildl. Res. 55: 455–463.

MOTYL T., 2007: Stan zwierzyny drobnej w obwodach łowieckich warszawskiego okręgu PZŁ. In: Nauka łowiectwu cz. 1. Kryzys zwierzyny drobnej i sposoby przeciwdziałania. Wydaw. Samorząd Województwa Mazowieckiego, Warszawa: 7–12.

NASIADKA P., ŚWITALSKA T., 2014: Ocena potencjalnych zasobów pokarmowych i osłon dla kuropatw (*Perdix perdix*) w miejscach ich reintrodukcji w warunkach rolnictwa ekstensywnego. Syl. 158 (7): 539–552.

OLECH B., 1971: Realized production, mortality and sex structure of a Partridge (*Perdix perdix* L.) population and its utilization for game purposes in Poland. Ekol. Pol. 19: 617–650.

PANEK M., 1992: The effect of environmental factors on survival of grey partridge (*Perdix perdix*) chicks in Poland during 1987–90. J. of Appl. Ecol. 29: 745–750.

PANEK M., 1997: The effect of agricultural landscape structure on food resources and survival of grey partridge *Perdix perdix* chicks in Poland. J. Appl. Ecol. 34 (3): 787–792.

PANEK M., 2002a: Space use, nesting sites and breeding success of grey partridge (*Perdix perdix*) in two agricultural management systems in western Poland. Game Wildl. Sci. 19: 313–326.

PANEK M., 2002b: Biologia lęgowa kuropatwy *Perdix perdix* w zachodniej Polsce na podstawie danych radiotelemetrycznych. Not. Ornitol. 43: 137–144.

PANEK M., 2005: Demography of grey partridges *Perdix perdix* in Poland in the years 1991–2004: reasons of population decline. Eur. J. Wildl. Res. 51: 14–18.

PANEK M., KAMIENIARZ R., 1998: Agricultural landscape structure and density of grey partridge (*Perdix perdix*) populations in Poland. Giber Faun. Sauv. Game Wildl. 15: 309–320.

PANEK M., KAMIENIARZ R., 1999: Relationships between density of brown hare *Lepus europaeus* and landscape structure in Poland in the years 1981–1995. Acta Theriol. 44: 67–75.

PANEK M., KAMIENIARZ R., 2000: Effect of landscape structure on nest site selection and nesting success of grey partridge *Perdix perdix* in western Poland. Pol. J. Ecol. 48: 239–247.

PANEK M., KAMIENIARZ R., BRESIŃSKI W., 2006: The effect of experimental removal of red foxes *Vulpes vulpes* on spring density of brown hares *Lepus europaeus* in western Poland. Acta Theriol. 51: 187–193.

PIELOWSKI Z., KAMIENIARZ R., PANEK M., 1993: Raport o zwierzętach łownych. Państwowa Inspekcja Ochrony Środowiska. Biblioteka Monitoringu Środowiska, Warszawa.

POPŁAWSKI L.,1962: Kuropatwa. PWRiL, Warszawa.
POTTS G.R., 1986: The Partridge: Pesticides, Predation and Conservation. Collins, London.
PULLIANEN E., 1965: Studies on the weight, ford and feeding behaviour of the partridge (*Perdix perdix* L.) in Finland. Ann. Acad. Scien. Fenn. A, 4: 3–24.
PULLIANEN E., 1968: Autumn weight of the partridge (*Perdix perdix* L.) in Finland. Ann. Zool. Fenn. 5: 241–244.
RANDS M.R.W. 1985: Pesticide use on cereals and the survival of grey partridge chicks: A field experiment. J. Appl. Ecol. 22 (1): 49–54.
SMITH R.K., JENNINGS N.V., HARRIS S., 2005: A quantitative analysis of the abundance and demography of European hares *Lepus europaeus* in relation to habitat type, intensity of agriculture and climate. Mamm. Rev. 35: 1–24.
SZWYKOWSKA M.M., 1969: Seasonal changes of the caloric value and chemical composition of the body of the partridge (*Perdix perdix* L.). Ekol. Pol. 17: 795–809.
WASILEWSKI M., 2007: Drapieżnictwo a zwierzyna drobna. In: Nauka łowiectwu, cz. 1. Kryzys zwierzyny drobnej i sposoby przeciwdziałania. Wyd. Samorząd Województwa Mazowieckiego, Warszawa: 34–38.
WITEK T., 1991: Warunki przyrodnicze produkcji rolnej, woj. lubelskie. IUNiG, Puławy.
WÜBBENHORST D., LEUSCHENER Ch., 2006: Vegetation structure at the breeding sites of the partridge (*Perdix perdix* L.) in central Europe and its possible importance for population density. Pol. J. Ecol. 54 (1): 57–67.

Streszczenie: *Struktura płci i wieku oraz masa ciała kuropatw (*Perdix perdix *L.) w okresach dużego i małego zagęszczenia ich populacji na Wyżynie Lubelskiej.* Badania struktury płci i wieku oraz masy ciała kuropatw prowadzone były na Wyżynie Lubelskiej w latach 1988 i 2015, w których był różny poziom zagęszczenia tych zwierząt. Wymienione cechy oceniono u 104 osobników odstrzelonych na początku października. Między porównywanymi okresami odnotowano prawie czterokrotny spadek poziomu zagęszczenia populacji tego gatunku. W tym samym okresie wystąpił ponad 80-krotny spadek łowieckiego pozyskania kuropatw. Stwierdzono także zmniejszenie się udziału samic w populacji po okresie rozrodu, co prawdopodobnie wynikało ze zwiększenia się śmiertelności tej płci w sezonie lęgowym. Proporcje liczby osobników młodych do dorosłych (2,4 i 1,7) oraz do dorosłych samców (4,0 i 1,7) w dwóch kolejnych latach badań, czyli wskaźniki produkcji młodych, nie różniły się istotnie, choć mogło to wynikać głównie z małej próby w drugim z tych lat. Struktura płci młodych kuropatw wynosiła 0,94 (samiec : samica) w 1988 roku oraz 0,71 (samiec : samica) i nie różniła się istotnie między okresami ($\chi^2 = 0{,}178$). Struktura płci osobników dorosłych wyniosła w 1988 roku 1,5 (samiec : samica). W 2015 roku odnotowano jedynie samce. Masa ciała młodych kuropatw zmniejszyła się między latami badań o 22,5 g, tj. 6%, zaś osobników dorosłych o 12,5 g. Wskazuje to na zmniejszenie wielkości ptaków, a tym samym kondycji osobniczej, prawdopodobnie na skutek ograniczenia zasobów pokarmowych. Różnice masy ciała między okresami były istotne tylko u osobników młodych. Tego rodzaju sytuacja może powodować zwiększoną śmiertelność młodych kuropatw jesienią i zimą, a tym samym pogłębiać trwający regres liczebności tego gatunku. Uzyskane wyniki potwierdziły, że wcześniejsze oceny przyczyn spadku liczebności kuropatw w Polsce, wskazujące wzrost strat w sezonie rozrodczym jako główny mechanizm demograficzny, były aktualne także w odniesieniu do drugiej dekady XXI wieku. Wynika stąd, że poprawianie warunków środowiskowych podczas sezonu rozrodczego, oprócz redukcji drapieżników, powinno być zasadniczą częścią programów czynnej ochrony kuropatw.

Słowa kluczowe: kuropatwa, masa ciała, wiek, płeć, zagęszczenie populacji, Wyżyna Lubelska

MS received 18.03.2016
MS accepted 10.10.2016

Authors' address:
Marian Flis
Katedra Zoologii, Ekologii i Łowiectwa
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13, 20-950 Lublin
Poland
e-mail: marian.flis@up.lublin.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 75–79
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.9

Effect of mating nucs spacing and subspecies of honey bee (*Apis mellifera*) on the drifting of queens returning from mating flights

JAKUB GĄBKA, MONIKA STAWICKA, BARBARA ZAJDEL, ZBIGNIEW KAMIŃSKI
Apiculture Division, Warsaw University of Life Sciences – SGGW

Abstract: *Effect of mating nucs spacing and subspecies of honey bee (*Apis mellifera*) on the drifting of queens returning from mating flights.* The loss of honeybee queens during mating flights increases the cost of their production. The aim of the study was to examine if the spacing of nucs influences the drifting of queen honey bees, which return from mating flights. The study also compared the drifting of Carniolan (*A. m. carnica*) and Italian (*A. m. ligustica*) queens. We examined the total of 89 queens which were placed in mating nucs together with about 1,000 workers. Some of the mating nucs were arranged in rows spaced 30 cm apart, without any landmarks, and other nucs were spaced a few meters apart, next to trees or bushes. Each group of nucs included Carniolan and Italian queens. The results show that significantly more queens failed to return from mating flights to nucs placed in rows without any landmarks (51%) than from those placed next to trees or bushes (7%). The study also showed that there is no significant differences between level of drifting of Carniolan and Italian queens.

Key words: beekeeping, honey bee, mating flights, drifting of honeybee queens, *Apis mellifera*

INTRODUCTION

During the mating flights, some honey bee queens are lost (Ratnieks 1990, Schlüns et al. 2005, Perez-Sato et al. 2008, Gąbka 2009). Losses of queens may reach 20% (Palmer and Oldroyd 2000, Medina and Gonçalves 2001) but in the colonies standing in a row at a close distance from one another, without any trees or bushes in the vicinity, the losses can be as high as 40% (Gąbka 2009). Also in case of workers about 40% of them drift away from colonies arranged in rows (Pfeiffer and Crailsheim 1998). One factor that is considered important in queen loss is the drifting of returning queens to a foreign hive in the apiary. Such mistakes are almost always fatal because workers kill any alien queens that they detect (Ribbands 1953). On the contrary, foreign foragers carrying a load of honey are accepted in bee colonies. Also drones gain admittance into strange colonies very easily (Washington 1967). Queen loss during mating is disastrous for the colony, because at the time of the queen's mating flight there is no open brood in the nest to rear a new queen. Besides, in mass production of egg laying honeybee queens, losses of queens during mating flights increase the cost of their production. Most of the bees, which drift, do so during their orientation flights

and before they become regular foragers (Free 1958). Also in the case of queens, drifting occurs on the maiden orientation flight. Queens on later flights do not drift (Perez-Sato et al. 2008).

Bees learn the colour of their hive in the close vicinity of the entrance, take little or no notice of colours above the lower brood chamber, and orient to a colour below the entrance more than to one above it. They do not learnt combinations of colours, but they distinguish between certain symbols placed immediately above the hive entrance. Moreover, foragers learn the height of their hive, and the height of its entrance above the ground (Free and Spencer-Booth 1961). Always more bees drift from a centre colony to the end colonies of a row than vice versa (Free 1958, Jay 1965, 1966a, b). There is no preference of drifting bees for related colonies (Pfeiffer and Crailsheim 1998). Neumann et al. (1997) found that drifters from neighbouring colonies prefer to go to other colonies rather than the neighbour. On the contrary, Pfeiffer and Crailsheim (1998) reported that most bees drift into the colony next to their original colony. According to Jay (1968, 1969a, b, 1971), three methods are effective in reducing drifting between hives: (i) the use of irregular or non-repetive layouts (patterns) of hives, and placing the hives within each layout to face different directions; (ii) the use of coloured hives, or coloured strips above or below the hive entrences (black, white, yellow, blue); (iii) the use of landmarks near hives (trees, bushes, fences etc.).

It is commonly believed that Italian bees are more prone to drift than the other subspecies. On the contrary, in Skowronek (1996) investigations level of drifting for Italian (*A. m. ligustica*) drones was less than 2%, whereas for middle European (*A. m. mellifera*) over 20%. Danka et al. (2006) found no differences in the drifting of yellow Italian bees and black Russian bees.

The aim of the study was to examine the influence of mating nucs spacing on the drifting of honeybee queens returning from mating flights. It also compares the drifting level of Carniolan and Italian queens.

MATERIAL AND METHODS

The experiment was performed in Apiculture Division at Warsaw University of Life Sciences – SGGW in 2012. Research was performed on 89 bee queens, including 46 Carniolan (*Apis mellifera carnica*) and 43 Italian (*Apis mellifera ligustica*) ones. Queens rearing was performed in two strong Carniolan nurse-colonies. First, the queens were taken away from these colonies, and after 9 days, when no more open brood was left, emergency queen cells were removed. This way the bees accepted more larvae introduced to be reared as queens (Gąbka et al. 2010). On the next day, 60 larvae from a purebred Carniolan queen and 60 larvae from a purebred Italian queen were grafted and introduced into the nurse-colonies. The larvae up to 1 day old were selected, as the age of brood used to rear queens affects their quality (Jordan 1960, Woyke 1971). After 10 days, queen cells were removed from the colonies and placed in three-comb trapezoidal mating nucs together with about one thousand of worker bees in each; 46 nucs had Carniolan queen cells, and 46 had Italian queen cells placed in them. For each subspecies, 25 nucs

were arranged in rows, 5 nucs in each, spaced about 30 cm apart, and 21 nucs were placed next to various landmarks (bushes or trees) spaced several meters apart. Rows of nucs were situated in two parallel lines. The distance between neighbouring rows was about 10 m. All nucs were faced in the same direction and painted in the same colour. After 3 days, the nucs were examined to see if queens had emerged from queen cells. All queens were individually marked by number tags. After 10 days, following the queens' mating flights, their presence in the nucs was controlled.

Statistical comparisons were made using the Chi^2 test of independence. Calculations were performed using SPSS 21 software.

RESULTS AND DISCUSSION

Overall, 46 Carniolan queens and 43 Italian queens were obtained from 92 queen cells introduced into mating nucs. In the case of nucs arranged in rows without landmarks, 11 (44%) of the 25 Carniolan queens and 14 (58%) of the 24 Italian queens were lost, whereas in the case of nucs placed next to trees or bushes several meters apart, 1 (5%) of the 21 Carniolan queens and 2 (11%) of the 19 Italian queens were lost.

A significant influence of the arrangement of mating nucs was observed on the losses of queens returning from mating flights, both among Carniolan queens ($\chi^2 = 5.63$, $df = 1$, $P = 0.018$) and Italian queens ($\chi^2 = 5.107$, $df = 1$, $P = 0.024$). In total, in the case of nucs arranged in rows without any landmarks, highly significantly more queens were lost compared to nucs placed next to trees or bushes ($\chi^2 = 10.736$, $df = 1$, $P = 0.001$) – Table 1. This confirms the results previously obtained by Gąbka (2009). In his studies, 40% of queens were lost from colonies arranged in rows without any landmarks, compared to 12.3% of queens from colonies arranged in rows next to trees or bushes, and to as few as 2.6% of queens from hives randomly placed close to trees or bushes. Perez-Sato et al. (2008) reported no significant differences in the drifting of queens from mating nucs spaced at various distances from one another: 5% of queens from colonies spaced 2 m apart and 4% of queens, from colonies spaced 5 m apart, were lost. Similarly, in our studies the losses of queens from nucs arranged at distances of several meters away from one another amounted to 7%. Pfeiffer and Crailsheim (1998) reported that about 40% of bees drift away from colonies arranged in rows, and in Boylan-Pett et al. (1991) investigations, on average, only 42% of the total foragers in a colony originated from that colony. It confirms our results for queens.

TABLE 1. Queen loss during flights depending on mating nucs arrangement

Specification	Number of queens before flights	Number of lost queens	% of lost queens
Nucs in rows without landmarks	49	25	51 a
Nucs in no rows with landmarks	40	3	7 b
Total	89	28	31

Different letters indicate significant differences between the groups (Chi^2 test: $P < 0.05$).

No significant impact of honeybee subspecies on the drifting of queens was found either in the case of nucs arranged in rows ($\chi^2 = 0.327$, $df = 1$, $P = 0.567$) or those placed next to landmarks ($\chi^2 = 0.41$, $df = 1$, $P = 0.522$). In total, differences between losses of Carniolan and Italian queens were not significant ($\chi^2 = 0.664$, $df = 1$, $P = 0.415$) (Table 2). Also, Danka et al. (2006) did not find higher level of drifting for Italian workers and Skowronek (1996) stated even more drifting middle European than Italian drones. In contrast to these results, it is commonly believed that Italian bees are more prone to drift than the other subspecies.

TABLE 2. Queen loss during flights depending on honeybee subspecies

Specification	Number of queens before flights	Number of lost queens	% of lost queens
Apis mellifera carnica	46	12	26 a
Apis mellifera ligustica	43	16	37a
Total	89	28	31

The same letters indicate no significant differences between the groups (Chi^2 test: $P > 0.05$).

CONCLUSION

Significantly more queen honey bees are lost during mating flights from nucs arranged in rows without landmarks compared to nucs placed next to trees or bushes.

The honeybee subspecies does not affect the drifting of queens returning from mating flights. Level of drifting for Italian and Carniolan queen honey bees do not differ.

REFERENCES

BOYLAN-PET W., RAMSDEL D.C., HOOPIN-GARNER R.A., HANCOCK J.F., 1991: Honeybee foraging behavior, in-hive survival of infectious, pollen-borne blueberry leaf mottle virus and transmission of the virus in highbush blueberry. Phytopathology 81 (11): 1407–1412.

DANKA R.G., SYLVESTER H.A., BOYKIN D., 2006: Environmental influences on flight activity of USDA-ARS Russian and Italian stocks of honey bees (Hymenoptera: Apidae) during almond pollination. J. Econ. Entomol. 99 (5): 1565–1570.

FREE J.B., 1958: The drifting of honey-bees. J. Agric. Sci. 51 (3): 294–306.

FREE J.B., SPENCER-BOTH Y., 1961: Further experiments on the drifting of honey-bees. J. Agric. Sci. 57 (2): 153–158.

GĄBKA J., 2009: Błądzenie matek pszczelich powracających z lotów weselnych. In: Materiały konferencyjne konferencji naukowej pt. Nauka praktyce – produkcja zwierzęca w zrównoważonym rozwoju obszarów wiejskich, Ciechanowiec: 7.

GĄBKA J., KAMIŃSKI Z., MADRAS-MAJEWSKA B., 2010: The influence of development stage of brood used for rearing honeybee queens on the number of obtained queen cells. Rocz. Nauk. PTZ 6 (4): 241–245.

JAY S.C., 1965: Drifting of honeybees in commercial apiaries. I. Effect of various environmental factors. J. Apicult. Res. 4 (3): 167–175.

JAY S.C., 1966a: Drifting of honeybees in commercial apiaries. II. Effect of various factors when hives are arranged in rows. J. Apicult. Res. 5 (2): 103–112.

JAY S.C., 1966b: Drifting of honeybees in commercial apiaries. III. Effect of apiary layout. J. Apicult. Res. 5 (3): 137–148.

JAY S.C., 1968: Drifting of honeybees in commercial apiaries. IV. Further studies of the effect of apiary layout. J. Apicult. Res. 7 (1): 37–44.

JAY S.C., 1969a: Drifting of honeybees in commercial apiaries. V. Effect of drifting on honey production. J. Apicult. Res. 8 (1): 13–17.

JAY S.C., 1969b: The problem of drifting in commercial apiaries. Am. Bee J. 109 (5): 178–179.
JAY S.C., 1971: How to prevent drifting. Bee Wld 52 (2): 53–55.
JORDAN R., 1960: Die Zucht der Königin, ausgehend vom Ei. Bienenvater 81 (1): 3–7.
MEDINA L.M., GONÇALVES L.S., 2001: Effect of weight at emergence of africanized (*Apis mellifera* L.) virgin queens on their acceptance and beginning of oviposition. Am. Bee J. 141: 213–215.
NEUMANN P., MORITZ R. F., MAUTZ D., 1997: Testing the reliability of the Schwarzenau honey bee performance apiary using single locus DNA-fingerprinting. Apidologie 28 (3–4): 216–217.
PALMER K.A., OLDROYD B.P., 2000: Evolution of multiple mating in the genus Apis. Apidologie 31: 235–248.
PEREZ-SATO J.A., HUGHES W.O.H., COUVILLON M.J., RATNIEKS F.L.W., 2008: Effects of hive spacing, entrance orientation, and worker activity on nest relocation by honey bee queens. Apidologie 39: 708–713.
PFEIFFER K.J., CRAILSHEIM K., 1998: Drifting of honeybees. Insect. Soc. 45: 151–167.
RATNIEKS F.L.W., 1990: The evolution of polyandry by queens in social hymenoptera: The significance of timing of removal of diploid males. Behav. Ecol. Sociobiol. 26: 343–348.
RIBBANDS C.R., 1953: The behaviour and social life of honeybees. Bee Research Association Limited, London, England.
SCHLÜNS H., MORITZ R.F.A., NEUMANN P., KRYGER P., KOENIGER G., 2005: Multiple nuptial flights, sperm transfer and the evolution of extreme polyandry in honeybee queens. Anim. Behav. 70: 125–131.
SKOWRONEK W., 1996: Drifting behaviour of drones of different races. Pszczeln. Zesz. Nauk. 40 (2): 249–250.
WASHINGTON R.J., 1967: Drones. Am. Bee J. 108: 7–9.
WOYKE J., 1971: Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. J. Apicult. Res. 10 (1): 45–55.

Streszczenie: *Wpływ ustawienia ulików weselnych oraz podgatunku pszczoły miodnej (*Apis mellifera*) na błądzenie matek powracających z lotów godowych.* Straty matek pszczelich podczas lotów weselnych zwiększają koszty ich produkcji. Celem pracy było zbadanie, czy sposób ustawienia ulików weselnych wpływa na błądzenie matek powracających z lotów godowych. Porównano również błądzenie matek kraińskich (*A. m. carnica*) i włoskich (*A. m. ligustica*). Ogółem zbadano 89 matek, które znajdowały się w ulikach weselnych, z około tysiącem robotnic. Część ulików ustawiono w rzędach w odległości około 30 cm od siebie, a część przy drzewach lub krzewach w odległości kilku metrów. W każdej grupie ulików były matki kraińskie i włoskie. Stwierdzono, że z ulików weselnych ustawionych w rzędzie, bez punktów orientacyjnych ginie podczas lotów godowych istotnie więcej matek (51%) niż z ulików ustawionych przy drzewach lub krzewach (7%). Nie stwierdzono istotnych różnic między błądzeniem matek kraińskich i włoskich.

Słowa kluczowe: pszczelarstwo, pszczoła miodna, loty godowe, błądzenie matek pszczelich, *Apis mellifera*

MS received 18.03.2016
MS accepted 16.02.2017

Authors' address:
Jakub Gąbka
Pracownia Pszczelnictwa
Wydział Nauk o Zwierzętach SGGW
ul. Nowoursynowska 166, 02-787 Warszawa
Poland
e-mail: jakub_gabka@sggw.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 81–89
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.10

Comprehensive microbiological evaluation of dry foods for growing dogs marketed in Poland

KAROLINA HOŁDA[1], WIOLETTA WICZUK[2],
ELŻBIETA HAĆ-SZYMAŃCZUK[2], ROBERT GŁOGOWSKI[1]
[1] Department of Animal Breeding and Production
[2] Department of Biotechnology Microbiology and Food Evaluation
Warsaw University of Life Sciences – SGGW

Abstract: *Comprehensive microbiological evaluation of dry foods for growing dogs marketed in Poland*. Microbiological safety is one of the most important parts of qualitative assessment and monitoring of commercially available products intended for canine nutrition. Twenty commercial dry dog foods formulated for growing dogs were surveyed for the prevalence of bacterial contamination. To assess total plate counts of mesophilic strains, yeasts and molds, *Enterobacteriaceae* family and *Enterococcus* ISO standards were applied. Moreover, the presence of major pathogenic bacteria was evaluated. The growth of molds was detected in five products. *Enterobacteriaceae* strains were identified in 12 examined foods. *Escherichia coli* was identified in four samples. Half of the analyzed foods showed apparent presence of enterococci. All analyzed samples were free from S*taphylococcus*, *Salmonella* and *Listeria* spp. contamination. During microscopic confirmation of suspicious colonies *Bacillus* spp. were identified in seven products. The results of our pilot study allowed to conclude that the principles of good manufacturing practice and hygienic regime were generally respected during factory processing, resulting in a relative low risk, with a clear necessity for continued control.

Key words: microorganism, dog, pet food, safety

INTRODUCTION

The evaluation of the microbiological status of pet foods is an important element of the nutritional safety for animals and health security for owners. Between the harvesting of pet food ingredients, handling and preparation at home, and finally the consumption of the product, there appear multiple opportunities for microbial populations to proliferate.

Modern techniques of dry dog food production aim at preventing any microbial contamination of the final product. But still numerous recalls are being announced, resulting in withdrawal (often voluntary) of particular batches of products, potentially contaminated with *Salmonella* sp., the main bacteriological concern of the pet food sector (Behravesh et al. 2010).

Not many reports have been published to date related to the general microbial safety of specifically dry kibble diets for dogs (Behravesh et al. 2010, Nemser et al. 2014). However, in Poland some compound feed surveys have been previously conducted (Wojdat et al. 2004).

In the literature, the prevailing experimental setup was to compare all types of pet food for both dogs and cats in one study. In our opinion, it is more clear to present results of comprehensive analysis of a particular category of products intended for one species, on the basis of randomly selected group of samples, similar to the approach recently proposed by van Rooijen et al. (2014).

The aim of the present, preliminary study was to ascertain the potential presence of multiple foodborne pathogens in commercial dry products intended for feeding growing dogs.

MATERIAL AND METHODS

Sample collection

Twenty dry dog food products of various brands were purchased in specialized retail stores and through an Internet distribution channel. All examined items were randomly chosen from the previously compiled inventory of extruded products, labeled as intended specifically for young and growing dogs, and are currently available on the market.

Weights of collected bags were from the range of 0.4 to 1.4 kg. After purchasing, all factory-sealed bags were stored in room temperature (approx. 18–22°C) until analysis. The remoteness of best before date from all labels was carefully checked before opening. Right before the moment of sampling, all bags were precautionary gently washed with alcohol according to PN-EN ISO 6887-2:2005.

Procedures of sample analysis

Preparation of test samples and dilutions for microbial examination. The general preparation for analyses of tested dog foods was performed basing on International Standard ISO regarding microbiology of food and animal feeding stuffs PN-EN ISO 6887-1:2000. Samples of 20 g were aseptically weighed consecutively from each bag and transferred with sterile spoon into 180 ml of aseptic peptone water (bioMérieux, Warsaw, Poland). Of each initial dilution 1 ml was taken for further processing and consequently inoculated into Petri's dishes.

Enumeration of the total count of aerobic microorganisms. This assay aimed to reveal the number of colonies grown on the plate count agar (PCA) (Bio-Rad Laboratories, Hercules, USA) medium after incubation under aerobic conditions in 30°C for 72 h. The procedure was conducted according to PN-EN ISO 4833-2:2013 standard.

Enumeration of the total count of yeasts and molds. Deep culture plate method using Saubouraud agar with chloramphenicol (BTL, Warsaw, Poland) was applied according to PN-ISO 21527--2:2009 standard. Inoculated plates were incubated at 25°C for 5 days. The morphological identification of colonies was performed following the description in Martins et al. (2003).

Enumeration of *Enterobacteriaceae*. These bacteria have ability to ferment glucose with acid production. Two sets of plates with Violet Red Bile Lactose (VRBL) medium (Bio-Rad Laboratories, Hercules, USA) were deep cultured in compliance with PN-ISO 21528-2:2005 standard. The counting was performed after 48 h incubation at 37°C.

Enumeration of coliforms. Endo agar (BTL, Warsaw, Poland) was used to enumerate coliforms in samples accord-

ing to PN-ISO 4832:2007 standard. Lactose fermenting bacteria after incubation at 37°C for 48 h present growth in deep red colonies.

Isolation and enumeration of *Enterococcus* spp. (*E. faecium*). In compliance with PN-EN 15788:2009 standard, Slanetz and Bartley medium (Oxoid, Basingstoke, UK) was used as a selective medium. Plates were incubated at 37°C for 24 h.

Detection of staphylococci. The differentiation of coagulase-positive bacteria is based on Baird Parker Agar with egg-yolk tellurite emulsion (bioMérieux, Warsaw, Poland) according to PN-EN ISO 6888-1:2001 standard. Species that contain lecithinase cause clear zones around the colonies whereas the reduction to elemental Te stains the bacteria black. The potential presence of *Staphylococcus aureus* was tested using catalase test.

***Salmonella* detection.** The following procedure was applied according to PN-EN ISO 6579:2003 standard. Carefully weighed 25 g of the sample was transferred to 225 ml of buffered peptone water and incubated in 37°C for 18 ±2 h, for pre-enrichment. Approximately 0.1 ml of the suspension was then incubated on Rappaport-Vassiliadis Soy broth (RVS) (Bio-Rad Laboratories, Hercules, USA) in 42.5°C for 24 ±3 h. Subsequently, the plating on the selective Brilliant Green Agar (BGA) (Oxoid, Basingstoke, UK) and Xylose Lysine Deoxycholate (XLD) (Bio-Rad Laboratories, Hercules, USA) was performed, followed by an incubation at 37°C for 48 ±3 h and 24 ±3 h, respectively.

***Listeria* detection.** The procedure for *Listeria* spp. detection was as follows (PN-EN ISO 11290-1:1999/A1:2005 modified standard): 25 g of the sample was transferred to 225 ml of Half-Fraser broth (Bio-Rad Laboratories, Hercules, USA) (for incubation in 30°C for 24 h), than transferring 1 ml into Fraser's broth (Bio-Rad Laboratories, Hercules, USA) (for incubation in 37°C for 48 h). Subsequently, one loopful from each tube was streaked onto Oxford Listeria Selective Agar Base supplemented with Oxford Listeria Selective Supplement (Merck, Darmstadt, Germany), (in amount of 1 vial/500 ml), and incubated at 37°C for 48 h.

Macroscopic and microscopic identifications. The following features were evaluated in all obtained cultures: color, size and surface type of the colony as well as description of color changes of the diagnostic (Martins et al. 2003, Pitt and Hocking 2009). Mold colonies and questionable bacterial cultures (subsequently to Gram stain test) were screened with MB300 microscope (OPTA-TECH, Warsaw, Poland).

Results were statistically modified using PS IMAGO 3.0 software.

RESULTS AND DISCUSSION

Twenty commercially available dry extruded products intended for young and growing dogs were analyzed for the potential presence of foodborne pathogens of bacterial and fungal origin (Fig.), showing the growth of at least one strain. The highest number of different microbial and/or fungal strains (4) was revealed in samples 4, 17 and 18. Subsequently, in products 1, 3, 8 and 12 the presence of three various pathogenic microorganisms was revealed. In the remaining

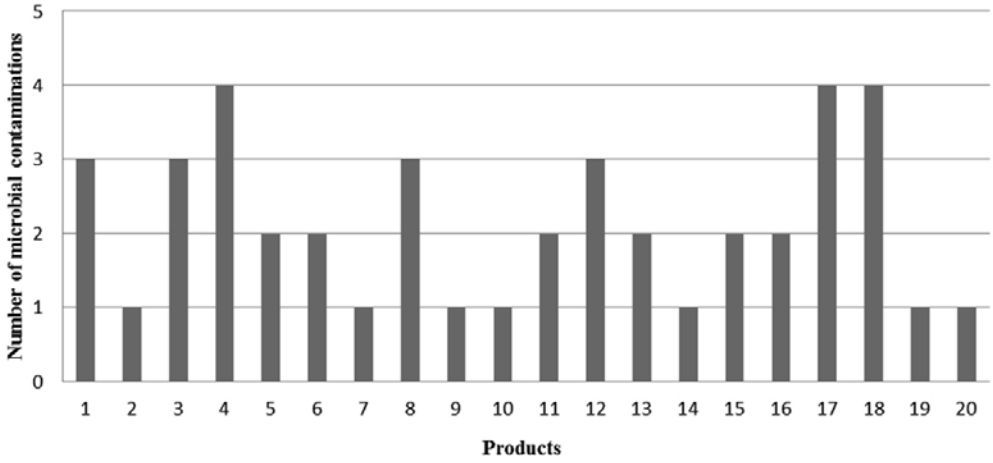

FIGURE. The number of microbiological contaminations detected in dry dog foods

group of products (13) one or two viable microbial strains were detected. In total, 10 different microbiological groups and genera were assayed in the samples.

Typical growth of aerobic bacteria was detected in 15 products studied ranging between 1.0×10^1 to 2.7×10^2 cfu/g (Table). The growth of moulds was detected only in 5 products (25%) with the highest number of 2.0×10^1 cfu/g. The microscopic examination identified *Aspergillus*, *Penicillium* and *Rhizopus* species within the observed colonies. Brewer's yeast were listed as an ingredient on the labels in 8 of 20 analyzed products but no growth of yeast-like colonies was observed. *Enterobacteriaceae* strains were identified in 12 examined products (60%) within the range of $1.0 \times 10^1 – 2.3 \times 10^2$ cfu/g. *Escherichia coli* was identified in 4 (20%) samples with relatively low count (10–50 cfu/g). Half of the analyzed foods showed apparent presence of enterococci, with the range of the bacterial presence from 1.0×10^1 to 7.0×10^1 cfu/g. Bacterial growth, indicating staphylococci presence was noted in 11 samples (55%). However, during macroscopic identification small size of colonies, no changes in the substrate color and the "in substrate" growth were observed. Subsequent negative catalase test results in all 11 samples suggested that the observed growth was more likely a result of anaerobic enterococci presence. All analyzed pet food samples were free from *Salmonella* contamination. No bacterial colony observed both on XLD and BGA media showed typical symptoms of the pathogenic growth. *Listeria* spp. were not isolated from any sample. During microscopic confirmation of suspicious bacterial colonies, stained according to Gram's method, *Bacillus* spp. were identified in 7 products.

It is estimated that currently most of the dry pet foods are produced worldwide with the implementation of extrusion technology. In dry expanded extruded products for pets, the final moisture content has to be lower than 10% to

TABLE. Microbiological presence in dry pet food samples (expressed as log cfu/g)

Product	TAMBC	Yeasts and molds	*Enterobacteriaceae*	*Escherichia coli*	*Enterococcus*
1	1.301	<1	2.113	<1	1.477
2	<1	<1	<1	<1	1.602
3	1.602	1.000	1.000	<1	<1
4	1.301	1.301	2.301	1.477	<1
5	2.301	<1	2.113	<1	<1
6	1.845	<1	2.361	<1	<1
7	<1	<1	2.361	<1	<1
8	1.954	<1	1.000	<1	1.301
9	<1	<1	1.602	<1	<1
10	<1	<1	<1	<1	1.000
11	2.000	<1	<1	<1	1.698
12	1.000	1.000	<1	1.000	<1
13	1.301	1.000	<1	<1	<1
14	1.954	<1	<1	<1	<1
15	1.301	<1	<1	<1	1.845
16	<1	<1	1.000	<1	1.301
17	1.903	<1	1.301	1.000	1.477
18	2.431	<1	1.477	1.698	1.000
19	1.000	<1	<1	<1	<1
20	1.602	<1	<1	<1	<1

TAMBC – total aerobic mesophilic bacteria count; <1 – under the detection limit (10 cfu/g).

prevent mold and bacterial growth. The hygienic quality of the final product is affected by the process of conditioning of raw materials with the use of heat, water, pressure and time (Thomas et al. 1997). The producer has to guarantee that within the shelf-life period that is declared on the label, the product will maintain its microbiological safety at specific storage conditions. This declaration is based on identified hazards for the product, heat or other preservation treatments and packaging methods and materials. Microbiological evaluation is a part of raw ingredients vendor control programs, routinely implemented in pet food producing plants, as well as finished product quality testing procedures.

The recent highly publicized outbreaks and recalls have caused a major review of microbiological control programs and reinforced the idea of going beyond traditional factory quality management processes.

Since 1920, various additives have been used in animal foods. The terms additive and preservative are often perceived synonymously. The latter has

highly negative implication for consumers (pet owners/parents) who are making purchase decisions. However, Annex I to the Regulation (EC) 1881/2006 on additives used in animal nutrition, lists preservatives in the category of "technological additives" defining them as substances or, when applicable, micro-organisms which protect feed against deterioration by micro-organisms or their metabolites'.

On the other hand, obligatory declaration of the content of micro-organisms that have a positive, stabilizing effect on the gut flora, added to the formulation must be placed on the label according to Regulation (EC) 767/2009.

In fact, regarding merely few additives with the maximum legal limits from "preservatives" and "micro-organisms" groups, the content declaration on the label is mandatory. Another Regulation (EC) 1831/2003 allows using exclusively names of functional groups on the label. Other feed additives used can be voluntary declared on the labels.

In the current study all positive samples of dog foods showed low levels of contamination regardless of the strain or species assayed. These results confirm earlier reports, describing the Polish compound feed market as generally safe (Wojdat et al. 2004). It has to be stated however, that precise determination of the microbiological safety in the category of dry dog foods based on combined results of the studies cited above is unfeasible. Extruded dry canine diets were previously considered not a good substrate for microbial growth (Adelantado et al. 2008).

Considering this, it could be anticipated that in majority of dry products commercially available, various additives preventing growth of bacterial and fungal cells were present.

An analysis of the labels content of foods assessed in the current study revealed few examples of voluntary declaration of such substances. On the majority of the labels (13 of 20; 65%) the presence of antioxidants and/or preservatives was declared. In two cases an additional note of UE approval of chemicals used was found. One producer declared citric acid used as a "natural" preservative and on another list of technological additives pentasodium triphosphate was reported.

In the current study two products listed "dried *Enterococcus faecium* fermentation product" after the ingredients heading. Interestingly, its presence was experimentally confirmed in only one of the two products. Moreover, the *E. faecium* strain was detected in 8 more products, most likely due to the cross contamination during processing or incorrect labeling.

Among the probiotic bacterial species, those of the genus *Bacillus* are not the most commonly used, but their use and effectiveness are documented in numerous reports (Biourge et al. 1998). Their apparent presence in 7 of the assayed products in this study may be the result of their ubiquity in the environment, as much as an apparent reflection of quality control point weaknesses in the processing plant.

It has to be noted, that the strict regulations overseeing maximum limits of particular bacterial and fungal contamination for pet foods have not been yet been established. For example the Regulation (EC) 183/2005 stating the general

rules of feed hygiene does not apply to retail pet food.

Apparently more relevant is the Commission Regulation (EU) 142/2011, concerning health rules of animal by-products and derived products not intended for human consumption. According to this document, in processed petfood *Salmonella* has to be absent in 25 g randomly-taken sample, whereas *Enterobacteriaceae* must not exceed 3.0×10^2 in 1 g. In the current study, contamination with *Enterobacteriaceae* was relatively high (2.3×10^2 in products 6 and 7) in 2 products, yet the threshold was not exceeded. This observation, along with the complete absence of *Salmonella* in the examined products likely confirms previously reported general ascertainment that dry dog foods pose low microbiological threat for animals and humans (Adelantado et al. 2008).

In the fungal flora category, one study reported the contamination of various dry pet foods (n = 20) within the range of 10^1–10^2 cfu/g underlining low levels of mold presence (Martins et al. 2003). Our assessments are even more encouraging considering that within the products for young and growing dogs in only one sample the cfu count for molds was above 10.

In the recently published paper, both human and pet *Salmonella* exposure associated with dry pet food was estimated. The strengths and weaknesses of current production processes and consumer handling in household were highlighted including a discussion of an ingredients-to-consumer quantitative assessment model. Authors pointed to an urgent need for more information on contamination levels in ingredients and validation studies of production steps critical to microbial reduction and in-plant cross-contamination prevention (e.g. pre-conditioning, oven drying), as well as increased understanding of cross-contamination routes were would likely improve exposure estimates. Maintaining proper hygienic behavior by consumers can also be crucial in lowering the likelihood or the extent of potential exposure. However, the accuracy, precision, and overall usefulness of these models is highly dependent on the availability and quality of input data. Therefore, conclusions from modeling exercises should be drawn with caution (Lambertini et al. 2016).

CONCLUSION

Microbiological safety control during the production of complete dog foods with low water activity requires scrutinizing the ingredient quality, numerous microbial reduction steps, avoidance of potential cross-contamination and a constant control of moisture. This report presents results that support a relative low risk linked with the dry complete foods for growing dogs. Similar methodological approach, applied to other categories like adult, senior or special pet foods, may improve quality control efficacy of in-plant processing steps that would accordingly improve pets and pet owners safety.

REFERENCES

ADELANTADO C., LÓPEZ S., INGLADA R., VILASECA L., CALVO M.A., 2008: Viability of micro-organisms involved in outbreaks of bacterial food borne diseases in dry extruded pet food. Biotechnology 7: 725–731.

BEHRAVESH C.B., FERRARO A., DEASY M., DATO W., MOLL C., SANDT C., REA N.K., RICKERT R., MARRIOTT C., WARREN K., URDANETA V., SALEHI E., VILLAMIL E., AYERS T., HOEKSTRA R.M., AUSTIN J.L., OSTROFF S., WILLIAMS I.T., 2010: Human *Salmonella* infections linked to contaminated dry dog and cat food, 2006–2008. Pediatrics 126: 477–483.

BIOURGE V., VALLET C., LEVESQUE A., SERGHERAERT R., CHEVALIER S., ROBERTON J.-L., 1998: The use of probiotics in the diet of dogs. J. Nutr. 128: 2730S–2732S.

Commission Regulation (EC) No 1831/2003 of 22 September 2003 on additives for use in animal nutrition. L 268: 29–43.

Commission Regulation (EC) No 183/2005 of 12 January 2005 laying down requirements for feed hygiene. L 35: 1–22.

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. L 364: 5–24.

Commission Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed. L 229: 1–28.

Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. L 54: 1–254.

LAMBERTINI E., BUCHANAN R.L., NARROD C., FORD R.M., BAKER R.C., PRADHAN A.K., 2016: Quantitative assessment of human and pet exposure to *Salmonella* associated with dry pet foods. Int. J. Food Microb. 216: 79–90.

MARTINS M.L., MARTINS H.M., BERNARDO F., 2003: Fungal flora and mycotoxins detection in commercial pet food. Rev. Port. Cienc. Vet. 98: 179–183.

NEMSER S.M., DORAN T., GRABENSTEIN M., McCONNELL T., McGRATH T., PAMBOUKIAN R., SMITH A.C., ACHEN M., DANZEISEN G., KIM S., LIU Y., ROBESON S., ROSARIO G., McWILLIAMS WILSON K., REIMSCHUESSEL R., 2014: Investigation of *Listeria*, *Salmonella* and toxigenic *Escherichia coli* in various pet foods. Foodborne Pathog. Dis. 11: 706–709.

PITT J.I., HOCKING A.D., 2009: Fungi and food spoilage. Springer Science, New York.

PN-EN 15788:2009. Animal feeding stuffs – Isolation and enumeration of *Enterococcus* (*E. faecium*) spp.

PN-EN ISO 11290-1:1999/A1:2005. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1. detection method.

PN-EN ISO 4833-2:2013. Microbiology of the food chain. Horizontal method for the enumeration of microorganisms. Part 2. Colony count at 30 degrees C by the surface plating technique.

PN-EN ISO 6579:2003. Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp.

PN-EN ISO 6887-1:2000. Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal solutions for microbiological examination.

PN-EN ISO 6887-2:2005. Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal solutions for microbiological examination. Part 2. Specific rules for the preparation of meat and meat products.

PN-EN ISO 6888-1:2001. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1. Technique using Baird-Parker agar medium.

PN-ISO 21527-2:2009. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds. Part 1. Colony count technique in products with water activity less than or equal to 0.95.

PN-ISO 21528-2:2005. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Enterobacteriaceae*. Part 2. Colony count method.

PN-ISO 4832:2007. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coliforms. Colony-count technique.

THOMAS M., Van ZUILICHEM D.J., Van Der POEL A.F.B., 1997: Physical quality of pelleted animal feed. 2. contribution of processes and its conditions. Anim. Feed Sci. Technol. 64: 173–192.

Van ROOIJEN C., BOSCH G., Van Der POEL A.F.B., WIERENGA P.A., ALEXANDER L., HENDRIKS W.H., 2014: Quantitation of Maillard reaction products in commercially available pet foods. J. Agric. Food Chem. 62: 8883–8891.

WOJDAT E., KWIATEK K., ZASADNY R., 2004: Microbiological quality of petfood in Poland. Pol. J. Vet. Sci. 7: 207–209.

Streszczenie: *Kompleksowa ocena jakości mikrobiologicznej dostępnych w Polsce suchych karm dla psów rosnących*. Jakość mikrobiologiczna to jedno z najistotniejszych kryteriów kontroli systemu produkcji i dystrybucji rynkowej produktów żywienia psów. Dwadzieścia dostępnych w sprzedaży produktów dla psów rosnących poddano ocenie czystości mikrobiologicznej. Analizy przeprowadzono ściśle według metodyki zgodnej z aktualnymi normami PN-ISO dotyczącymi oznaczania bakterii mezofilnych, drożdży i pleśni, przedstawicieli rodzin *Enterobacteriaceae* oraz *Enterococcus*. W badaniu oznaczono również obecność ważniejszych organizmów patogennych. Wzrost pleśni stwierdzono w pięciu przypadkach. W dwunastu produktach zaobserwowano bakterie z rodziny *Enterobacteriaceae*. Połowa spośród ocenianych karm okazała się pozytywna pod względem enterokoków. Wszystkie oceniane produkty były wolne od *Staphylococcus*, *Salmonella* i *Listeria* spp. Dla siedmiu produktów przeprowadzono obserwacje mikroskopowe podejrzanych kolonii, identyfikując je jako *Bacillus* spp. Uzyskane w niniejszym, pilotażowym badaniu wyniki pozwalają stwierdzić, że kluczowe elementy dobrych praktyk produkcyjnych są na ogół zachowywane podobnie jak kryteria higieniczne podczas procesów technologicznych. Ze stosowaniem karm suchych wiąże się relatywnie małe ryzyko wystąpienia u psów poważnych schorzeń o podłożu mikrobiologicznym, co nie wyklucza konieczności stałego monitoringu jakości produktów dostępnych na polskim rynku.

Słowa kluczowe: mikroorganizm, pies, karma dla pupila, bezpieczeństwo

MS received 04.01.2017
MS accepted 08.04.2017

Authors' address:
Robert Głogowski
Zakład Hodowli Zwierząt Futerkowych
Towarzyszących i Koni
Katedra Szczegółowej Hodowli Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8
02-786 Warszawa
Poland
e-mail: robert_glogowski@sggw.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 91–102
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.11

Respecting EU cross-compliance requirements as an indicator of animal welfare on dairy farms in Poland

MONIKA JANOCHA, TADEUSZ KOŚLA, EWA M. SKIBNIEWSKA
Department of Animal Environment Biology, Warsaw University of Life Sciences – SGGW

Abstract: *Respecting EU cross-compliance requirements as an indicator of animal welfare on dairy farms in Poland.* The aim of the study was to determine the level of animal welfare on dairy farms in Poland and to compare the measured parameters with the cross-compliance requirements. The study was conducted on 46 farms in the winter season. The microclimate measurements such as air moisture content, concentrations of selected air pollutants and the brightness of the room has been taken. A comparison of cattle housing systems on bedding and on the slatted floor has been examined. The barns were divided into four groups, depending on the size of the herd: 10–20 cows (12 barns), 21–40 cows (22 barns), 41–60 cows (7 barns), and 100–180 cows (5 barns), respectively. The largest group consisted of haerds housing 21–40 cows, which represented 48% of all herds involved in the study. Of all the barns that used slatted floors or bedding, we selected three largest herds each in order to compare hygienic parameters between both types of housing. The main findings were as follows: (1) the concentration of selected air pollutants in most of the barns did not deviate from the recommendations of the (Polish) National Research Institute of Animal Production; (2) the concentration of selected air pollutants was lower in barns where the cattle was kept on slatted floors; (3) the cattle housed on slatted floors also had better lighting conditions; (4) smaller herds of dairy cows were found to have relative air humidity conditions; (5) in 81% of farms, air humidity in the premises remained within the animal welfare standards.

Key words: dairy cows, housing, environment, microclimate, EU requirements

INTRODUCTION

Thanks to the Common Agricultural Policy of the European Union, farmers are able to produce food at prices affordable to consumers in exchange for direct payments that support the viability of production (ARiMR 2012). This direct aid is paid subject to the condition that the farm owners to all the standards within the scope of rules called cross-compliance (ARiMR 2012, Nowak 2013). Animal welfare standards have been in force since 1 January 2013 (Pośniak-Sobczyńska 2011). Farmers who keep animals must ensure that the humidity and concentration of selected target gases in the housing facilities are below acceptable levels and that lighting is appropriate. Regulated parameters are stocking density of animals, proper size of the stalls and their appropriate technical standards, sufficient manure removal, adequate care provided to animals and appropriate placement of technological equipment used in the production process (Sundrum et al. 1994, Bartussek 2000, Veissier et al. 2008, Bartussek et

al. 2011). Failure to comply with cross-compliance requirements will result in reduction or even withdrawal of direct EU payments (Kuczaj 2010, Pośniak-Sobczyńska 2011, Nowak 2013).

The cross-compliance are supervised by the Agency for Restructuring and Modernisation of Agriculture and the Veterinary Inspection. Checks by the Veterinary Inspection include the quality of animal welfare (ARiMR 2012). In the case of dairy cows, cross-compliance requirements do not specify the size of the stalls (Kuczaj 2010). These are dealt with by the Regulation of the Minister of Agriculture and Rural Development No 56, item 344 from 2010 although not all of the provisions contained in the Regulation are cross-compliance requirements (ARiMR 2012).

Dairy cattle housing premises should be equipped with fixed or portable lighting. The construction and arrangement of the stalls should eliminate the risk of injuries for the animals and should be made of materials facilitating their cleaning and disinfection. Sick or injured animals should be adequately taken care of and separated from the herd. They should be managed on straw bedding. The cattle must have freedom of movement. The farmer is obliged to ensure optimum microclimate conditions inside the livestock facilities (Averós et al. 2013), involving the control of particulate matter in the air, humidity, temperature, and concentration of selected gases (www.mrirw.gov.pl, Wyrębski and Reklewski 2000). The microclimate in the barn can be conditioned by means of ventilation systems and air humidification or dehumidification equipment. Animals themselves emit heat, moisture and gases, which has a major impact on the climate in the barn (Radoń 2005, Kołacz and Dobrzański 2006, Kośla 2011).

A dairy cow cubicle must fit to the size of the animal and to the type of tether. The breed of the cow must also be taken into account (Lenard 1993). The material used for the cubicle construction must be safe for the animals, without sharp edges that could cause injury. Cubicle separations should not be too long so as not to interfere with the passageway. It is recommended that they be approx. 30 cm shorter than the resting place of the cow. The curb between the alley and the stall should be 20–25 cm high. The cow is then unable to enter backwards into the stall, or to lie with her hind legs on the passageway (Romaniuk et al. 2004, Litwińczuk and Szulc 2005). Cubicles are mainly made of steel pipes. An important element is the neck rail, which should be installed at a height allowing cows to lie down and get up without risk of injuries. In the USA and Italy, the neck rail is installed at a height of 140 cm. In France, an adjustable neck rail has been designed, which allows changing in the range of from 125 to 130 cm, depending on the height at the withers (Dagorn 2008).

According to Czerniawska-Piątkowska et al. (2008), cows housed in free-stall system barns attain higher yields and produce better milk quality in terms of fat and protein, as compared with cows managed in the tie-stall system. Free-stall barn cows also demonstrate higher fertility. Moreover, Kaczor and Paschma (2008) and Kaczor et al. (2013) report that free-stall housing of heifers and cows promotes better cleanliness of animals, as compared with stanchion or tie-stall barns.

The aim of the study was to determine the level of animal welfare in dairy farms and to compare the measured parameters with the cross-compliance requirements. Specifically, parameters such as carbon dioxide, ammonia, hydrogen sulfide, relative humidity and light intensity we measured and compared.

MATERIAL AND METHODS

The herds

The data were collected in 46 barns divided into 4 groups, depending on the size of the herd: 10–20 cows (12 barns), 21–40 cows (22 barns), 41–60 cows (7 barns), 100–180 cows (5 barns). Due to the lack of barns housing between 60 and 100 cows, no such group has been created. The largest group consisted of herds housing 21–40 cows, which represented 48% of all herds involved in the study.

Survey methods

The survey was carried out during the winter of 2012–2013, in dairy cattle farms located in the Tomaszów Mazowiecki County, Łódź Voivodship, Poland. All measurements were performed between 9:30 and 15:00. In all the evaluated barns, we carried out a single measurement of the concentration of selected gases: carbon dioxide (CO_2), ammonia (NH_3) and hydrogen sulfide (H_2S). The measurements were done using the Gas Hunter IR (Alter, Poland), which allows simultaneous measuring of three gases (ppm). Relative humidity (%) was measured using a DT-8820 multifunctional environment measuring instrument. Light intensity (lx) was measured at a cow head height. Information on the floor type was also collected, whether cows were accommodated on slatted floor or bedding. The parameters were compared in relation to the floor type (slatted floor – bedding). The results were processed using the Statistica 12.0™ software (StatSoft, Inc., Kraków, Poland) package and the differences were compared by a non-parametric test.

RESULTS AND DISCUSSION

Concentration of selected gases

According to the Information Bulletins of the National Research Institute of Animal Production (Karta informacyjna IZ 10101), also other literature confirm these data (Kośla 2011, Majchrzak and Mazur 2012, Kaczor et al. 2013, Nowak 2013), the concentration of selected gases in the premises for cattle should not exceed the following levels: CO_2 0.3% (3,000 ppm), NH_3 0.0026% (26 ppm), and H_2S 0.001% (10 ppm).

Cross-compliance requirements for adult cattle do not specify acceptable concentration levels of gases; instead, one can learn that the animals must be kept in conditions that are not "harmful" (Journal of Laws 2010 No 116, item 778, with amendments).

Our study has shown (Table 1 and Fig. 1) that the average concentration of CO_2 inside the barns was 2,269 ppm, ranging from 730 to 4,500 ppm. Figure 1 shows the means of the values measured in four groups of barns (with standard deviations). The lowest CO_2 levels were measured in barns housing 100–180 cows.

The concentration of CO_2 should not exceed 3,000 ppm (Karta informacyjna

TABLE 1. The concentration of selected gases in the cowshed (ppm)

Gas	Group	Mean	*N*	*SD*	Min	Max	Q25	Median	Q75
CO_2	1	2 570a	10	1 044	1 500	4 500	1 600	2 400	3 100
	2	2 240	21	865	730	4 000	1 500	2 000	2 800
	3	2 486	7	778	1 500	3 500	1 500	2 800	3 100
	4	1 490b	5	911	700	3 000	850	1 400	1 500
	all	2 269	43	926	700	4 500	1 500	2 200	3 000
NH_3	1	7.30a	10	5.46	3.00	17.00	3.00	5.00	11.00
	2	5.43	21	4.55	3.00	20.00	3.00	4.00	5.00
	3	3.00b	7	1.63	0.00	5.00	2.00	3.00	4.00
	4	1.80b	5	1.64	0.00	3.00	0.00	3.00	3.00
	all	5.05	43	4.48	0.00	20.00	3.00	3.00	5.00
H_2S	1	0.19	10	0.27	0.00	0.70	0.00	0.05	0.50
	2	0.27	21	0.65	0.00	3.00	0.00	0.00	0.30
	3	0.20	7	0.20	0.00	0.50	0.00	0.30	0.30
	4	0.02	5	0.05	0.00	0.10	0.00	0.00	0.00
	all	0.21	43	0.48	0.00	3.00	0.00	0.00	0.30

1 – herd 10–20 cows, 2 – herd 21–40 cows, 3 – herd 41–60 cows, 4 – herd 100–180 cows.

N = cowshed, ab – the differences statistically significant $P \leq 0.05$.

IZ 10101, Kośla 2011, Nowak 2013) our survey shows, however, that the CO_2 concentration exceeds the recommended standards in 22% of herds. Mazur (2011), who conducted a spring survey in 10 multi-stall barns, observed that CO_2 concentration in most rooms exceeded 1,000 ppm. The measured values remained in the range of from 500 to 2,960 ppm, which conforms with the standards of the National Research Institute of Animal Production (Karta informacyjna IZ 10101). However, according to other studies (Mazur 2012) conducted in six free-stall housing systems with natural air ventilation through ridge exhaust, CO_2 concentration in winter ranged from 677 to 1,428 ppm. Majchrzak and Mazur (2012) on seven tested beef cattle barns investigated average concentration of the carbon dioxide did not exceed 1,000 ppm.

The mean concentration of NH_3 (Table 1) in the barns was 5.05 ppm, ranging from 0 to 20 ppm. With reference data indicate, that this concentration should not exceed 26 ppm (Karta Informacyjna IZ 10101, Kośla 2011, Nowak 2013). Figure 2 shows that mean air NH_3 concentration (and standard deviation) in large herds is much lower compared to small barns. The worst CO_2 and NH_3 concentration levels were found in barns with usable attic, those equipped with mechanical fans and natural draft chimney vents. According to the studies by Mazur (2012), conducted in six free-stall barns with natural air ventilation

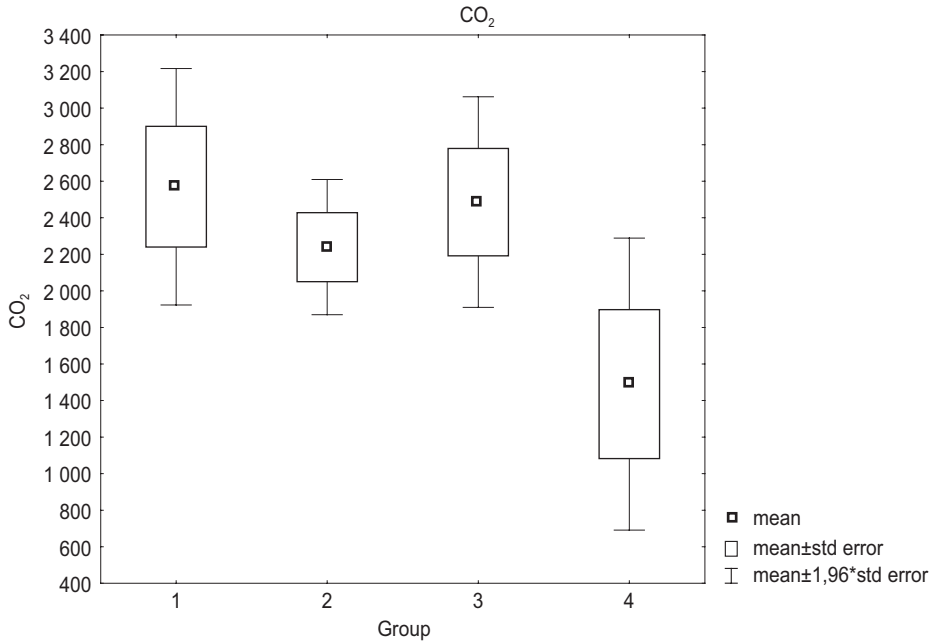

FIGURE 1. Mean carbon dioxide concentration in barns (ppm). Herd as in Tables 1 and 2

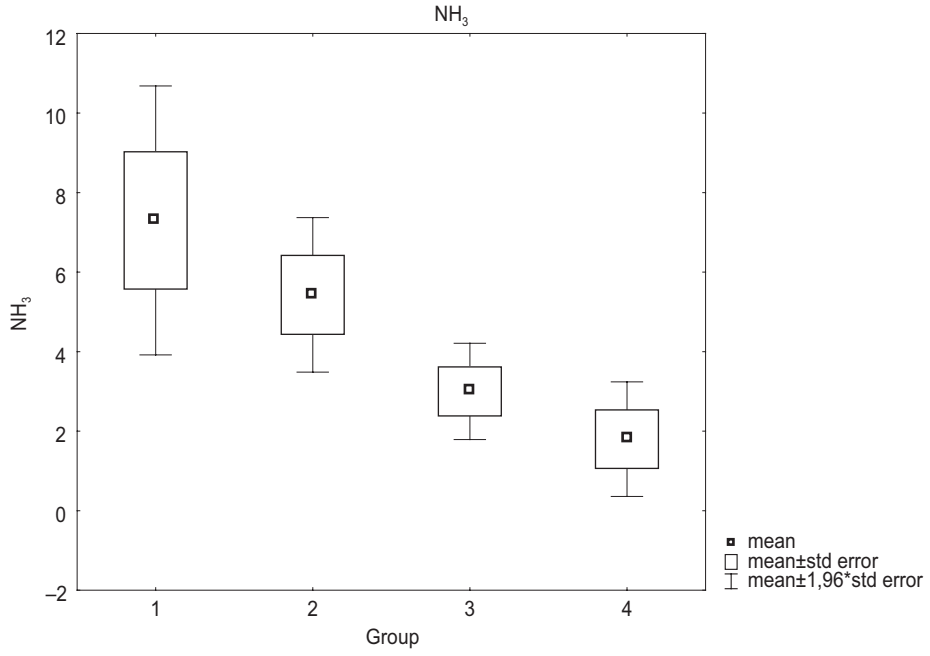

FIGURE 2. Ammonia concentration in barns (ppm). Herd as in Tables 1 and 2

through ridge exhaust, NH_3 concentration remained in the range from 7.8 to 13.2 ppm. In the results of Majchrzak and Mazur (2012) on seven tested beef cattle barns determined the average ammonia concentration from 1.08 to 4.02 ppm.

In our study, the mean concentration of H_2S in the barns was 0.21 ppm (Table 1 and Fig. 3). Figure 3 shows that the concentration of this gas in barns housing 100 cows or more is much lower, as compared with smaller herds. The National Research Institute of Animal Production (Karta informacyjna IZ 10101) recommends that the concentration of H_2S should not exceed 10 ppm; more recent studies suggest 5 ppm, though (Kośla 2011, Nowak 2013). The highest concentration observed in the surveyed barns was 3 ppm.

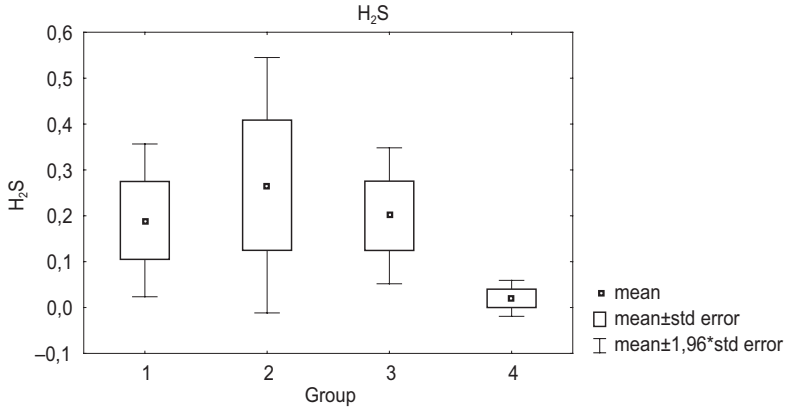

FIGURE 3. Hydrogen sulfide concentration in barns (ppm). Herd as in Tables 1 and 2

Illumination

Information Cards of the National Research Institute of Animal Production (Karta Informacyjna IZ 10101) recommend that light intensity in cow premises be at least 15–30 lx. The exception is the milking parlor, where light intensity should not be lower than 30–40 lx. A lack of good illumination, be it natural or artificial, adversely affects reproduction of cows. Cows kept in such conditions demonstrate poor heat symptoms, which results in longer calving intervals (Kołacz and Dobrzański 2006).

Regulation of Minister of Agriculture and Rural Development (Journal of Laws No 116, item 778 from 2010) recommends farmers to ensure that animals have adequate access to natural or artificial light. In our study, the intensity of light in the premises for dairy cows ranged from 16 to 400 lx. The measurements were performed at daylight, between 9:00 and 14:00. Given the averaged results shown in the Table 2 and diagram in Figure 4, considerable differences in lighting can be seen by individual barns. Large barns (100–180 cows) had better illumination parameters compared to other barns. In the literature (Majchrzak and Mazur 2012) it reported illumination over 30 lx which was in line with animal welfare requirements.

TABLE 2. Relative air humidity and illumination in the cowshed

Item	Group	Mean	*N*	*SD*	Min	Max	Q25	Median	Q75
Humidity (%)	1	68.75a	12	10.89	52.00	85.00	60.00	71.00	77.50
	2	78.05b	22	10.35	60.00	95.00	70.00	78.00	85.00
	3	72.29	7	12.85	55.00	96.00	65,.00	70.00	80.00
	4	71.00	5	7.42	60.00	80.00	70.00	70.00	75.00
	all	73.98	46	11.07	52.00	96.00	70.00	73.50	80.00
Illumination (lx)	1	81.92B	12	51.58	20.00	200.00	50.00	60.00	100.00
	2	88.19B	22	59.53	16.00	200.00	50.00	60.00	150.00
	3	87.71B	7	79.09	16.00	200.00	18.00	60.00	200.00
	4	280.00A	5	83.66	200.00	400.00	200.00	300.00	300.00
	sll	107.33	46	86.51	16.00	400.00	50.00	60.00	150.00

1 – herd 10–20 cows, 2 – herd 21–40 cows, 3 – herd 41–60 cows, 4 – herd 100–180 cows.

N = cowshed, ab – the differences statistically significant $P \leq 0.05$, AB – the differences statistically significant $P \leq 0.01$.

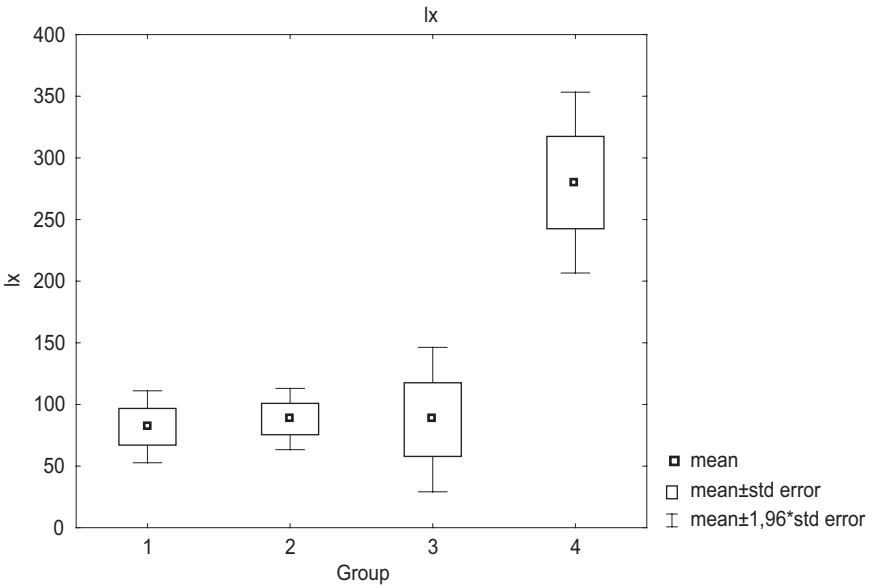

FIGURE 4. Illumination in barns (lx). Herd as in Tables 1 and 2

Sunlight is the best type of lighting for animals. This light kills bacteria and has a beneficial effect on productivity and wellbeing of animals. Solar radiation involves ultraviolet, which enhances the production of vitamin D_3, essential for proper development of young animals. It is advisable that the ratio of window area to floor area be 1 : 12–16 (Kośla 2011).

In the close proximity of windows, there should be no trees, silos or other buildings. The windows are usually installed above the level of the animals. It is recommended that they are placed as high as possible. Also, it must be ensured that door frames, window frames, roof eaves, or thick walls do not limit the flux of incoming light. Artificial lighting in cattle premises is complementary to natural light. With artificial lighting, adequate light intensity must be ensured. The lamps should be distributed so as to provide an equal level of visibility throughout the barn (Kołacz and Dobrzański 2006). Artificial lighting is particularly useful in winter, as the extension of daylight, which has a positive effect on the productivity of dairy cows. It is also recommended to use lighting at night so that the animals may retain orientation in space (Romaniuk et al. 2004).

Air humidity

According to the Regulation by the Minister of Agriculture and Rural Development 116, item 778 from 2010, cattle should be housed in air humidity that is safe for animals. According to Information Bulletins of the National Research Institute of Animal Production (Karta informacyjna IZ 10101), the optimum air humidity levels in dairy cow barns should remain within the range of 60–80%. Air humidity in a barn, depends on the ventilation system and the air exchange in the building, wall thermal insulation, ambient temperature ad relative humidity, the number and size of animals, manure removal system, the substrate on which the cows are kept, and the water content in the feed (Kuczaj 2010). Exhaled air and sweat of the cows are the main source of humidity, producing up to 75% of the total humidity of the premises (Kołacz and Dobrzański 2006, Kośla 2011). In some livestock buildings, humidity is so high, that vapor in the air condenses on the ceiling and the walls. The main reason for this is the lack of wall insulation (Litwińczuk and Szulc 2005). High air humidity, especially in combination with low temperatures, has a negative

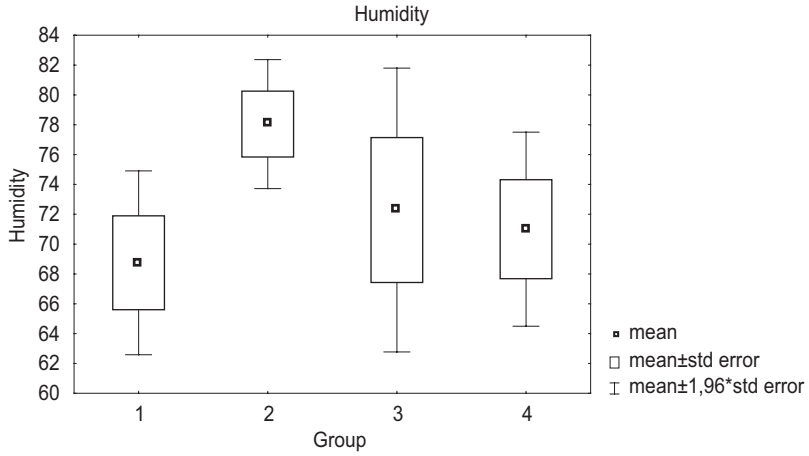

FIGURE 5. Relative air humidity (%) in barns. Herd as in Tables 1 and 2

impact on the animals (Kośla 2011). Under such conditions, animals show lower feed intake, a decrease in milk yields, and have problems with breathing. In winter, this can lead to colds, pneumonia, as well as muscular and articular rheumatism. High humidity in combination with high temperatures, on the other hand, can cause problems with body thermoregulation. This may result again in reduced yields, digestive tract disorders and apathy (Litwińczuk and Szulc 2005). In our study, air humidity in the barns ranged from 52 to 96%. As can be seen in Table 2 and Figure 4, 28% of barns failed to meet the standards. Relative air humidity in the study by Mazur (2011), carried out on 10 multi-stall barns, remained in the range from 32.2 to 99.9%. The high values indicated poorly functioning ventilation. Majchrzak and Mazur (2012) on seven tested beef cattle barns determined relative humidity of air oscillated between 56.13 and 76%, but in the two of them exceeded optimum value (70%). Kaczor et al. (2013) they found in barns open relative humidity from 60.3 to 85.9%, with average 76.5%, which results in the investigated barns in own research regardless of their size were similar (Table 2). While Daniel (2008) during the summer said relative humidity in barns for dairy cows during the afternoon hours in the field, which is below the recommended standards (Karta informacyjna IZ 10101, Kośla 2011, Nowak 2013).

Comparing hygienic parameters in barns with bedding versus barns with slatted floor

Of all the barns that used slatted floors or bedding, we selected three largest herds each in order to compare hygienic parameters between both types of housing. In barns with straw bedding, CO_2 concentration was much higher than in barns with slatted floors (Table 3). According to Kołacz and Dobrzański (2006), CO_2 levels in slatted-floor barns is lower in the part occupied by the cows compared to the upper space inside the barn. Although CO_2 is heavier than air, the warm air exhaled by animals lifts it up.

Ammonia concentration was low in both housing systems. The concentration of this gas to a large extent depends on the hygiene in the barn (Table 3). Barns with slatted floors, where manure sinks to the gutter under the floor, the air concentration of NH_3 was low (Kołacz and Dobrzański 2006).

TABLE 3. Measured concentrations of gases in selected barns with bedding and slatted floors

Herd size	Type of barn	CO_2 concentration (ppm)	NH_3 concentration (ppm)	H_2S concentration (ppm)
100 cows	bedding	3 000	3	0.1
100 cows	bedding	1 400	3	0
56 cows	bedding	3 100	0	0
150 cows	slatted floor	850	0	0
180 cows	slatted floor	700	3	0
110 cows	slatted floor	1 500	0	0

No H_2S has been detected in any of the three studied slatted-floor barns (Table 3). This may have resulted from a well designed gutter system in the building. Slurry tanks must be tight so that gases are not able to drift back through the gutter into the barn (Kołacz and Dobrzański 2006).

Measurements show that the slatted-floor barns had a lower humidity indicator. In both types of barns, however, humidity was maintained within the relevant standards (Table 4). The results of humidity measurements conducted in winter in two multiple-stall barns equipped with side curtains was 66–85% (Daniel 2008). Similar results in humidity studies were reported by Kaczor and Paschma (2008). Relative humidity was measured in a heifer shed in the barn and remained in the range of 60–85%.

According to the Regulation of the Minister of Agriculture and Rural Development 166, item 778 from 2010, cows should be provided with natural or artificial light. Our study (Table 4) shows that the light intensity in bedded barns was 60–200 lx, whereas in the barns with slatted floors 300–400 lx. Slatted-floor barns included in the study were equipped with side curtains and ridge skylights that let in much light. In the case of bedded floor barns, often old or adapted from other buildings, the intensity of the entering light was much lower.

TABLE 4. Results of humidity and illumination measurements in selected barns with bedding and slatted floors

Herd size	Type of barn	Humidity (%)	Illumination (lx)
100 cows	bedding	80	200
100 cows	bedding	70	200
56 cows	bedding	70	60
150 cows	slatted floor	75	300
180 cows	slatted floor	70	300
110 cows	slatted floor	60	400

CONCLUSION

The 46-barn survey enabled evaluation of the welfare of dairy cows in Poland. The main findings were as follows:

1. The concentration of selected gases in most of the barns did not deviate from the recommendations of the National Research Institute of Animal Production.
2. The cattle housed on slatted floors also had better lighting conditions.
3. Smaller herds of dairy cows were found to have poorer air humidity conditions.
4. In 81% of farms, air humidity in the premises remained within the animal welfare standards.

REFERENCES

ARiMR, 2012: Zasada wzajemnej zgodności (cross-compliance). Centrala ARiMR, Warszawa.

AVERÓS X., APARICIO M.A., FERRARI P., GUY J.H., HUBBARD C., SCHMID O., VLATKO ILIESKI V., SPOOLDER H.A.M., 2013: The Effect of Steps to Promote Higher Levels of Farm Animal Welfare across the EU. Societal versus Animal Scientists' Perceptions of Animal Welfare, Animals 3: 786–807: doi:10.3390/ani3030786.

BARTUSSEK H., 2000: Haw to measure welfare? Proceedings of the Second NAHWOA Workshop, Cordoba. In: M. Hovi, R.G. Trujillo (Eds) Diversity of Livestock Systems and Definition of Animal Welfare: 135–142.

BARTUSSEK H., LEEB C., HELD S., 2011: Animal Needs Index for cattle – ANI 35L/2000 – cattle. Federal Research Institute for Agriculture in Alpine Regions BAL Gumpenstein, Irdning, Austria. Available at http://www.bartussek.at/pdf/anicattle.pdf [accessed: 10.11.2011].

CZERNIAWSKA-PIĄTKOWSKA E., SZEWCZUK M., SNOPKOWSKA M., 2008: Porównanie użytkowości mlecznej krów rasy polskiej holsztyńsko-fryzyjskiej w różnych systemach utrzymania. Zesz. Nauk. UP Wrocław 56: 25–33.

DAGORN T., 2008: Lagistalle – boksy legowiskowe zgodne z dobrostanem krów. Bydło 11: 48–51.

DANIEL Z., 2008: Wpływ mikroklimatu obory na mleczność krów. Inż. Roln. 9: 67–73.

KACZOR A., PASCHMA J., 2008: Wpływ systemu utrzymania na warunki bytowania jałówek. Rocz. Nauk. Zoot. 35 (2): 195–202.

KACZOR A., PARAPONIAK P., MANDECKI A., 2013: Warunki termiczno-wilgotnościowe w pomieszczeniach typu otwartego dla krów. Rocz. Nauk. Zoot. 40 (2): 183–196.

Karta informacyjna IZ 10101, 1977: Wskaźniki zoohigieniczne, bydło. Instytut Zootechniki w Krakowie, Kraków.

KOŁACZ R., DOBRZAŃSKI Z., 2006: Higiena i dobrostan zwierząt gospodarskich. Wydaw. AR, Wrocław.

KOŚLA T., 2011: Metodyka badań z higieny zwierząt i prewencji weterynaryjnej. Wydaw. SGGW, Warszawa.

KUCZAJ M., 2010: Hodowla bydła. Standardy unijne i krajowe. Wydaw. UP, Wrocław.

LENARD J., 1993: Budownictwo wiejskie. Wydaw. SGGW, Warszawa.

LITWIŃCZUK Z., SZULC T., 2005: Hodowla i użytkowanie bydła. PWRiL, Warszawa.

MAJCHRZAK M., MAZUR K., 2012: Analiza mikroklimatu w oborach dla bydła mięsnego w kontekście spełnienia wymagań dobrostanu zwierząt. Probl. Inż. Rol. 4: 131–139.

MAZUR K., 2011: Badania parametrów mikroklimatu w oborach wolnostanowiskowych w okresie wiosennym. Probl. Inż. Roln. 1: 111–122.

MAZUR K., 2012: Emisja amoniaku i dwutlenku węgla z obór przeznaczonych dla krów mlecznych. Probl. Inż. Roln. 2: 141–150.

NOWAK D., 2013: Warunki utrzymania bydła w świetle obowiązujących przepisów. Centrum Doradztwa Rolniczego w Brwinowie, Oddział w Poznaniu, Poznań.

POŚNIAK-SOBCZYŃSKA J., 2011: Wzajemna zgodność. Hodowla i Chów Bydła 7–8: 18–20.

RADOŃ J., 2005: Prognozowanie kształtowania się mikroklimatu budynków inwentarskich – możliwości i ograniczenia. Inż. Roln. 7: 245–253.

ROMANIUK W. et al. (Eds), 2004: Systemy utrzymania bydła. Poradnik. IBMER, Duńskie Służby Doradztwa Rolniczego, Warszawa.

Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi w sprawie wymagań i sposobu postępowania przy utrzymaniu gatunków zwierząt gospodarskich, dla których normy ochrony zostały określone w przepisach UE (Journal of Laws 2010 No 56, item 344).

Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi w sprawie minimalnych warunków utrzymania gatunków zwierząt gospodarskich innych niż te, dla których normy ochrony zostały określone w przepisach UE (Journal of Laws 2010 No 116, item 778).

SUNDRUM A., ANDERSSON R., POSTLER G., (Eds), 1994: Tiergerechtheitsindex 200/1994 – Ein Leitfaden zur Beurteilung von Haltungssystemen [Animal needs index 200/1994 – A manual for the assessment of housing systems]. Köllen Druck und Verlag, Bonn.

VEISSIER I., BUTTERWORTH A., BOCK B., ROE E., 2008: European approaches to ensure good animal welfare. Appl. Anim. Behav. Sci. 113: 279–297.

WYRĘBSKI J., REKLEWSKI Z., 2000: Wymagania środowiskowe bydła mlecznego. In: Poradnik producentów mleka. Warszawa: 81–96.

Streszczenie: *Respektowanie unijnych wymogów wzajemnej zgodności jako wskaźnik dobrostanu zwierząt w gospodarstwach krów mlecznych w Polsce.* Celem pracy było określenie poziomu wskaźników dobrostanu zwierząt i porównanie ich w wymogami wzajemnej zgodności w gospodarstwach utrzymujących krowy mleczne. Badania zostały przeprowadzone w 46 gospodarstwach w okresie zimowym. Przeprowadzono pomiary mikroklimatyczne: wilgotności, stężenia wybranych gazów, oświetlenia. Porównano systemy utrzymania bydła na ściółce i na ruszcie. Obory podzielono na cztery grupy w zależności od liczebności stada krów: 10–20 sztuk (12 obór), 21–40 sztuk (22 obory), 41–60 sztuk (7 obór), 100–180 sztuk (5 obór). Największą grupę tworzą stada liczące 21–40 sztuk krów. Stanowią one 48% wszystkich stad uwzględnionych w badaniach. Spośród wszystkich obór rusztowych i ściółkowych wybrano po trzy stada o największej liczebności w celu porównania parametrów zoohigienicznych w obu typach obór. Badania pozwoliły na ocenę warunków utrzymania krów mlecznych. Stwierdzono co następuje: (1) stężenia gazów szkodliwych w większości obór mieszczą się w normach Instytutu Zootechniki; (2) krowy utrzymywane w oborach na ruszcie mają lepsze warunki oświetleniowe; (3) w stadach o mniejszej liczebności krów stwierdzono gorsze warunki wilgotnościowe; (4) w 81% gospodarstw wilgotność w pomieszczeniach jest utrzymana w normach zootechnicznych.

Słowa kluczowe: krowy mleczne, obory, środowisko, mikroklimat, wymogi UE

MS received 19.04.2016
MS accepted 14.03.2017

Authors' address:
Tadeusz Kośla
Katedra Biologii Środowiska Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8
02-786 Warszawa
Poland
e-mail: tadeusz_kosla@sggw.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 103–112
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.12

Meat quality in pigs fed mixtures with low-tannin faba bean

ANNA MILCZAREK, MARIA OSEK
Department of Animal Nutrition and Feed Management, Siedlce University of Natural Sciences and Humanities

Abstract: *Meat quality in pigs fed mixtures with low-tannin faba bean.* The aim of the study was to evaluate slaughter values of carcasses and meat (*Musculus longissimus lumborum*, *Musculus semimembranosus*) quality in pigs fed mixtures with faba beans. Research included 48 fatteners, which were divided into three feeding groups. Pigs in group I were fed mixtures in which extracted soybean meal was used as the only high-protein raw material, whereas animals in groups II and III were fed mixtures with 5/10% or 10/20% of low-tannin faba bean in grower/finisher mixtures respectively. It was proved, that introduction of faba bean into mixtures increased of meatiness and loin "eye" area and decreased of carcass fatness. The significant increase in the share of the most valuable exogenous fatty acids ($C18:2_{n-6}$, $C18:3_{n-3)}$ in muscles of animals from groups II and III were found. *Musculus longissimus lumborum* in pigs fed mixtures with faba beans were characterized by significantly better water holding capacity WHC ($P \leq 0.01$).

Key words: faba beans, carcass yield, pork, physical properties, chemical composition

INTRODUCTION

High-protein raw materials are essential components in feed mixtures for desirable pig growth. The extracted soybean meal is the most commonly used source of protein in animal feeding. It is estimated that approximately 98% of soybean comes from genetically modified seeds. Some research proved that genetic modification did not affect not only the nutritional value of soybean meal, but also fattening and slaughter performance and meat quality (Flachovsky et al. 2005, Hanczakowska and Świątkiewicz 2014). Furthermore, Świątkiewicz et al. (2011) did not prove that the transgenetic DNA can be transferred from feeds to animal tissues. Despite these findings, consumers in Poland and many other countries are unwilling to accept products made from genetically modified plants. The price of imported soybean meal and the possibility of banning the application of the genetically modified feeds in animal feeding are good reasons to start searching for alternative protein sources in pig feeding. Underestimated *Fabaceae* seeds, including faba bean seeds, can be the basic domestic source of protein in feed mixtures. Low-tannin varieties of faba bean give an opportunity to apply them more widely in pig feeding (Jezierny et al. 2010, Milczarek and Osek 2016). Every change in the composition of feed mixtures for fattener pigs can influence both productive indices and meat quality, including its dietary and

gustatory values (Gatta et al. 2013, Milczarek and Osek 2014b, Milczarek and Osek 2016). The previous studies (Zijlstra et al. 2008, Smith et al. 2013, Hanczakowska and Świątkiewicz 2014, Milczarek and Osek 2016) did not lead to the unambiguous conclusion that low-tannin faba bean had positive effect on the parameters mentioned above and did not answer the question what the optimal level of the feed can be applied in mixtures.

The research, therefore, was undertaken and its aim was to evaluate slaughter values of carcasses and meat quality in slow-growing breed pigs, which were fed mixtures with different contents of low-tannin faba bean Amulet variety.

MATERIAL AND METHODS

The feeding experiment was conducted on 48 slow-growing fatteners with a mean body weight 28 kg that were divided into three equal feeding groups (I, II and III), each of 16 pigs. Pigs were raised using a two-phase dietary feeding program where the first-phase diets (grower) were fed for 64 days and the second-phase diets (finisher) were fed for 55 days. The animals were fed mixtures that contained barley, extracted soybean meal, oil and mineral-vitamin additives. The extracted soybean meal was the only high-protein raw material in mixtures for fatteners in the first group (control), whereas in experimental mixtures in groups II and III, the soybean meal was partially replaced by 5 and 10% of low-tannin faba bean meal Amulet variety in grower mixtures as well as 10 and 20% in finisher mixtures, respectively. The Nutritional Recommendations for Pigs (2015) was used to formulate isoprotein (160/147 g protein) and isocaloric (13,2/12,8 MJ ME) grower/finisher feed mixtures.

The fatteners were slaughtered at the average body weight 120 kg according to the technology required in meat processing plants and carcass meatiness was estimated using the Ultra-Fom 300 apparatus. The initial acidity (pH_1) in the *longissimus* muscles (between the last pectoral vertebra and the first lumbar vertebra) was measured 45 min after the slaughter by means of the portable pH-meter that was equipped with a glass electrode. Next the carcasses were chilled for 24 h at a temperature of 0–4°C. After that the pH_{24} of *longissimus* muscles were measured then carcass length, backfat thickness and height and width of loin were measured using the right halves of carcass. The carcass length was measured from the anterior edge of the symphysis pubis to the recess of the first rib. The backfat thickness were five points on the right lying half-carcasses: in the thickest point above the shoulder blade, on the back above the joint between the last pectoral vertebra and the first lumbar vertebra, and on three points on the lower back (at the level of sacral vertebrae: I, II, III).

Next the samples of *musculus longissimus lumborum* and *musculus semimembranosus* were determined for the physicochemical and organoleptic properties. Water holding capacity (WHC) of muscles were determined according to Grau and Hamm's (1953) method. Moreover, drip loss after 48 and 72 h *post mortem* were determined using methods practiced by Prange et al. (1977). The instrumental evaluation of

meat colour was performed by means of the photocolorimeter in the system CIE L*a*b*, where L* represented the lightness of a colour that was the spatial vector, whereas a* and b* were trichromatic coordinates. The chroma index (C) and the colour hue (H) were calculated using the results of colour parameters a* and b* (Strzyżewski et al. 2008, Milczarek and Osek 2016). The content of basic nutrients (dry matter, crude ash, crude protein, crude fat) were tested according to AOAC (2005). Fatty acids profile of the lipid fraction determined by gas chromatography of methyl esters in a Varian 450-GC fitted with a flame ionisation detector (air-hydrogen).

The obtained results were statistically analyzed with the Statistica software ver. 12 (StatSoft Inc., Tulsa, USA). The arithmetic means and standard deviations (SD) for all features in groups were calculated. One-way analysis of variance were used. The significance of differences between means were evaluated with the Duncan's test.

RESULTS AND DISCUSSION

Introducing low-tannin faba bean to mixtures for fatteners did not affect the dressing percentage (Table 1). Better meatiness and larger loin "eye" area were found in pigs fed mixtures with faba bean, however, statistically significant differences were proved only between groups III and I (control). The carcass fatness of fatteners II and III groups was lower than of the control group, due to the fact that the carcasses were characterized by significantly thinner backfat that was measured above the

TABLE 1. Post-slaughter results of fatteners

Item	Groups						*P*
	I		II		III		
	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	
Dressing percentage (%)	77.02	2.39	74.89	2.09	75.07	2.01	>0.05
Meatiness (%)	51.59b	1.25	52.05ab	1.27	52.95a	1.28	≤0.05
Carcass length (cm)	84.60	1.25	86.80	1.30	85.90	1.24	>0.05
Backfat thickness (mm)							
over the shoulder	32.13A	1.50	28.50B	1.79	28.00B	1.36	≤0.01
mid back	40.00	1.30	39.25	1.57	40.75	1.44	>0.05
sacrum I	35.38a	1.23	31.88b	1.33	32.75ab	1.46	≤0.05
sacrum II	30.50A	1.26	24.63B	1.39	22.38B	1.26	≤0.01
sacrum III	32.13a	1.49	27.88b	1.64	27.63b	1.45	≤0.05
$\bar{x}$ from 5 measurements	34.03A	1.27	30.23B	1.38	29.60B	1.22	≤0.01
Lard weight (kg)	2.55	0.42	2.48	0.35	2.37	0.29	>0.05
Loin "eye" area (cm^2)	50.40B	1.53	50.46B	1.58	52.95A	1.48	≤0.01

A, B or a, b – means within the same rows with different letter differ significantly $P \leq 0.01$ or $P \leq 0.05$.

shoulder blade as well as in lower back II and III, which resulted in lower backfat thickness mean from five measurements by approximately 4 mm ($P \leq 0.01$). Zijlstra et al. (2008) and Smith et al. (2013) stated that feed mixtures with different contents of faba bean meal did not significantly influence carcass fatness, however, larger content of faba bean (30%) in mixtures significantly ($P < 0.05$) decreased the dressing percentage and the backfat thickness (Zijlstra et al. 2008). On the other hand, Smith et al. (2013) did not indicate the influence of the faba bean meal (7.5, 15, 22.5 and 30%) on either dressing percentage or carcass meatiness. The results found in the present studies, i.e. lower carcass fatness and better carcass meatiness after applying low-tannin faba bean in mixtures for pigs, were consistent with the results presented in earlier studies by Milczarek and Osek (2014a).

Many physical parameters (pH, colour, water holding capacity, drip loss) determine the meat quality characteristics of pork. The partial substitution of extracted soybean meal by faba bean meal with a low level of tannins in feed mixtures for fatteners did not influence the muscle acidity measured at 45 min and 24 h after slaughter (Table 2).

Scheffler and Gerrard (2007) stated that the ultimate meat quality and its technological properties depend on the rate and range of the decrease in the pH value *post mortem*. Acidification of muscles 24 h *post mortem* (pH_{24}) amounted to 5.6–5.7, which was typical of the highest quality meat – the *longissimus lumborum* and *semimembranosus* muscles (Tomović et al. 2013). The high acidification (pH <5.5) of the muscle tissues 24 and 48 h *post mortem* indicates worse culinary and technological properties of the meat and results in receiving acid meat – the kind of meat is characterized by large losses during the heat treatment.

Strzyżewski et al. (2008) proved that the pH value greatly influenced meat colour, which undoubtedly is an essential meat quality characteristic that determines the appearance of meat and its attractiveness in the eyes of consumers. No significant effects of the application of faba bean in experimental mixtures on lightness of a colour (L*) and the saturation of a red colour (a*) were proved. On the other hand, the supplementation of feed mixtures with faba bean meal increased ($P \leq 0.05$) the values of parameters such as b* and H in both tested muscles. It could be connected with higher antioxidant (lutein, zeaxantin, tocopherols) amount in muscles (Dal Bosco et al. 2013). The lack of the influence of faba bean on the parameters of the L* and a* colour in the *longissimus* muscles was confirmed by Gatta et al. (2013) as well as Milczarek and Osek (2014a, 2016).

Another crucial element to evaluate meat quality in fatteners' tissues is the analysis of water holding capacity (WHC) and drip loss (WN) during the meat storage. The *longissimus lumborum* muscles in pigs fed mixtures with faba bean were characterized by significantly better water holding capacity ($P \leq 0.01$) and more desirable values of drip loss measured at 48 and 72 h *post mortem*, compared to the control group.

The results of drip loss proved in the present research showed high quality of

TABLE 2. Physical characteristics of muscles

Item	Groups						*P*
	I		II		III		
	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	
Musculus longissimus lumborum							
pH_1	6.25	0.19	6.10	0.25	6.13	0.26	>0.05
pH_{24}	5.58	0.09	5.65	0.08	5.66	0.18	>0.05
Water holding capacity (%)	21.75A	3.88	19.64B	2.77	19.67B	3.88	≤0.01
Drip loss after 48 h (%)	1.96	0.33	1.70	0.29	1.78	0.30	>0.05
Drip loss after 72 h (%)	3.07	0.51	2.71	0.32	2.97	0.48	>0.05
L*	49.06	1.84	46.87	0.98	49.43	3.41	>0.05
a*	6.73	0.62	7.16	0.99	7.01	0.93	>0.05
b*	2.03a	0.60	3.04b	0.76	2.42ab	0.47	≤0.05
$C = [(a^*)^2 + (b^*)^2]^{0.5}$	7.03	0.48	7.77	1.02	7.42	0.90	>0.05
$H = b^*/a^*$	0.30	0.12	0.42	0.11	0.35	0.08	>0.05
Musculus semimembranosus							
L*	45.34	1.28	42.35	2.70	44.44	3.74	>0.05
a*	9.40	0.54	9.64	0.59	9.50	1.56	>0.05
b*	1.82a	0.47	3.46ab	1.32	2.41b	0.65	≤0.05
$C = [(a^*)^2 + (b^*)^2]^{0.5}$	9.57	0.50	10.24	0.90	9.80	1.63	>0.05
$H = b^*/a^*$	0.19a	0.13	0.36b	0.12	0.25ab	0.05	≤0.05

A, B or a, b – means within the same rows with different letter differ significantly $P \leq 0.01$ or $P \leq 0.05$.
L* – lightness of colour, a* – redness, b* – yellowness, C – chroma index, H – hue of colour.

the meat, since Joo et al. (1999) stated that drip loss in meat of the highest quality amounted to less than 6.0%, whereas Bertram et al. (2000) suggested more rigorous classification, in which the drip loss amounted to less or equal 4.0%. Gatta et al. (2013) as well as Milczarek and Osek (2014a, 2016) did not prove any significant effects of applying mixtures with low-tannin faba bean on water holding capacity. According to Jennen et al. (2007), worse values of WHC parameters during the meat processing were associated with large financial loss (due to the decrease in the meat weight), worse dietetic value of meat, lower consumer acceptance (limited possibility to sell it as culinary meat), as well as worse properties and technological yield of the meat.

The contents of basic nutrients (protein, fat and minerals) determine the nutritive value of meat. No significant effects of feeding on the nutrient content in both tested muscles were found in the present studies (Table 3).

Moreover, the previous research by Gatta et al. (2013), as well as Milczarek

TABLE 3. Basal nutrients (g/100 g) of muscles

Item	Groups						*P*
	I		II		III		
	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	
Musculus longissimus lumborum							
Dry matter	26.62	0.37	26.57	0.28	26.48	0.56	>0.05
Crude ash	1.11	0.02	1.09	0.03	1.12	0.05	>0.05
Crude protein	22.99	0.33	22.89	0.35	22.81	0.43	>0.05
Crude fat	2.60	0.23	2.55	0.18	2.45	0.17	>0.05
Musculus semimembranosus							
Dry matter	26.83	0.54	26.65	0.69	26.52	0.65	>0.05
Crude ash	1.11	0.04	1.09	0.03	1.10	0.04	>0.05
Crude protein	22.32	0.35	22.03	0.36	21.91	0.51	>0.05
Crude fat	3.63	0.27	3.57	0.40	3.57	0.29	>0.05

and Osek (2014b, 2016), did not show the influence of faba bean in animal mixtures on the nutrient content. The intramuscular fat content in the tested muscles was 1.35–1.67% and it can be admitted to be a recommended level, since the fat content lower than 1% decreased gustatory values of meat (Schwörer et al. 2000). According to Danish and American researchers, 2–3% of the intramuscular fat content was proved to be the optimal content that can be acceptable for consumers and also from the quality pork meat point of view (Wood et al. 1994, Przybylski et al. 2007). Such fat content positively influenced tenderness, tastiness and juiciness of meat and also decreased losses during the heat treatment (cooking, grilling). Larger content of intramuscular fat (above 3.5%), however, can cause lower acceptance by consumers due to noticeable fat deposits in the meat (Czarniecka-Skubina et al. 2007).

The dietetic value of meat also depends on the profile of fatty acids in the intermuscular fat. The supplementation of feed mixtures for fatteners with low-tannin faba bean positively modified the lipid profile in muscles (Tables 4 and 5).

The significant increase in the content of the most valuable exogenous fatty acids, i.e. linoleic acid C18:2_{n-6} and linolenic acid C18:3_{n-3}, was found. Furthermore, the ratio of PUFA n-3 to PUFA n-6 was more desirable and narrower in pigs that were fed mixtures with faba bean.

The acids mentioned above are essential for human development and to keep the body in good health, but there is often a lack of the acids in human nutrition (Williams 2000). According to WHO (2003), 5–4 : 1 is the most favourable ratio of n-6 to n-3 PUFAs in human diets. The ratio usually is much wider (Kunachowicz et al. 2005), but it is possible to improve the ratio

TABLE 4. Fatty acids profile (% of sum FA) of *Musculus longissimus lumborum*

Item	Groups						*P*
	I		II		III		
	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	
C 12:0	0.03	0.004	0.03	0.009	0.03	0.003	>0.05
C 14:0	0.84	0.08	0.73	0.13	0.77	0.07	>0.05
C 16:0	27.91^{a}	0.91	26.77^{b}	0.83	26.55^{b}	0.91	≤0.05
C 16:1	3.53^{a}	0.25	3.15^{ab}	0.52	3.00^{b}	0.15	≤0.05
C 18:0	9.69	0.34	9.74	0.65	9.88	0.66	>0.05
C 18:1	52.92	1.09	53.33	0.85	52.20	0.95	>0.05
C 18:2 $_{n-6}$	4.35^{B}	0.52	5.43^{AB}	0.64	6.59^{A}	0.52	≤0.01
C 18:3 $_{n-3}$	0.17^{Bb}	0.04	0.27^{ABa}	0.07	0.33^{Aa}	0.12	≤0.01
C 20:0	0.04	0.02	0.07	0.03	0.07	0.03	>0.05
C 20:1	0.20	0.007	0.21	0.04	0.19	0.03	>0.05
C 20:2	0.04	0.01	0.04	0.004	0.05	0.005	>0.05
C 20:3 $_{n-6}$	0.02	0.03	0.01	0.005	0.02	0.007	>0.05
C 20:4 $_{n-6}$	0.13^{ab}	0.02	0.11^{b}	0.02	0.18^{a}	0.08	≤0.05
SFA	38.52	1.17	37.33	1.30	37.30	1.34	>0.05
UFA	61.36	1.16	62.54	1.31	62.55	1.34	>0.05
MUFA	56.65	1.01	56.68	0.80	55.39	0.96	>0.05
PUFA	4.71B	0.56	5.86AB	0.62	7.15^{A}	0.66	≤0.01
PUFA $_{n-6:n-3}$	26.53A	0.85	21.11B	0.63	21.01^{B}	0.73	≤0.01
DFA	71.05^{b}	0.46	72.28^{a}	0.88	72.43^{a}	0.88	≤0.05
OFA	28.75^{a}	0.95	27.49^{b}	0.92	27.32^{b}	0.93	≤0.05

A, B or a, b – means within the same rows with different letter differ significantly $P \leq 0.01$ or $P \leq 0.05$.

SFA – saturated fatty acids, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, DFA – neutral or hypocholesterolemic fatty acids = MUFA + C18:0, OFA – hypercholesterolemic fatty acids = C14:0 + C16:0.

by modifying animal diets (Milczarek and Osek 2014b). The statistically significant and favourable increase in the contents of neutral fatty acids and hypocholesterolemic acids (DFA) as well as the decrease in the content of hypercholesterolemic acids (OFA) in lipids in both tested muscles were found in fatteners after the supplementation of feed mixtures with faba bean. The increase (P >0.05) in the linoleic acid content and the PUFA content, and the decrease in the OFA content in pigs of Pulawska breed fed mixtures with 10% of low-tannin faba bean were proved by Milczarek and Osek (2014b).

TABLE 5. Fatty acids profile (% of sum FA) of *Musculus semimembranosus*

Item	Groups						*P*
	I		II		III		
	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	$\bar{x}$	±*SD*	
C 12:0	0.03	0.006	0.03	0.005	0.03	0.004	>0.05
C 14:0	0.71	0.07	0.74	0.06	0.74	0.07	>0.05
C 16:0	25.58	0.82	24.55	0.71	25.23	0.50	>0.05
C 16:1	3.74a	0.40	3.46ab	0.29	3.34b	0.15	≤0.05
C 18:0	8.14b	0.60	9.07a	0.78	8.77ab	0.49	≤0.05
C 18:1	54.53	1.34	54.18	1.10	54.09	1.09	>0.05
C 18:2 $_{n-6}$	6.35Bb	1.13	6.84Aa	1.31	6.81ABa	0.80	≤0.01
C 18:3 $_{n-3}$	0.21A	0.14	0.29B	0.12	0.26B	0.08	≤0.01
C 20:0	0.05	0.03	0.07	0.03	0.05	0.03	>0.05
C 20:1	0.18b	0.01	0.23a	0.05	0.19 ab	0.02	≤0.05
C 20:2	0.05Bc	0.007	0.07Aa	0.02	0.06ABb	0.009	≤0.01
C 20:3 $_{n-6}$	0.02	0.01	0.03	0.01	0.02	0.007	>0.05
C 20:4 $_{n-6}$	0.26	0.12	0.28	0.06	0.25	0.11	>0.05
SFA	34.50	1.02	34.46	0.79	34.82	0.83	>0.05
UFA	65.34	1.02	65.37	0.79	65.01	0.83	>0.05
MUFA	58.45	1.53	57.86	1.14	57.62	1.12	>0.05
PUFA	6.89B	1.35	7.51A	1.14	7.39A	0.95	≤0.01
PUFA $_{n-6:n-3}$	31.63A	1.27	26.85B	0.78	29.70AB	1.09	≤0.01
DFA	73.48B	0.84	74.87A	0.73	73.78A	0.54	≤0.01
OFA	26.28A	0.87	25.29B	0.74	25.97B	0.55	≤0.01

A, B or a, b, c – means within the same rows with different letter differ significantly $P \leq 0.01$ or $P \leq 0.05$.

SFA – saturated fatty acids, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, DFA – neutral or hypocholesterolemic fatty acids = MUFA + C18:0, OFA – hypercholesterolemic fatty acids = C14:0 + C16:0.

CONCLUSION

The research showed that low-tannin faba bean up to 10% in grower and 20% finisher mixtures can be recommended as a partial substitution of extracted soybean meal for slow growing pigs due to the fact that the faba bean meal decreased carcass fatness, increased meatiness and improved water holding capacity. As far as human feeding is concerned, mixtures with low-tannin faba bean meal favourably increased the content of essential unsaturated fatty acids and decreased the

content of hypercholesterolemic acids in the lipid profile in the *longissimus lumborum* and *semimembranosus* muscles of pigs.

REFERENCES

AOAC, 2005: Association of Official Analytical Chemists, Official Methods of Analysis. 18th Edition by AOAC International, Revision II 2007, USA.

BERTRAM H.C., PETERSEN J.S., ANDERSEN H.J., 2000: Relationship between RN-genotype and drip loss in meat from Danish pigs. Meat Sci. 56: 49–55.

CZARNIECKA-SKUBINA E., PRZYBYLSKI W., JAWORSKA D., WACHOWICZ I., URBAŃSKA I., NIEMYJSKI S., 2007: Charakterystyka jakości mięsa wieprzowego o zróżnicowanej zawartości tłuszczu śródmięśniowego. ŻNTJ 6 (55): 285–294.

Dal BOSCO A., RUGGERI S., MATTIOLI S., MUGNAI C., SIRRI F., CASTELLINI C., 2013: Effect of faba bean (*Vicia faba* var. *minor*) inclusion in starter and growing diet on performance, carcass and meat characteristics of organic slow-growing chickens. Ital. J. Anim. Sci. 76: 472–478.

FLACHOVSKY G., CHESSON A., AURLICH K., 2005: Animal nutrition with feeds from genetically modified plants. Arch. Anim. Nutr. 59: 1–40.

GATTA D., RUSSO C., GIULIOTTI L., MANNARI C., PICCIARELLI P., LOMBARDI L., GIOVANNINI L., CECCARELLI N., 2013: Influence of partial replacement of soya bean meal by faba beans or peas in heavy pigs diet on meat quality. residual anti-nutritional factors and phytoestrogen content. Arch. Anim. Nutr. 67 (3): 235–247.

GRAU R., HAMM R., 1953: A simple method for the determination of water binding in muscles. Naturwissenschaften 40: 29–30.

HANCZAKOWSKA E., ŚWIĄTKIEWICZ M., 2014: Legume seeds and rapeseed press cake as replacers of soybean meal in feed for fattening pigs. Ann. Anim. Sci. 14 (4): 921–394.

JENNEN D.G.J., BRINGS A.D., LILU G., JÜNDST H., THOLEN E., JONAS E., TESFAYE D., SCHELANDER K., PHATSARA C., 2007: Genetic aspects conceming drip loss and water-holding capacity in porcine *longissimus* muscle. Meat Sci. 52: 291–297.

JEZIERNY D., MOSENTHIN R., BAUER E., 2010: The use of grain legumes as a protein source in pig nutrition: A review. Anim. Feed Sci. Technol. 157: 111–128.

JOO S.T., KAUFFMAN R.G., LEE S., KIM B.C., PARK G.B., 1999: The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine *longissimus* muscle. Meat Sci. 52: 291–297.

KUNACHOWICZ H, NADOLNA I., PRZYGODA B., IWANOW K., 2005: Tabele składu i wartości odżywczej żywności. PZWL, Warszawa.

MILCZAREK A., OSEK M., 2014a: Effectiveness of using faba bean seeds and corn distillers grains with soubles as a partial replacement the soybean meal in feeding of pulawska pigs. Acta Sci. Pol. Zootech. 13 (4): 55–66.

MILCZAREK A., OSEK M., 2014b: Jakość mięsa świń rasy puławskiej żywionych mieszankami zawierającymi różne źródła białka. Ann. Univ. Mariae Curie Sklodowska Sect. EE Zootech. 32: 43–50.

MILCZAREK A., OSEK M., 2016: Partial replacement of soya bean with low-tannin faba bean varieties (Albus or Amulet): effects on growth traits, slaughtering parameters and meat quality of Pulawska pigs. Ann. Anim. Sci. 16 (2): 477–487.

Nutritional Recommendations for Pigs, 2015. The Kielanowski Institiute of Animal Physiology and Nutrition PAS, Jabłonna.

PRANGE H., JUGRRT L., SCHARNER E., 1977: Untersuchungen zur Muskel Fleischqualität beim Schwein. Archiv für Experimentelle Veterinär Medizin. Leipzig 31 (2): 235–248.

PRZYBYLSKI W., KAJAK-SIEMASZKO K., JAWORSKA D., CZARNIECKA-SKUBINA E., WACHOWICZ I., URBAŃSKA I., 2007: Influence of different level of intramuscular fat on pork quality. Anim. Sci. (1): 112–113.

SCHEFFLER T.L., GERRARD D.E., 2007: Mechanisms controlling pork quality development: The biochemistry controlling post mortem energy metabolism. Meat Sci. 77: 7–16.

SCHWÖRER D., HOFER A., LORENZ D., REBSAMEN A., 2000: Selection progress of intramuscular fat in Swiss pig production.

EAAP Publication 100. Zurich, Switzerland. 25 August 1999: 69–72.
SMITH L.A., HOUDIJK J.G.M., HOMER D., KYRIAZAKIS I., 2013: Effects of dietary inclusion of pea and faba bean as a replacement for soybean meal on grower and finisher pig performance and carcass quality. J. Anim. Sci. 91: 3733–3741.
STRZYŻEWSKI T., BLISKA A., KRYSZTOFIAK K., 2008: Zależność pomiędzy wartością pH mięsa a jego barwą. Nauka Przyroda Technologie 2 (2): 1–9.
ŚWIĄTKIEWICZ M., HANCZAKOWSKA E., TWARDOWSKA M., MAZUR M., KWIATEK K., KOZACZYŃSKI W., ŚWIĄTKIEWICZ S., SIERADZKI Z., 2011: Effect of genetically modified feeds on fattening results and transfer of transgenic DNA to swine tissues. Bull. Vet. Inst. Pulawy 55: 121–125.
TOMOVIĆ V.M., JOKANOVIĆ M.R., PETROWIĆ L.S., TOMOVIĆ M.S., TASIĆ T.A., IKONNIĆ P.M., ŃUMIĆ Z.M., ŃOJIĆ B.V., ŃKALJAC S.B., ŃOSO M.M., 2013: Sensory, physical and chemical characteristics of cooked ham manufactured from rapidly chilled and earlier deboned *M. semimembranosus*. Meat Sci. 93: 46–52.
WHO, 2003: Technical Report 916. Diet, nutrition and prevention of chronic diseases. Genewa.
WILLIAMS C.M., 2000: Dietary fatty acids and human health. Ann. Zootech. 49: 165–180.
WOOD J.D., WSEMAN J., COLE D.J.A., 1994: Control and manipulation of meat quality. In: Principles of Pig Science. Nottingham University Press: 433–456.
ZIJLSTRA R.T., LOPETINSKY K., BELTRANENA E., 2008: The nutritional value of zero-tannin faba bean for grower-finisher pigs. Can. J. Anim. Sci. 88 (2): 293–302.

Streszczenie: *Jakość mięsa świń żywionych mieszankami z niskotaninowym bobikiem.* Celem badań była ocena wartości rzeźnej i jakości mięsa (*musculus longissimus lumborum*, *musculus semimembranosus*) świń żywionych mieszankami z bobikiem. Badaniami objęto 48 tuczników pochodzących z trzech grup żywieniowych. Świnie grupy I żywiono mieszankami zawierającymi śrutę poekstrakcyjną sojową jako jedyny surowiec wysokobiałkowy, a tuczniki grup II i III otrzymywały mieszanki grower/finiszer z 5/10% lub 10/20% udziałem bobiku niskotaninowego. Wykazano, że wprowadzenie bobiku do mieszanek dla tuczników zwiększyło ich mięsność i powierzchnię „oka" polędwicy oraz zmniejszyło otłuszczenie tusz. Ponadto mięśnie świń grup II i III zawierały więcej niezbędnych nienasyconych kwasów tłuszczowych ($C18:2_{n-6}$, $C18:3_{n-3}$). Istotnie ($P \leq 0{,}01$) lepszym wskaźnikiem wodochłonności (WHC) cechował się *musculus longissimus lumborum* świń żywionych mieszankami z bobikiem.

Słowa kluczowe: bobik, wartość rzeźna, wieprzowina, właściwości fizyczne, skład chemiczny

MS received 15.11.2016
MS accepted 13.03.2017

Authors' address:
Anna Milczarek
Katedra Żywienia Zwierząt i Gospodarki Paszowej
Uniwersytet Przyrodniczo-Humanistyczny w Siedlcach
ul. B. Prusa 14, 08-110 Siedlce
Poland
e-mail: anna.milczarek@uph.edu.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 113–120
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.13

The changes in the milk composition and its lipid fraction during the rearing of lambs in non-milked sheep

AURELIA RADZIK-RANT, WITOLD RANT, URSZULA JANKOWSKA, EWA KUŹNICKA
Department of Animal Breeding, Warsaw University of Life Sciences – SGGW

Abstract: *The changes in the milk composition and its lipid fraction during the rearing of lambs in non-milked sheep*. Studies regarding the effect of lactation stage on milk content and lipid fraction composition are mostly conducted on milked animals. The aim of this study was to analyze the changes in the basic milk composition and content of fatty acids in the fat fraction during the rearing of lambs in non-milked sheep. The study was carried out on 22 ewes of Polish lowland sheep of Żelazneńska strain, which reared lambs sold at low weight classes (up to 22 kg). Milk samples were collected at 10th (period 1), 25th (period 2) and 52nd (period 3) day of lactation. There were no differences in the amount of the basic components of milk in the studied periods of lactation beside the fat ($P \leq 0.05$) content. There were also no difference in the content of fatty acid groups in the fat fraction of ewe's milk examined at 10th, 25th and 52nd day of lactation. Although, in the third period of lactation, the content of saturated fatty acids (SFA) was slightly higher compared to first period. In turn, the content of oleic acid ($P \geq 0.30$) and C18:3 ($P \leq 0.05$) was higher in 10th day of rearing then in 52nd day. A slightly larger share of essential C18 unsaturated fatty acids in ewe's milk in early lactation may suggest the involvement of adipose tissue in the formation of milk fat.

Key words: non-milked sheep, milk composition, stage of lactation

INTRODUCTION

Sheep's milk is one of the most valuable product of animal origin. In addition to basic nutrients it contains many biologically active compounds, which ensure the proper development of lambs, and can affect the quality and health-promoting properties of their meat after slaughter. They have also a positive impact on human health. Content of sheep's milk and the milkfat composition may depend on many factors, i.e. breed, diet, age of ewes, as well as the stage of lactation (Atti et al. 2006, De La Fuente et al. 2009, Rozbicka-Wieczorek et al. 2015).

There are not many research on changes in the composition of sheep's milk during lactation especially in non-milked sheep. During lactation, secretory cells of mammary gland utilize 80% of the blood circulating metabolites for milk synthesis, depending on the speed of infiltration of precursors of milk compounds (i.e. free amino acids, glucose or fatty acids). The reduction of lipogenesis and increase of fatty acid mobilization from adipose tissue at the beginning

of lactation, induces an increase in the activity of enzymes of mammary gland, to provide substrates for milk fat synthesis. The increase in protein catabolism in the serum with the progress of lactation, provides a steady increase substrates for the synthesis of milk protein (Nazifi et al. 2002, Krajnicakova et al. 2003, Darwesh et al. 2013). Changes in these processes during lactation may cause changes in the content of the milk components depending on its stage. Major changes in the composition of milk fat during lactation are recorded in dairy animals producing more milk as cows or goats (Darwesh et al. 2013, Billal et al. 2014). Also in sheep, stage of lactation may differently influence the composition of milk in milked sheep and those, which only rearing lambs.

The aim of this study was to analyze the changes in the basic milk composition and content of fatty acids in the fat fraction during the rearing of lambs in non-milked sheep.

MATERIAL AND METHODS

Animals, treatment and sampling

The study was carried out on ewes of Polish lowland sheep of Żelazneńska strain, which reared lambs sold at low weight classes. Reared lambs required weight reached about 60 days of age. Chosen 22 ewes at the age of 3–4 years, which were fed according to standards for lactating ewes (Osikowski et al. 1998). The diet was based on meadow hay (3.89 MJ/kg of dry matter [DM], 11.8% of total protein [CP]/kg DM, 29.32% crude fiber [CF]/kg SM) and concentrate (7.02 MJ/kg DM, 18% CP/kg DM) consisting of: oat meal (30.5%) wheat meal (23%), rapeseed (30.5%), wheat bran (15%) and compound mineral (1%). Ewes were fed twice a day, morning and evening. Fresh water was available ad libitum. All the ewes lambing in approximately the same day.

Milk samples were collected at 10th (period 1), 25th (period 2) and 52nd (period 3) day of lactation. The lambs were separated from their dams 2 h before milk collection. Then ewes after injection 5 units of oxytocin were hand milked. A representative milk sample (100 ml) was taken from the full udder together with residual milk of each ewe and placed in a sterile bottle with a preservative (Mlekostat CC). Immediately after collection, milk samples were transferred to the Milk Testing Laboratory in order to determine the chemical composition and the content of fatty acids in the fat fraction.

Chemical analysis

The basic chemical composition, i.e. protein, fat, lactose and total solid (TS) amount were determined by infrared spectrophotometry using Milkoscan FT-120 (Foss Electric, Hillerod, Denmark).

Milk fat was extracted according to Röse-Gotlieb method (AOAC 1990). Methylation of the fatty acids was made by transesterification according to EN-ISO 5509:2000. The separation and quantification of fatty acid methyl esters (FAMEs) were carried out by gas chromatography using a Hewlett Packard 5890 with FID detector equipped with capillary column (length – 60 m; internal diameter – 0.25 mm; film thickness 0.25 μm; Agilent Technologies, Wald-

bronn, Germany). Operating conditions were as follows: carrier helium flow 20 cm per 1 s; detector temperature at 240°C, injector temperature at 220°C. The temperature program was as follow: 130°C for 1 min; 130–210°C at 10°C for 1 min; 210°C for 25 min, 210–230°C at 2.5°C for 1 min and 230°C for 18 min. On the basis of retention time relative to the palmitic acid C16:0 selected fatty acids was identified. By the method of the external calibration for both their total fatty positional and geometric isomers of quantitative analysis was carried out using reference Supelco and Sigma.

Statistical analysis

Statistical analysis of the data was performed using the SPSS 23.0 software (2016) using paired t-test for dependent samples.

RESULTS AND DISCUSSION

The content of the basic ingredients of milk in the studied periods of rearing lambs are presented in Table 1. The differences in the content of the most components were not statistically significant, although as expected during the peak of lactation, which in sheep falls to four weeks, the fat content in milk was the lowest ($P \leq 0.05$) in comparison to period 3.

Similar changes in the chemical composition of milk during lactation in Corriedale and Friesian sheep were registered by Niżnikowski et al. (1999). In Wrzosówka sheep, unlike in the present study, milk fat content was lower at the beginning of lactation. The increase in fat content was observed from the fourth week of milk secretion (Nowak and Niżnikowski 1994). In another study on the effect of lactation stage on milk composition in the local goats breeds, also the lowest part in fat amount in its middle stage has been reported (Strzałkowska et al. 2010, Darwesh et al. 2013, Mahmoud et al. 2014). The decrease in the content of this component during the major part of lactation may be related with the effect of dilution due to the increase in milk volume and a decrease in fat mobilization from adipose tissue that decreases the availability of plasma non-esterified fatty acid (NEFA) for mammary lipid synthesis (Chilliard et al. 2003).

No difference in the content of fatty acid groups in the fat fraction of ewe's

TABLE 1. The content of basic ingredients in the milk of examined lactation periods (%)

Item	Period 1		Period 2		Period 3	
	AVG	*SD*	*AVG*	*SD*	*AVG*	*SD*
Dry matter	18.86	2.66	18.22	2.46	19.18	1.46
Protein	4.48	0.61	4.56	0.81	4.05	1.40
Fat	8.11	2.16	7.57[a]	1.13	9.31[b]	1.54
Lactose	4.83	0.61	5.08	0.39	4.71	0.60
Ash	0.76	0.13	0.84	0.08	0.79	0.13

Means with different letters in rows (a, b) differ significantly at $P \leq 0.05$.

TABLE 2. The content of fatty acids group in fat milk of examined lactation periods (g/100 g fat)

Item	Period 1		Period 2		Period 3	
	AVG	*SD*	*AVG*	*SD*	*AVG*	*SD*
SFA	58.19	2.99	58.78	1.48	59.13	1.09
MUFA	29.12	2.39	29.09	2.28	28.61	0.70
PUFA	3.51	0.24	3.52	0.27	3.45	0.26
UFA	32.63	2.51	32.61	2.48	32.06	0.72
n-6	1.98	0.17	2.01	0.17	1.95	0.19
n-3	1.06	0.07	1.05	0.08	1.00	0.06

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, UFA – unsaturated fatty acids.

milk examined at 10th, 25th and 52nd day of lactation was recorded (Table 2). Although, the content of saturated fatty acids (SFA) was higher ($P \geq 0.40$) in the third period of lactation, especially when compared to the first, while the UFA content was lower ($P \geq 0.50$) in period 3 compared to the first. It can only suggest, a slight decrease in the *de novo* synthesis of fatty acids in early stage of lactation and greater mobilization of lipids, mainly NEFA, from adipose tissue for the synthesis of milk fat in connection with possible negative energy balance in this period. The significantly higher concentration of UFA and lower SFA in fat of cow's milk during early lactation has been reported by many researchers (Auldist et al. 1998, Stoop et al. 2009, Arnould et al. 2010).

In fat milk of studied ewes the largest share in SFA group constituted medium chain fatty acids (MCFA) (C12–C16), and their content at 10th, 25th and 52nd day of the milk secretion was 61.5, 62.4, 62.5% respectively. Of these acids, the special attention is paid to palmitic acid C16:0, whose content can reflect the involvement of the mammary gland in the synthesis of milk fat (Barber et al. 1997). In the first stage of lactation the content of that acid was lower by about 3% when compared to the second and third period, which may indicate a slight reduction in the *de novo* synthesis in the early lactation (Table 3). The second, regarding amount, in SFA group was acid C18:0. Its highest level was recorded in third period considered. The increase in C16:0 and C18:0 in the final stage of lactation in Churra sheep breed also have been obtained by De La Fuente et al. (2009). It should be noted that the above-mentioned sheep are used for milk production, whose lactation is much longer.

In the group of monounsaturated fatty acids the acid C18:1*cis*9 was dominated. In the first studied period, its quantity represented 77% of the total MUFA, in the second and third period 76 and 75%, respectively. This acid which is one of the most important fatty acids in the fat fraction of milk, may come from various sources, including adipose tissue. Its slightly higher content ($P \geq 0.30$) in the first period especially when compared to the third, may suggest that transferred

TABLE 3. The content of fatty acids in fat milk of examined lactation periods (g/100 g fat)

Fatty acids	Period 1		Period 2		Period 3	
	AVG	*SD*	*AVG*	*SD*	*AVG*	*SD*
C6:0	2.27	0.25	2.16	0.25	2.25	0.23
C8:0	1.29	0.11	1.25	0.10	1.24	0.09
C10:0	5.73	1.03	5.61	1.11	5.47	0.99
C12:0	3.14	0.25	3.19	0.26	3.07	0.15
C14:0	8.25	1.41	8.50	1.19	8.79	0.82
C15:0	1.16	0.19	1.14	0.20	1.18	0.16
C16:0	23.26	1.17	23.83	0.98	23.90	1.38
C17:0	1.04	0.15	1.06	0.12	1.05	0.15
C18:0	12.05	0.72	12.04	1.16	12.18	0.76
C10:1	0.14	0.03	0.13	0.02	0.14	0.02
C14:1	1.26	0.22	1.22	0.45	1.25	0.31
C15:1	0.22	0.05	0.21	0.03	0.22	0.04
C16:1	2.38	0.36	2.59	0.21	2.51	0.21
C18:1*t*11	1.85	0.35	1.93	0.43	2.08	0.55
C18:1	0.74	0.09	0.73	0.09	0.8 0	0.05
C18:1*c*9	22.41	2.64	22.16	2.42	21.48	0.86
C20:1	0.12	0.03	0.12	0.03	0.14	0.02
C18:2	1.81	0.17	1.83	0.16	1.77	0.18
C18:2*c*9*t*11	0.45[a]	0.07	0.47[a]	0.10	0.51[b]	0.12
C18:3	0.68[a]	0.04	0.66[a]	0.05	0.63[b]	0.03
C20:3	0.08	0.03	0.08	0.01	0.07	0.01
C20:4	0.17	0.02	0.17	0.01	0.18	0.02
C20:5	0.09	0.01	0.09	0.01	0.10	0.01
C22:5	0.15	0.04	0.15	0.03	0.14	0.03
C22:6	0.07	0.01	0.07	0.01	0.06	0.01

Means with different letters in rows (a, b) differ significantly at $P \leq 0.05$.

from a pool of plasma NEFA replaced acids *de novo* synthesized by the mammary gland (Table 3). Negative correlation –0.73 ($P \leq 0.05$) between oleic acid and palmitic acid tested on 10th day of rearing lambs and positive (0.62) correlation with a total fat content seems to confirm this. Due to the deficit of energy in early lactation enzymes activity involved in the synthesis of milk fat may be somewhat reduced even in not milked animals characterized by lower milk production (Chiliard et al. 2003). Similar relationships between the above-

mentioned acids was obtained by De La Fuente et al. (2009) in milk of Churra ewes, by Chilliard et al. (2003) in goats and Billal et al. (2014) in cows.

The differences in the content of polyunsaturated fatty acids in tested periods of lactation, besides linolenic acid (C18:3) and conjugated linoleic acid (C18:2*c*9*t*11) have been not confirmed statistically. The content of C18:3 acid in 10th day of rearing was higher ($P \leq 0.05$) compared to 52nd day of lactation (Table 3). It is known that these fatty acids in milk fat does not come from endogenous synthesis, but only from the feed or adipose tissue after their release by lipoprotein lipase in the blood (Clegg et al. 2001). In the study conducted by De la Fuente et al. (2009) on the influence of the stage of lactation in dairy Churra breed in contrast to the results obtained in this study the content of C18:3 increased with advancing lactation. In the present study together with advancing lactation increased ($P \leq 0.05$) the C18:2*c*9*t*11 acid content of which the presence in the milk is mainly related to endogenous synthesis from vaccenic acid.

CONCLUSION

The period of lactation of studied ewes during the rearing lambs did not affect the content of the basic components of milk.

No clear effect was noted of lactation stage on the contents of groups and individual fatty acids in the fat fraction in milk of studied ewes. An exception was the linolenic acid (C18:3 n-3) which higher contents was registered in the early phase of the rearing lambs.

Both fatty acids content in milk and relationships between some fatty acids only suggest trends for the occurrence of negative energy balance in early lactation in ewes whose milk is used only for rearing lambs. A slightly larger share of essential C18 unsaturated fatty acids in milk in early lactation may suggest the involvement of adipose tissue in the formation of milk fat.

Acknowledgement

This study was carried out as a part of research project N N311081240, supported by the National Science Center of Poland.

REFERENCES

AOAC (Association of Official Analytical Chemists)., 1990: Food Composition Additives, Natural Cotaminants, 15th edn. Arlington, TX, USA.

ARNOULD V.M.R., HAMMANI H., SOYEURT H., GENGLER N., 2010: Short communication: Genetic variation of saturated fatty acids in Holsteins in the Walloon region of Belgium. J. Dairy Sci. 93: 4391–4397.

ATTI N., ROUISSI H., OTHMANE M.H., 2006: Milk production, milk fatty acid composition and conjugated linolenic acid (CLA) content in dairy ewes raised on feedlot or greazing pasture. Livest. Sci. 104: 121–127.

AULDIST M.J., WALSH B.J., THOMSON N.A., 1998: Seasonal and lactational influences on bovine milk composition in New Zealand. J. Dairy Resch. 65: 401–411.

BARBER M.C., CLEGG R.A., TRAVERS M.T., VERNON R.G., 1997: Lipid metabolism in the lactating mammary gland. Biochimica et Biophysica Acta 1347: 101–126.

BILLAL G., CUE R.J., MUSTAFA A.F., HAYES J.F., 2014: Effect of parity, age at calving and stage of lactation on fatty acid composition of milk in Canadian Holsteins. Can. J. Animal Sci. 94 (3): 40–41.

CHLIARD Y., FERLAY A., ROUEL J., LAMBERET G., 2003: A review of nutritional and physiological factors affecting goat milk synthesis and lipolysis. J. Dairy Sci. 86: 1571––1570.

CLEGG R.A., BARBER M.C., POOLEY L., ERNENS I., LARONDELLE Y., TRAVERS M.T., 2001: Milk fat synthesis and secretion molecular and cellular aspects. Livest. Prod. Sci. 70: 3–14.

De La FUENTE L.F., BARBOSA E., CARRIEDO J.A., GONZALO C., ARENAS R., FRESNO J.M., 2009: Factors influencing variation of fatty acid content in ovine milk. J. Dairy Sci. 92: 3791–3799.

DARWESH K.A., MERKHAN K.Y., BUTI E.T.S., 2013: Impact of lactation stage on the body condition and milk quality of black goat. J. Agric. Food Res. 2 (2): 48–52.

EN-ISO 5509:2000. Animal and vegetable fats and oils. Preparation of methyl esters of fatty acids.

KRAJNICAKOVA M., KOVAC G., KOSTECKY M., VALOCKY I., MARACEK I., SUTIAKOVA I., LENHARDT L., 2003: Selected clinical-biochemical parameters in the puerperal period of goats. Bulletin Vet. Inst. Puławy. 47: 177–182.

MAHMOUD N.M.A., El ZUBEIR I.E.M., FADLELMOULA A.A., 2014: Effect of stage of lactation on milk yield and composition of first kinder damascus does in the Sudan. J. Anim. Prod. Adv. 4 (3): 355–362.

NAZIFI S., SAEB M., GHAVAMI S.M., 2002: Serum lipid profile in Iranian fat-tailed sheep in late pregnancy, at parturition and during the post-parturition period. J. Vet. Med. 49: 9–12.

NIŻNIKOWSKI R., RANT W., RADZIK-RANT A., SZTYCH D., KUŹNICKA E., 1999: Poziom mleczności oraz możliwości użytkowania mlecznego owiec różnych genotypów na podstawie badań wykonanych w Zakładzie Hodowli Owiec i Kóz SGGW. In: III Owczarska Szkoła Wiosenna „Alternatywne kierunki wykorzystania krajowego pogłowia owiec" Krynica, 12–14 kwietnia: 29–45.

NOWAK W., NIŻNIKOWSKI R., 1996: Wpływ wybranych czynników na cechy mleczności maciorek rasy wrzosówka karmiących jagnięta. Zesz. Nauk. PTZ 23: 145–160.

OSIKOWSKI M., PORĘBSKA W., KORMAN K., 1998: Normy żywienia owiec. In: Normy żywienia bydła i owiec systemem tradycyjnym, R. Ryś (Ed.). 12th edn. Wydaw. IŻ, Kraków: 29–57.

ROZBICKA-WIECZOREKA.J., RADZIK-RANT A., RANT W., KUCZYŃSKA B., CZAUDERNA M., 2015: Characterization of the milk lipid fraction in non-dairy sheep breeds. Arch. Anim. Breed. 58: 395–401.

STOOP W.M., BOVENHUIS H., HECK J.M.L., ARENDONK J.A.M., 2009: Effect of lactation stage and energy status on milk fat composition of Holstein-Friesien cows. J. Dairy Sci. 92: 1469–1478.

STRZAŁKOWSKA N., JÓŹWIK A., BAGNICKA E., KRZYŻEWSKI J., HORBAŃCZUK K., PYZEL B., SŁONIEWSKA D., HORBAŃCZUK J.O., 2010: The concentration of free fatty acids in goat milk as related to the stage of lactation, age and somatic cell count. Anim. Sci. Papers and Reports 28, 4: 389–395.

Streszczenie: *Zmiany w składzie mleka i jego frakcji tłuszczowej w trakcie odchowu jagniąt u owiec nieużytkowanych mlecznie*. Badania dotyczące wpływu laktacji na składniki mleka i skład frakcji lipidowej są w większości prowadzone na zwierzętach użytkowanych mlecznie. Celem tych badań było przeanalizowanie zmian w podstawowym składzie mleka i w zawartości kwasów tłuszczowych w jego frakcji tłuszczowej w trakcie odchowu jagniąt u owiec niedoskonalonych w kierunku użytkowania mlecznego. Badania prowadzono na 22 maciorkach nizinnych odmiany żelazneńskiej, które odchowywały jagnięta sprzedawane w niskich standardach wagowych (do 22 kg). Próby mleka pobierano w 10. (okres 1), 25. (okres 2) i 52. (okres 3) dniu laktacji. Nie znaleziono różnic w zawartości podstawowych składników mleka w badanych okresach laktacji, oprócz różnic w zawartości tłuszczu. Różnice nie występowały także w zawartości grup kwasów tłuszczowych w frakcji tłuszczowej mleka maciorek badanych w 10., 25. i 52. dniu laktacji, chociaż zawartość nasyconych kwasów tłuszczowych (SFA) była nieznacznie większa w trzecim okresie laktacji w porównaniu z okresem pierwszym. Z kolei zawartość kwasu oleinowego ($P \geq 0{,}30$) i C18:3 ($P \leq 0{,}05$) była większa w 10. dniu od-

chowu jagniąt niż w 52. dniu. Niewiele większy udział niezbędnych nienasyconych kwasów tłuszczowych C18 w mleku maciorek we wczesnej laktacji może wskazywać na zaangażowanie tkanki zapasowej w tworzenie tłuszczu mleka.

Słowa kluczowe: owce niedojone, skład mleka, stadium laktacji

MS received 14.11.2016
MS accepted 03.03.2017

Authors' address:
Aurelia Radzik-Rant
Katedra Szczegółowej Hodowli Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8, 02-786 Warszawa
Poland
e-mail: aurelia_radzik_rant@sggw.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 121–127
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.14

Meat quality of fattening pigs fed yellow lupin-based diets

MARCIN SOŃTA, ANNA REKIEL, JUSTYNA WIĘCEK,
BEATA KUCZYŃSKA, WIOLETA KNIŻEWSKA
Department of Animal Breeding and Production, Warsaw University of Life Sciences – SGGW

Abstract: *Meat quality of fattening pigs fed yellow lupin-based diets*. The 30 crossbred pigs [(Landrace × Yorkshire) × Duroc] were fattened in three-phase fattening period. In their nutrition as a source of protein was used soybean meal (Group C) or soybean meal and seeds of yellow lupine in the amount of 7.5% (Group E1) and 15% (Group E2). After achieving body weight of about 117.5 kg animals were slaughtered. The samples of *musculus longissimus lumborum* collected from all slaughtered pigs. Significant differences were found in drip loss percentage between groups C and E1 ($P \leq 0.05$). As regards the fatty acids, there were lower proportions of C18:2 in group E1 vs C ($P \leq 0.05$) and of C18:2 and C20:4 in group E2 vs C ($P \leq 0.01$). Differences in PUFA percentage, PUFA/SFA ratio, and proportion of n-6 fatty acids were significant, with lower values of the traits in group E1 vs C ($P \leq 0.05$) and in group E2 vs C ($P \leq 0.01$), which shows that the dietetic value of pork has slightly deteriorated. The chemical composition and the physical parameters of the meat samples were normal and at a similar level in all the groups.

Key words: fattening pigs, feeding, yellow lupin, meat quality

INTRODUCTION

Production results as well as the quality of raw materials and products are subject to thorough analysis as part of the field-to-fork programmes. These also account for the nutrition, quantity and quality of compound feeds for animals, and the acquisition of feed materials for their production. For years studies have been conducted and attempts have been made to replace soybean meal with legume seeds in livestock production (Roth-Maier et al. 2004, Froidmont et al. 2005, Pisařiková et al. 2008, Płazak et al. 2012, Kasprowicz-Potocka et al. 2014). The results obtained are thoroughly evaluated and analysed. Feeds with legume seeds are used to investigate the growth and slaughter parameters of monogastric animals (Zralý et al. 2006, 2007, Płazak et al. 2012, Sońta et al. 2015, 2016) and the qualitative parameters of the slaughter material obtained and the products made (Zralý et al. 2006, 2007, Kim et al. 2011, Mordenti et al. 2012, Hanczakowska and Świątkiewicz 2014, Milczarek and Osek 2014, Sirtori et al. 2015). The results of the studies cited above and many other experiments have confirmed the nutritional suitability of legumes for pigs. The analyses of economic efficiency are the final stage of research concerning the utility of legumes for production. The studies performed by Sońta et al.

(2015, 2016) have confirmed the appropriateness of using legumes in live pig production.

Advances in breeding, the new quality of the produced plant material, and the implementation of novel processing technologies for legume seeds have advantageously affected the value and nutritional suitability of different legume species and varieties. There have also been changes in the breeding and productive value of animals. In view of the above, and after analysing various opinions concerning the nutritional suitability of legumes in the feeding of monogastric animals, the present study was undertaken to determine the effect of partial replacement of soybean meal with yellow lupin meal in growing pig diets on pork quality.

MATERIAL AND METHODS

Thirty crossbred weaners, gilts and barrows [♀ (Landrace × Yorkshire) × × ♂ Duroc] were fattened from 27.2 to 117.5 kg of body weight. Animals were divided into three groups: control C and experimental E1 and E2 (each having 10 animals, 1 : 1 sex ratio) and placed in pens (10 animals per pen) under uniform conditions (Regulation of the Ministry of Agriculture and Rural Development of 15 February 2010). During three fattening stages, animals were fed *ad libitum* complete diets with constant access to water, as described in Sońta et al. (2016). In the control group, protein was provided by soybean meal, and in the experimental groups E1 and E2 by yellow lupin (7.5 and 15%, respectively), which partially replaced soybean meal. At the end of fattening (approx. 117.5 kg of body weight), animals were slaughtered and after 24-hour chilling of the carcasses at 4°C, a muscle sample (approx. 0.5 kg) was collected from the right half-carcasses for quality analysis. Ground samples of meat from *musculus longissimus lumborum* were analysed for the content of water, protein, fat and collagen (PN-A-82109:2010) using a Food--Scan Lab meat analyser (Foss). Fatty acid profile was determined. Fatty acid methylation was performed according to the trans esterification method EN-ISO 5509:2000). Identification of individual fatty acids in crude fat was conducted using an Agilent 7890A GC (Agilent, Waldbronn, Germany) with flame–ionization detector (FID), HP Chem software and Varian Select FAME column (100 m length, 0.25 mm diameter, 0.25 µm film thickness; Varian/Agilent Technologies, Waldbronn, Germany). The separation was performed at pre-programmed temperature: 130°C for 1 min; 130–170°C at 6.5°C/min; 170–215°C at 2.75°C/min; 215°C for 12 min, 215–230°C at 20°C/min and 230°C for 3 min. Each peak was identified using pure methyl ester standards: PUFA 1, Lot LB 75066; PUFA 2, Lot LB 83491; FAME Mix RM-6, Lot LB 68242; Supelco 37 Comp. FAME Mix, Lot LB 68887 (Supelco, Bellefonte, PA, USA). The following fatty acids were determined in the profile: C14:0 – myristic acid, C16:0 – palmitic acid, C16:1 – palmitoleic acid, C18:0 – stearic acid, C18:1 – oleic acid, C18:2 – linoleic acid, C18:3 – linolenic acid, C20:4 – arachidonic acid, C20:5 – eicosapentaenoic acid, C22:4 – docosatetraenoic acid, as well as SFA, MUFA, PUFA, n-3 and n-6. The atherogenic index (AI) and the thrombogenic

index (TI) were calculated according to Ulbricht and Southgate (1991):

$$IA = (4 \times C14:0 + C16:0 + C18:0) / / (MUFA + PUFA)$$

$$IT = (C14:0 + C16:0 + C18:0) / / (0.5 \times MUFA + 0.5 \times n\text{-}6\ PUFA + + 3 \times n\text{-}3PUFA + n\text{-}3/n\text{-}6\ PUFA)$$

Meat colour parameters were measured in the CIE L*a*b* space with a Chroma Meter CR-400/410 colorimeter (Konica Minolta). The meat colour determination procedure consisted of taking an approx. 2-cm muscle slice and making the measurements at 3 points (the result was averaged). Hue (b*/a*) and chroma [$\sqrt{(a^{*2} + b^{*2})}$] were calculated according to Mordenti et al. (2012).

In order to determine drip loss, an approx. 300 g sample of meat was placed in a polyethylene bag and kept under cold storage conditions (4°C) for 24 h. After this time, the exudate was poured off and its amount was expressed as a percent of the sample weight.

Water holding capacity was determined using the method described by Grau and Hamm (1952) as modified by Pohja and Ninivaara (1957).

Shear force was measured with a Zwick 1120 (Germany) tensiometer equipped with a Warner–Bratzler blade. The samples of meat (approx. 150 g) were roasted at 180°C until the internal temperature reached 76°C in the geometrical center of the sample. The samples were cooled at room temperature (18–22°C) and placed into a cold storage room (4°C). Three cube-shaped samples (20 × 20 × 20 mm) were cut from the slice after 24 h. Determinations were made transversely across the muscle fibres until the sample was cut completely. The maximum force needed to shear the sample was taken as the shear force value. A crosshead speed of 30 mm/min was applied until an initial tension of 2 N was reached, and 50 mm/min was used during the test proper.

The results were statistically analysed with IBM SPSS Statistics 21 software. The normality of data distribution was verified by the Shapiro–Wilk test. Differences between the groups were tested using the Kruskal–Wallis test.

RESULTS AND DISCUSSION

No statistically significant differences were found in the main chemical components of *musculus longissimus lumborum* (MLL) samples from pigs in groups C, E1 and E2 (Table 1). Hue and chroma were comparable. A difference of 1 percentage point ($P \leq 0.05$) was noted for drip loss (group C vs E1). Shear force was highest for the meat samples from pigs in group C, and lower by 3.62 and 9.41% in groups E1 and E2, respectively. The highest drip loss was noted for group C, with 6.55 and 6.67% lower values for groups E1 and E2, respectively.

Zralý et al. (2006, 2007), when feeding pigs with diets containing white lupin, obtained slightly higher moisture content, and lower or comparable protein content in meat samples, compared to our study. Sobotka and Antoszkiewicz (2002), who replaced soybean meal in pig diets with field bean, peas and rapeseed meal, also observed higher moisture content and lower protein content of the meat. Milczarek and Osek (2014), when using field bean and DDGS as a replace-

TABLE 1. Chemical and physical parameters of *musculus longissimus lumborum*

Item	Groups						*P*
	control		experimental 1		experimental 2		
	AVG	*SE*	*AVG*	*SE*	*AVG*	*SE*	
Content of the main chemical components (%)							
Water	71.78	0.24	71.51	0.20	71.43	0.29	0.470
Protein	23.23	0.11	23.38	0.13	23.05	0.10	0.159
Fat	3.81	0.22	3.54	0.24	3.83	0.31	0.788
Collagen	0.97	0.06	0.84	0.07	0.83	0.06	0.196
Physical properties							
CIE colour coordinates							
L*	51.49	0.94	52.29	0.53	51.71	0.86	0.816
a*	7.71	0.49	7.38	0.24	7.74	0.36	0.628
b*	5.12	0.33	5.13	0.25	5.12	0.16	0.993
Hue	0.66	0.03	0.70	0.04	0.66	0.04	0.545
Chroma	5.07	0.16	5.00	0.08	5.07	0.08	0.668
WHC (cm^2/g)	16.95	1.02	15.84	0.97	15.82	1.07	0.712
Drip loss (%)	1.23[a]	0.27	2.23[a]	0.34	2.10	0.92	0.040
Shear force (N)	92.30	8.29	88.96	13.94	83.61	12.23	0.709

a, a – means in rows with the same small letters differ significantly at $P \leq 0.05$.

ment, found slightly higher protein and lower fat content in MLL and in ham compared to the control group. Mordenti et al. (2012) reported no negative effect of removing soybean meal from pig diets on hue and chroma compared to the control group. Sirtori et al. (2015) showed lower hue values for samples of *musculus longissimus lumborum* muscle in the groups supplemented with peas or vetch in comparison with the control group (by 14.71 and 20.59%, respectively). The same authors obtained similar results for chroma, which suggests that it is appropriate to study the above physical parameters of meat when feeding pigs with diets containing vegetable protein replacers of soybean meal. In our study, the colour coordinates, hue and chroma were at a similar level regardless of the group.

Statistically significant differences were only found for C18:2, C20:4 and PUFA, for the PUFA/SFA ratio, and for n-6 fatty acids (Table 2).

Froidmont et al. (2005) studied the fatty acid content of muscle tissue and backfat from pigs fed diets in which soybean meal or white lupin, also supplemented with α-galactosidase, served as protein source. When comparing the content of fatty acids between the groups for both studied tissues, the authors observed many more significant differences for backfat. In the muscle tissue of the pigs fed the white lupin diet, they

TABLE 2. Fatty acid profile (%) of *musculus longissimus lumborum*

Item	Groups						*P*
	control		experimental 1		experimental 2		
	AVG	*SE*	*AVG*	*SE*	*AVG*	*SE*	
C14:0	1.25	0.02	1.32	0.04	1.34	0.03	0.199
C16:0	25.10	0.32	25.81	0.21	26.13	0.26	0.106
C16:1	3.95	0.14	3.79	0.09	3.87	0.13	0.558
C18:0	12.26	0.28	12.51	0.08	12.67	0.36	0.531
C18:1	47.41	0.45	48.38	0.36	48.40	0.61	0.286
C18:2	8.86^{Aa}	0.54	7.07^{a}	0.27	6.48^{A}	0.22	0.001
C18:3	0.29	0.08	0.19	0.01	0.17	0.01	0.335
C20:4	0.47^{A}	0.02	0.54	0.02	0.58^{A}	0.01	0.003
C20:5	0.08	0.01	0.11	0.01	0.09	0.01	0.064
C22:4	0.22	0.03	0.29	0.03	0.27	0.03	0.173
SFA	38.82	0.50	39.65	0.24	40.13	0.61	0.263
MUFA	51.36	0.42	52.17	0.33	52.27	0.58	0.269
PUFA	9.92^{Aa}	0.59	8.21^{a}	0.29	7.60^{A}	0.22	0.002
PUFA/SFA	0.26^{Aa}	0.02	0.21^{a}	0.01	0.19^{A}	0.01	0.002
n-6	9.55^{Aa}	0.52	7.90^{a}	0.27	7.33^{A}	0.21	0.001
n-3	0.37	0.07	0.31	0.02	0.27	0.02	0.322
n-6/n-3	30.48	3.14	26.06	1.14	27.79	1.31	0.275
IA	0.69	0.01	0.72	0.01	0.74	0.02	0.174
IT	1.22	0.03	1.28	0.01	1.31	0.03	0.219

a, a – means in rows with the same small letters differ significantly at $P \leq 0.05$, A, A – means in rows with the same capital letters differ significantly at $P \leq 0.01$.

found a lower proportion of SFA and PUFA, and a higher MUFA proportion. In our study, the direction of change for PUFA and MUFA was similar. In the study by the researchers cited above (Froidmont et al. 2005), the PUFA/SFA ratio was more favourable in the control groups, while the n-6 to n-3 fatty acids ratio was lowest in samples of meat from pigs fed the lupin-based diet. A similar direction of change was observed in our study. Zralý et al. (2007) found a higher proportion of saturated fatty acids in the meat of pigs receiving a white lupin diet (experimental group), and a higher percentage of mono- and polyunsaturated fatty acids in the control group. Our results for SFA and PUFA were similar to the findings of Zralý et al. (2007). Values of AI and TI did not differ among the groups (Table 2) and were comparable to those reported by Okrouhlá et al. (2013). These authors hold that for both meat and the meat product, the AI and TI

values can be modified be dietary means. The authors who fed broiler chickens (Laudadio et al. 2012) and young slaughter cattle (Cutrignelli et al. 2008, Vicenti et al. 2009) with different protein feed materials, including soybean meal, peas, white lupin and field bean, found no fundamental differences in the IA and IT values.

CONCLUSION

Partial replacement of soybean meal with yellow lupin meal had no effect on chemical and physical parameters of *musculus longissimus lumborum*, but caused a slight deterioration in the dietetic value of pork.

REFERENCES

CUTRIGNELLI M.I., CALABRO S., BOVERA F., TUDISCO R., D'URSO S., MARCHIELLO M., PICCOLA V., INFASCELLI F., 2008: Effects of two protein sources and energy level of diet on the performance of young Marchigiana bulls. 2. Meat quality. Ital. J. Anim. Sci. 7: 271–285.

EN-ISO 5509:2000. Animal and vegetable fats and oils. Preparation of methyl esters of fatty acids.

FROIDMONT E., WATHELET B., BECKERS Y., ROMNEÉ J.M., DEHARENG F., WAVREILLE J., SCHOELING O., DECAUWERT V., BARTIAUX-THILL N., 2005: Improvement of lupin seed valorisation by the pig with the addition of α-galactosidase in the feed and the choice of a suited variety. Biot. Agron. Soc. Envir. 9 (4): 225–235.

GRAU R., HAMM R., 1952: Eine einfache Methode zur Bestimmung der Wasserbindung in Fleisch. Fleischwirtschaft 4: 295–297.

HANCZAKOWSKA E., ŚWIĄTKIEWICZ M., 2014: Legume seeds and rapeseed press cake as replacers of soybean meal in feed for fattening pigs. Ann. Anim. Sci. 14 (1): 921–934.

KASPROWICZ-POTOCKA M., ZAWORSKA A., MIKUŁA R., MORAWSKI A., RUTKOWSKI A., 2014: Krajowe źródła białka w żywieniu świń – wyniki badań w gospodarstwach produkcyjnych i ekologicznych. Cz. 1. Trz. Chlew. 9: 36–39.

KIM J.C., MULLAN B.P., NICHOLLS R.R., PLUSKE J.R., 2011: Effect of Australian sweet lupin (*Lupinus angustifolius* L.) inclusion levels and enzyme supplementation on the performance, carcass composition and meat quality of grower/finisher pigs. Anim. Prod. Sci. 51 (1): 37–43.

LAUDADIO V., NAHASHON S.N., TUFAREL-LI V., 2012: Growth performance and carcass characteristics of guinea fowl broilers fed micronized-dehulled pea (*Pisum sativum* L.) as a substitute for soybean meal. Poultry Sci. 91 (11): 2988–2996.

MILCZAREK A., OSEK M., 2014: Jakość mięsa świń rasy puławskiej żywionych mieszankami zawierającymi różne źródła białka. Ann. Univ. M.C. Skłod. Sec. EE: Zoot. 32 (4): 43–50.

MORDENTI A., MARTELLI G., BROGNA N., NANNONI E., VIGNOLA G., ZAGHINI G., SARDI L., 2012: Effects of a soybean-free diet supplied to Italian heavy pigs on fattening performance, and meat and dry-cured ham quality. Ital. J. Anim. Sci. 80 (11): 459-465.

OKROUHLÁ M., STUPKA R., ČITEK J., ŠPRYSL M., BRZOBOHATÝ L., 2013: Effect of dietary linseed supplementation on the performance, meat quality, and fatty acid profile of pigs. Czech J. Anim. Sci. 58 (6): 279–288.

PÍSAŘÍKOVÁ B., ZRALÝ Z., BUŇKY F., TRAČKOVÁ M., 2008: Nutritional value of white lupine cultivar Butan in diets for fattening pigs. Vet. Med. 53: 124–134.

PŁAZAK E., GAJEWCZYK P., AKIŃCZA J., KOSKA M., 2012: Wartość tuczna i rzeźna tuczników żywionych mieszankami pełnoporcjowymi o zróżnicowanym pochodzeniu komponentów białka. Zesz. Nauk UP we Wrocławiu, Biol. Hod. Zw. 64 (586): 37–49.

PN-A-82109:2010. Mięso i przetwory mięsne. Oznaczanie zawartości tłuszczu, białka i wody. Metoda spektrometrii transmisyjnej w bliskiej podczerwieni (NIT) z wykorzystaniem kalibracji na sztucznych sieciach neuronowych (ANN).

POHJA N.S., NINIVAARA F.P., 1957: Die Bestimmung der Wasserbindung des Fleisches mittels der Konstandruckmethode. Fleischwirtschaft 9: 193–195.

Regulation of the Ministry of Agriculture and Rural Development of 15 February 2010 w sprawie wymagań i sposobu postępowania przy utrzymaniu gatunków zwierząt gospodarskich, dla których normy ochrony zostały określone w przepisach Unii Europejskiej. Journal of Laws 2010 No 56, item 344.

ROTH-MAIER D.A., BÖHMER B.M., ROTH F.X., 2004: Effects of feeding canola meal and sweet lupin (*L. luteus*, *L. angustifolius*) in amino acid balanced diets on growth performance and carcass characteristics of growing-finishing pigs. Anim. Res. 53 (1): 21–34.

SIRTORI F., CROVETTI A., ACCIAIOLI A., BONELLI A., PUGLIESE C., BOZZI R., CAMPODONI G., FRANCI O., 2015: Effect of replacing a soy diet with *Vicia faba* and *Pisum sativum* on performance, meat and fat traits of Cinta Senese pigs. Ital. J. Anim. Sci. 14 (1): 99–104.

SOBOTKA W., ANTOSZKIEWICZ Z., 2002: Skład chemiczny i właściwości fizykochemiczne mięsa tuczników w zależności od pochodzenia białka w mieszankach paszowych. Biul. Nauk. UWM w Olsztynie 16: 70–73.

SOŃTA M., REKIEL A., WIĘCEK J., 2015: Efektywność stosowania mieszanek z udziałem łubinu wąskolistnego w żywieniu świń rosnących. Roczn. Nauk. PTZ 11 (1): 35–46.

SOŃTA M., REKIEL A., WIĘCEK J., 2016: Efektywność tuczu świń mieszankami z udziałem łubinu żółtego *Lupinus luteus*. Roczn. Nauk. PTZ 12 (2): 9–18.

ULBRICHT T.L.V., SOUTHGATE D.A.T., 1991: Coronary heart disease: seven dietary factors. Lancet 338: 985–992.

VICENTI A., TOTEDA F., DI TURI L., COCCA C., PERRUCCI M., MELODIA L., RAGNI M., 2009: Use of sweet lupin (*Lupinus albus* L. var. Multitalia) in feeding for Podolian young bulls and influence on productive performances and meat quality traits. Meat Sci. 82: 247–251.

ZRALÝ Z., PÍSAŘÍKOVÁ B., TRČKOVÁ M., HERZIG I., JŮZL M., SIMEONOVOVÁ J., 2006: Effect of lupine and amaranth on growth efficiency, health and carcass characteristics and meat quality of market pigs. Acta Vet. Brno 75 (3): 363–372.

ZRALÝ Z., PÍSAŘÍKOVÁ B., TRČKOVÁ M., HERZIG I., JŮZL M., SIMEONOVOVÁ J., 2007: The effect of white lupine on the performance, health, carcass characteristics and meat quality of market pigs. Vet. Med. 52 (1): 29–41.

Streszczenie: *Jakość mięsa tuczników żywionych mieszankami z udziałem łubinu żółtego*. Przeprowadzono trójfazowy tucz 30 świń mieszańców [(Landrace × Yorkshire) × Duroc]. W ich żywieniu stosowano jako źródło białka poekstrakcyjną śrutę sojową (grupa C) lub poekstrakcyjną śrutę sojową i nasiona łubinu żółtego w ilości 7,5% (grupa E1) i 15% (grupa E2). Po osiągnięciu masy ciała ok. 117,5 kg zwierzęta ubito. Od wszystkich tuczników pobrano po uboju próby *musculus longissimus lumborum*. Różnice istotne odnotowano w procencie wycieku swobodnego między grupami C i E1 ($P \leq 0,05$). W profilu kwasów tłuszczowych stwierdzono: mniejszy udział kwasu C18:2 w grupie E1 względem C ($P \leq 0,05$) oraz C18:2 i C20:4 w grupie E2 względem C ($P \leq 0,01$). Różnice w udziale PUFA, stosunku PUFA/SFA i udziale kwasów z grupy n-6 potwierdzono statystycznie; wartości cech były mniejsze w grupie E1 względem C ($P \leq 0,05$) oraz E2 względem C ($P \leq 0,01$), co świadczy o nieznacznym pogorszeniu wartości dietetycznej wieprzowiny. Skład chemiczny oraz parametry fizyczne prób mięsa były prawidłowe i na podobnym poziomie we wszystkich grupach.

Słowa kluczowe: tuczniki, żywienie, łubin żółty, jakość mięsa

MS received 09.11.2016
MS accepted 14.03.2017

Authors' address:
Anna Rekiel
Katedra Szczegółowej Hodowli Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8, 02-786 Warszawa
Poland
e-mail: anna_rekiel@sggw.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 129–136
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.15

Comparison of meat quality of the Polish Red-and-White and Simmental young bulls

EWA SOSIN-BZDUCHA
Department of Animal Genetic Resources Conservation, National Research Institute of Animal Production

Abstract: *Comparison of meat quality of the Polish Red-and-White and Simmental young bulls*. The experiment used meat from 16 bulls of the Polish Red-and-White (ZR) (n = 8) and Simmental (SM) (n = 8) breeds. Samples of the *longissimus lumborum* muscle (MLL) were analyzed for basic chemical composition and fatty acid profile of intramuscular fat. Physicochemical and organoleptic properties of meat were evaluated. No differences were found in basic chemical composition of the meat. Intramuscular fat from ZR bulls had a notably lower proportion of polyunsaturated fatty acids (PUFA), but a more favorable n-6/n-3 fatty acids ratio in MLL than meat from Simmental breed. Sensory assessment showed that meat from the conservation breed received higher scores due to greater juiciness, more delicate texture and better aroma.

Key words: cattle, Polish Red-and-White, Simmental, meat, quality

INTRODUCTION

The Polish Red-and-White breed conservation programme, launched in 2007, aims to reestablish dual-purpose cattle suitable for relatively high milk production and capable of profitable rearing of calves and fattening of bulls based on farm-produced feedstuffs. This goal is implemented by gradually decreasing the percentage of Holstein-Friesian blood. Research on the quality of products from local breeds increases the added value, which, in turn, may help to improve the profitability and increase the number of animals from endangered populations. Researchers in many countries have worked on modifying the health-promoting properties through nutrition, management or the use of prolonged fattening (Sargentini et al. 2010 – Maremmana breed), or the physicochemical and organoleptic properties (Marino et al. 2014 – Podolian breed). In recent years, novel research has been done on dairy performance and milk quality (Litwińczuk et al. 2012, Adamska et al. 2014), and fattening capacity and meat quality of conservation breeds, mainly the White-backed and Polish Red (Litwińczuk et al. 2014a, Litwińczuk et al. 2014b, Dymnicki et al. 2014).

Beef from Polish Red-and-White cow was included on 27 June 2013 on the list of traditional products by the Ministry of Agriculture and Rural Development. Although the Polish Red-and-White breed has the best fattening capacity of all conservation breeds of cattle, there are relatively few studies on the meat quality of this breed, despite the changes

that have taken place over the years in research methods, the environment and the genotype of the animals, mainly as a result of the breeding policy.

Simmental cattle are a multipurpose breed that is improved for both milk and meat production and according to Choroszy et al. (2009), it complies with the requirements of the meat industry.

The objective of the study was to determine and compare the meat quality of two dual-purpose breeds: the Polish Red-and-White, included in the conservation programme, and the Simmental.

MATERIAL AND METHODS

Animals and feeding

Subjects were 8 Polish Red-and-White and 8 Simmental bulls. Polish Red-and--White bulls descended from parents qualified for inclusion in the genetic resources conservation programme. Bulls were kept and fed in breed groups. Bulls were fed maize silage and hay, or haylage supplemented with concentrate 2.0 kg per day (88.5% DM, 0.96 UFL, 120 g PDIN, 108 g PDIE). The concentrates fed to the bulls contained soybean meal, rapeseed cake, barley, wheat, triticale, ground limestone, and dicalcium phosphate. Rations were formulated to meet IZ-INRA requirements for weight gains of approx. 1,000 g/day (IZ-INRA 2009). The experiment was terminated after 24 months in summer season and the bulls were subjected to experimental slaughter after 24-hour feed withdrawal. Animals were slaughtered in a commercial EU-licensed abattoir, stunned using captive-bolt pistol and dressed according to commercial practice.

Samples and analysis

Samples of *musculus longissimus lumborum* (MLL) were collected from right side of the carcass chilled at 4 ±1°C for 24 h. The muscle samples were analysed for basic chemical composition using standard AOAC procedures (AOAC 1997). The composition of fatty acids was determined as described by Folch et al. (1957) as methyl esters in hexane by gas chromatography with Shimadzu GC-2010 with Rtx 2330 capillary column (105 m lenght × 0.32 mm internal diameter × 0.2 µ film thickness); injection volume 1.0 µl; temperature programme 60–240°C; injector temperature 250°C; detector temperature 250°C; helium as the carrier gas; according to ISO 12966-2:2011, with slight modifications. Atherogenic index was calculated, according to Ulbricht and Southgate (1991), based on the equation:

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / / (MUFA + PUFA)$$

Cholesterol was determined by gas chromatography with Shimadzu GC-2010 with ZB column (30 m lenght × 0.25 mm internal diameter × 0.5 µ film thickness); injection volume 1.0 µl; temperature programme 100–360°C; injector temperature 250°C; detector temperature 300°C; helium as the carrier gas. The vitamin E was determined using using the high-performance liquid chromatography HPLC technique with fluorescent detection on Merck-Hitachi HPLC system (Darmstadt, Germany). A reverse phase LiChroCART™ 250-4 Superspher™ 100 RP-18 column was used for chromatographic separation. A mixture of methanol and H_2O (96.5 : 3.5 v/v) was used as the eluent (1 mL/min).

The analyses were performed after spectrophotometric standardization of standard ethanol solutions.

Physicochemical and organoleptic evaluation of meat

Physicochemical and organoleptic analysis of meat was performed 48 h *post mortem*. Meat pH was measured with a penetrating electrode (Hanna Instruments FC232D) connected to a pH meter (Hanna Instruments HI 99163) after calibration with two buffers (pH 4.01 and pH 7.01). The pH-meter automatically corrected pH values, taking into account muscle temperature. Meat colour was determined using the model CR-310 Minolta chroma meter fitted with a 50 mm orifice (Boccard et al. 1981) according to CIE-L*a*b* system. The samples of meat were freshly cut into 3-cm thick steaks and were evaluated after 30 min of bloom time. Heating loss was determined on 1.5-cm thick steaks in an electric cooker at 165°C to an internal temperature of 70°C according to Boccard et al. (1981).

The maximum shear force (F_{max} expressed in N) was measured with a Warner–Bratzler V-blade on texture analyzer Model TA-XT2 plus (Stable Micro Systems, Godalming, Surrey, England). Meat samples were cut (1.5 mm/s crosshead speed) into 10-mm^2 cubes (minimum of 5 per sample) parallel to muscle fibre orientation. Results of shear force measurements were analysed using testXpert II software.

Sensory evaluation (i.e. colour, aroma, flavor, texture, juiciness and delicacy) following heat treatment (roasting) was performed on a 5-point scale (1 point – the worst, 5 points – the best) according to the procedures described by Baryłko-Pikielna and Matuszewska (2014). The organoleptic evaluation was performed by a panel of 9 trained judges.

Statistical analysis

The data obtained during the study were statistically analysed with Statistica ver. 9.1 (2009) using Student's t-test for independent samples.

RESULTS AND DISSCUSION

Chemical composition of meat

Breed had no effect (P >0.05) on basic meat composition (Table 1). As reported by Geay et al. (2001), the chemical composition of muscles is relatively constant, especially for dry matter or protein. The content and composition of lipids stored in muscle depends on genotype, type of ration, its energy, digestion, intestinal absorption, hepatic metabolism and lipid transport systems to muscle (Geay et al. 2001). The meat of ZR bulls was charac-

TABLE 1. Chemical composition of *musculus longissimus lumborum* from Polish Red-and-White (ZR) and Simmental (SM) bulls ($\bar{x}$ ±SD)

Item	Breed	
	ZR	SM
Moisture (%)	75.3 ±0.98	75.81 ±1.00
Crude protein (%)	22.10 ±1.16	22.38 ±0.23
Crude ash (%)	1.07 ±0.03	1.09 ±0.02
Crude fat (%)	1.99 ±0.48	1.50 ±0.64
Cholesterol (mg/g)	0.52 ±0.02	0.51 ±0.05
Vitamin E (µg/g)	2.51 ±0.70	2.04 ±0.57

terized by a higher fat content compared to the meat of SM bulls (1.99 vs 1.5%), but the differences were not significant. Genotype was found to have no effect on the level of vitamin E and cholesterol in the meat. Regardless of the breed, the meat of bulls was characterized by a relatively high vitamin E content (more than 2 μg/g) and low cholesterol content (0.51–0.52 mg/g). Węglarz et al. (2000) found the meat of Red-and-White Lowland and Simmental bulls to have a higher cholesterol content (0.575 and 0.597 mg/g, respectively).

Fatty acid profile

Our study found no effect of breed on the fatty acid profile of MLL fat except for the saturated fatty acids myristic (C14) and palmitic (C16), the proportion of which was higher in the fat of Polish Red-and-White bulls (Table 2, $P \leq 0.05$). Studies conducted to date on the relationship between fatty acid profile of intramuscular fat and genotype allow a conclusion that genotype-related variation in fatty acids is more noticeable in saturated (SFA) and monounsaturated fatty acids (MUFA) than in polyunsaturated fatty acids (PUFA) (Scollan et al. 2006, 2014). Laborde et al. (2001) found Simmental cattle to be characterized by higher activity of Δ-9 desaturase that converts vaccenic acid (C18:1, n-7) to CLA as well as by higher MUFA content. Fat from SM bulls had a higher content of polyunsaturated fatty acids (PUFA), in particular n-6 fatty acids, but the differences were not significant ($P > 0.05$) (Table 2). Enser et al. (1998) report that in ruminants, polyunsaturated fatty acids are preferentially deposited in phospholipids and for this reason less fatty

TABLE 2. Fatty acid composition (% total acid) of intramuscular fat of *musculus longissimus lumborum* from Polish Red-and-White (ZR) and Simmental (SM) bulls ($\bar{x} \pm SD$)

Item	Breed	
	ZR	SM
C10:0	0.03 ±0.01	0.05 ±0.01
C12:0	0.17 ±0.02	0.18 ±0.04
C14:0	1.97 ±0.22[a]	1.22 ±0.34[b]
C16:0	25.03 ±2.56[a]	17.59 ±3.12[b]
C16:1	2.28 ±0.25	1.77 ±0.14
C18:0	21.03 ±1.96	22.09 ±2.48
C18:1	33.16 ±2.8	30.83 ±3.71
C18:2, n-6	8.90 ±1.25	13.06 ±2.28
C20:0	0.12 ±0.03[a]	0.21 ±0.05[b]
C20:4, n-6	4.44 ±1.12	9.27 ±2.56
C18:3, n-3	1.80 ±0.17	2.05 ±0.2
CLA	0.27 ±0.08	0.32 ±0.09
CLA c9-t11	0.20 ±0.02	0.29 ±0.04
CLA c9-c11	0.05 ±0.00	0.00 ±0.00
CLA t9-t11	0.02 ±0.00	0.03 ±0.01
C22:0	0.30 ±0.02	0.41 ±0.04
C22:1	0.01 ± 0.00	0.02 ±0.00
C20:5, EPA, n-3	0.47 ±0.08	0.93 ±0.1
C22:6, DHA, n-3	0.02 ±0.00	0.00 ±0.00
SFA	48.64 ±4.78	41.76 ±4.02
UFA	51.40 ±5.06	58.24 ±4.45
MUFA	35.46 ±2.23	32.62 ±4.12
PUFA	15.90 ±3.89	25.63 ±4.16
PUFA, n-6	13.34 ±3.39	22.33 ±4.85
PUFA, n-3	2.29 ±0.35	2.98 ±0.47
PUFA/SFA	0.35 ±0.08	0.72 ±0.12
PUFA n-6/n-3	5.83 ±1.96	7.49 ±2.32
AI*	0.67 ±0.15[a]	0.41 ±0.13[b]

* Atherogenic index (C12:0 + 4 × C14:0 + C16:0) / / (MUFA + PUFA).

a, b – values in rows with different letters are significantly different at $P \leq 0.05$.

breeds are generally characterized by a higher proportion of PUFA. The atherogenic index was higher in meat from the Polish Red-and-White compared to the Simmental breed (0.67 vs 0.41), which was due to the higher content of saturated fatty acids C12, C14, C16 and the notably lower proportion of PUFA. The atherogenic index alone could suggest that intramuscular fat from the meat of ZR bulls has lower health-promoting value, but of equal importance to the consumers is the n-6 to n-3 ratio, which was lower (more favorable) for the ZR breed (5.83 vs 7.49). As recommended by ISSFAL (2004), it is desirable that the human diet should include fat with low atherogenic index and a low n-6 to n-3 fatty acids ratio (2 : 1).

Physicochemical and organoleptic characteristics of meat

Meat from Polish Red-and-White bulls was characterized by lower shear force ($P \leq 0.05$), greater juiciness ($P \leq 0.01$), more delicate texture ($P \leq 0.05$), and better overall score ($P \leq 0.05$) (Table 3). However, the meat pH_{48h} *post mortem* was relatively high for both ZR and SM breeds, but still around recommended range for good quality of meat, i.e. between 5.4 and 5.8 (Węglarz 2010). Elevated pH is mainly due to various types of stress to which animals are exposed (transport, fasting, temperature). Węglarz (2010) found that only 12% of meat from young bulls slaughtered in the summer season had normal pH values of 5.4–5.8, 30% of meat exceeded 6.2, and more than half exhibited intermediate values. Cattle show the signs of physiological stress when temperatures are slightly over 20°C (Davis and Mader 2001).

TABLE 3. Physicochemical and organoleptic characteristics of *musculus longissimus lumborum* from Polish Red-and-White (ZR) and Simmental (SM) bulls ($\bar{x}$ ±SD)

Item	ZR	SM
Heating loss (%)	35.08 ±4.13	38.13 ±4.20
pH_{48h}	5.86 ±0.19	5.84 ±0.05
Colour		
L	33.44 ±1.17	34.13 ±1.18
a*	19.71 ±0.98	20.02 ±0.73
b*	5.40 ±0.74	5.24 ±1.11
C*	20.44 ±1.04	20.71 ±0.88
Shear force (N)	99.33 ±37.08^{a}	123.07 ±18.5^{b}
Colour	4.67 ±0.10	4.14 ±0.43
Aroma – intensity	3.57 ±0.05	3.69 ±0.12
Aroma – desirability	4.29 ±0.09^{a}	3.96 ±0.17^{b}
Flavour – intensity	3.77 ±0.19	3.52 ±0.23
Flavour – desirability	3.79 ±0.09	3.52 ±0.21
Texture – tenderness	2.55 ±0.14	2.65 ±0.02
Juiciness	3.76 ±0.14^{A}	3.10 ±0.16^{B}
Delicacy	2.90 ±0.23^{a}	2.73 ±0.06^{b}
Overall score	3.65 ±0.03^{a}	3.41 ±0.12^{b}

L* – colour lightness, a* – redness, b* – yellowness, C* – colour intensity.

a, b – values in rows with different letters are significantly different at $P \leq 0.05$, A, B – values in rows with different letters are significantly different at $P \leq 0.01$.

In our study, bulls were slaughtered in the summer season when temperatures exceeded 25°C, which could affect the pH values.

Simmental meat was characterized by higher shear force compared to meat

from the Polish Red-and-White breed ($P \leq 0.05$). It is in line with the study of Litwińczuk et al. (2014b) who reported higher shear force value (no statistical differences) for Simmental meat (97.7 N) than for meat of native breed: Polish Red (92.2 N), Polish Black-and-White (89.0 N). Significant differences ($P \geq 0.05$) were obtained between meat of White-backed cattle (108.9 N) and Polish Holstein (84.9 N). Sochor et al. (2005), comparing the Simmental breed (SM) with Charolais (CH), Czech Pied (CP) and Blonde d'Aquitaine (BA) obtained for this breed high shear force values which were similar to those obtained in our study (SM 110.98 N; CH 86.63 N; CP 84.58 N; BA 119.95 N). Between-breed differences in meat tenderness, due to various course of meat protein degradation, were observed by Iwanowska et al. (2010). The relatively high shear force value (more than 110 N on average), observed in the experiment in comparison with the findings of other authors, results from the difference in the age of animals. The increased toughness of meat with age is strictly associated with the changes occurring in the connective tissue and myofibrils, which become more compact and resistant to the influence of physicochemical factors, including temperature.

Meat colour depends on the concentration and chemical form of myoglobin, the main heme pigment. No significant differences were found in the colour of meat from the breeds under comparison. The results obtained for colour lightness are lower than those reported for the Italian Maremmana bulls slaughtered at both 18 and 24 months of age (Sargentini et al. 2010 – 33.79 vs 38.4 and 41.1 on average). The dark meat colour could also result from the relatively high acidity of the meat.

The organoleptic traits are already observed to improve when the intramuscular fat content ranges between 3.5 and 5% (Mandell et al. 1997). In our study, the intramuscular fat content was not that high, but tests of the organoleptic properties showed that the meat of Polish Red-and-White bulls was more delicate, juicy and had more desirable aroma compared to the meat of Simmental bulls.

CONCLUSION

The Polish Red-and-White and Simmental bulls did not differ in basic chemical composition of the meat, although the conservation breed bulls had a slightly higher content of intramuscular fat. Differences between the breeds occurred for the fatty acid profile of intramuscular fat, but they mostly concerned saturated fatty acids. Meat from Simmental bulls was characterized by lower atherogenic index, while meat from Polish Red-and-White bulls had a lower n-6/n-3 PUFA ratio. The meat of the conservation breed bulls earned higher sensory panel ratings due to its higher juiciness, delicate texture and more desirable aroma.

REFERENCES

ADAMSKA A., RUTKOWSKA J., TABASZEWSKA M., BIAŁEK M., 2014: Milk of Polish Red and White cows as a source of nutritionally valuable fatty acids. Arch. Tierz. 57 (10): 1–10.

AOAC, 1997: Official methods of analysis. Association of official analytical chemists. 15th edn. Washington. DC.

BARYŁKO-PIKIELNA N., MATUSZEWSKA I., 2014: Sensory testing of food. Fundamentals. Applications. 2nd edn. Scientific Publishing PTTZ, Kraków.

BOCCARD, R., BUCHTER L., CASTEELS E., COSENTINO E., DRANSFIELD E., HOOD D.E., JOSEPH R.L., McDOUGALL D.B., RHODES D.N., SCHÖN I., TIMBERGEN B.J., TOURAILLE C., 1981: Procedures for measuring meat quality characteristics in beef production experiments. Report of a Working Group in the Commission of the European Communities (CEC) beef production research programme. Livest. Prod. Sci. 8: 385–397.

DAVIS M.S., MADER T.L., 2001: Effects of water application to feedlot mounds during the summer. In: Proceedings of the ASAE Sixth International Livestock Environment Symposium. Louiville, KY, USA: 165–173.

DYMNICKI E., SOSIN-BZDUCHA E., REKLEWSKI Z., 2014: Relationships between body weight of Polish Red suckler cows and their progeny (in Polish). Rocz. Nauk. Zoot. 41 (1): 9–19.

ENSER M., HALLET K.G., HEWETT B., FURSEY G.A., WOOD J.D., HARRINGTON G., 1998: Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. Meat Sci. 49: 329–341.

FOLCH J., LEES M., SLOANE-STANLEY G.H., 1957: A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509.

GEAY Y., BAUCHART D., HOCQUETTE J.F., CULIOLI J., 2001: Effect of nutritional factors on biochemical structural and metabolic characteristic of muscles in ruminants, consequences on dietetic value and sensorial qualities of meat. Reprod. Nutr. Dev. 41: 1–26.

ISO 12966-2:2011. Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters. Part 2. Preparation of methyl esters of fatty acids.

ISSFAL, 2004: The 6th Congress of the International Society for the study of fatty acids and lipids. Brighton (UK).

IWANOWSKA A., IWAŃSKA E., GRZEŚ B., MIKOŁAJCZAK B., POSPIECH E., ROSOCHACKI S., JUSZCZAK-KUBIAK E., ŁYCZYŃSKI A., 2010: Changes in protein and tenderness of meat from young bulls of four different breeds at three ages over 10 days of cold storage. Anim. Sci. Pap and Rep. 28 (1): 13–25.

IZ-INRA, 2009: Standards for Cattle, Sheep and Goat Nutrition. National Research Institute of Animal Production, IZ-PIB, Kraków.

LABORDE F.L., MANDELL J.N., TOSH J.J., WILTON J.W., BUCHMAN-SMITH J.G., 2001: Breed effects on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in finishing steers. J. Anim. Sci. 79: 355–365.

LITWIŃCZUK Z., BARŁOWSKA J., CHABUZ W., BRODZIAK A., 2012: Nutritional value and technological suitability of milk from cows of three Polish breeds included in the genetic resources conservation programme. Ann. Anim. Sci. 12 (3): 423–432.

LITWIŃCZUK Z., ŻÓŁKIEWSKI P., FLOREK M., CHABUZ W., DOMARADZKI P., 2014a: Semi-intensive suitability and slaughter value of young bulls of three Polish native breeds in comparison with Polish Holstein-Friesian and Simmental. Ann. Anim. Sci. 14 (2): 453–460.

LITWIŃCZUK Z., FLOREK M., DOMARADZKI P., ŻÓŁKIEWSKI P., 2014b: Właściwości fizykochemiczne mięsa buhajków trzech rodzimych ras-polskiej czerwonej, białogrzbietej i polskiej czarno-białej oraz simentalskiej i polskiej holsztyńsko-fryzyjskiej. ŻNTJ 5 (96): 53–62.

MANDELL I.B., GULLET E.A., WILTON J.W., ALLEN O.B., OSBORNE V.R., 1997: Effect of diet and slaughter endpoint on growth performance, carcass composition and beef quality traits in Limousin and Charolais steers. Can. J. Anim. Sci. 77: 23–32.

MARINO R., ALBENZIO M., DELLA MALVA A., BRAGHIERI A., SEVI A., 2014: Nutraceutical properties of meat from grazing Podolian young bulls. Food and Nutr. Sci. 5: 618–625.

SARGENTINI C., BOZZI R., LORENZINI G., DEGI'INNOCENTI, MARTINI A., GIORGETTI A., 2010: Productive performances of Maremmana young bulls reared following organic rules and slaughtered at 19 and 24 months of age. Italian J. Anim. Sci. 9 (31): 163–168.

SCOLLAN N.D., HOCQUETTE J.F., NUERENBERG K., DANNENBERGER D., RICHARDSON R. I., MOLONEY A., 2006: Innova-

tions in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. 74 (1): 17–33.
SCOLLAN N.D., DANNENBERGER D., NUERENBERG K., RICHARDSON I., MacKINTOSH S., HOCQUETTE J.F., MOLONEY A.P., 2014: Enhencing the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. 97 (3): 384–394.
SOCHOR J., SIMEONOVOVA J., ŠUBRT J., BUCHAR J., 2005: Effect of selected fattening performance and carcass value traits on textural properties of beef. Czech. J. Anim. Sci. 50 (2): 81–88.
ULBRICHT T.L.V. SOUTHGATE D.A.T., 1991: Coronary heart disease: seven dietary factors. The Lancet 19 (338): 985–992.
WĘGLARZ A., GARDZINA E., ORMIAN M., 2000: Fizykochemiczna i organoleptyczna jakość mięsa buhajków rasy cb, czb, simental i mieszańców F1 (cb × simental). Rocz. Nauk. Zoot. supl. 5: 52–58.
WĘGLARZ A., 2010: Meat quality defined based on pH and colour depending on cattle category and slaughter season. Czech. J. Anim. Sci. 55 (12): 548–556.

Streszczenie: *Porównanie jakości mięsa młodych buhajków ras polskiej czerwono-białej i simentalskiej.* Materiał doświadczalny stanowiło mięso pochodzące od 16 buhajków ras polskiej czerwono-białej (ZR) (n = 8) i simentalskiej (SM) (n = 8). W próbkach mięśnia najdłuższego grzbietu (MLL) oznaczono podstawowy skład chemiczny oraz profil kwasów tłuszczowych tłuszczu śródmięśniowego. Przeprowadzono ocenę fizykochemiczną i organoleptyczną mięsa. W podstawowym składzie chemicznym mięsa nie stwierdzono różnic. W tłuszczu śródmięśniowym ZR stwierdzono wyraźnie mniejszy udział kwasów wielonienasyconych (PUFA), lecz korzystniejszy stosunek kwasów n-6/n-3 w tłuszczu śródmięśniowym MLL. Mięso rasy zachowawczej zostało wyżej ocenione w badaniu sensorycznym z uwagi na większą soczystość, delikatność oraz lepszy zapach.

Słowa kluczowe: bydło, rasa polska czerwono-biała, rasa simentalska, mięso, jakość

MS received 10.06.2016
MS accepted 10.01.2017

Author's address:
Ewa Sosin-Bzducha
Dział Ochrony Zasobów Genetycznych Zwierząt
Instytut Zootechniki – Państwowy Instytut Badawczy
ul. Krakowska 1, 32-083 Balice
Poland
e-mail: ewa.sosin@izoo.krakow.pl

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 137–145
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.16

Green synthesis of silver nanoparticles by using aqueous mint (*Mentha piperita*) and cabbage (*Brassica oleracea* var. *capitata*) extracts and their antibacterial activity

MALWINA SOSNOWSKA, MARTA KUTWIN, ADRIAN ADAMIAK, KAMIL GAWIN, ŻANETA BUGAJSKA, KAROLINA DANILUK
Department of Animal Nutrition and Biotechnology, Warsaw University of Life Science – SGGW

Abstract: *Green synthesis of silver nanoparticles by using aqueous mint (*Mentha piperita*) and cabbage (*Brassica oleracea *var.* capitata*) extracts and their antibacterial activity*. The objective of this study was the synthesis of silver nanoparticles (Ag-NP) using leaves of mint and cabbage extracts as the reducing and stabilising agents. The presence of nanoparticles was initially confirmed by the obtained colour and next by transmission electron microscope (TEM). Analysis of TEM of obtained Ag-NP indicated that their size ranged 5–50 nm for mint and 10–150 nm for cabbage. The antibacterial activity of nanoparticles against pathogenic strains *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* were assessed by evaluation of metabolic activity, using the PrestoBlue and XTT test. The higher inhibition of bacterial viability was observed against Gram-negative (*E. coli*, *S. enterica*) than Gram-positive (*S. aureus*) bacteria.

Key words: silver nanoparticles, green synthesis, bioreduction, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*

INTRODUCTION

In recent years, an increasing number of studies considering alternative and more eco-friendly processes for the synthesis of nanoparticles has been observed. The main methods used for silver nanoparticles (Ag-NP) synthesis are physical, chemical and biological methods (Prabhu and Poulose 2012). The problem with physiochemical methods is that the synthesis is expensive (necessity to use high pressure, energy and temperature) and involve the use of toxic and harsh chemicals (Kalishwaralal et al. 2008).

Green synthesis of nanomaterials is based on extracts from biological organisms such as plants, bacteria, yeasts, fungi and algae. Silver nanoparticles can be synthesised using different parts of plants and their extracts such as leaf (*Euphorbia hirta*), seed (*Jatropha curcas*), fruit (*Carica papaya*), root (*Morinda citrifolia*), flower (*Tagetes erecta*), steam and peel (Kuppusamy et al. 2016). Plant extracts are natural sources of non-toxic reducer/stabiliser agents, so they may be useful for manufacturing of nanoparticles. *Mentha piperita* is also good sources of menthol, limonene, pulegone, carvophyllene and pinene. Menthol reacts in many aspects as a normal secondary alcohol and also can be useful in biosynthesis of Ag-NP by alcohol reduction of $AgNO_3$ (Kamatou et al. 2013). Cabbage is a good source of vitamins, especially

ascorbic acid (30–36 mg in 100 g), minerals, electrolytes, sulforaphane, indoles, zea-xanthin and lutein (Tamileswari et al. 2015).

Silver nanoparticles have high antibacterial, antimalarial, antidiabetic, antioxidant and anticancer activity (Jeyaraj et al. 2013, Chung et al. 2016). They were also used to explore their antibacterial potential against resistant pathogens (Chung et al. 2016). The objective of this study was to determine the efficiency of Ag-NP synthesis, using mint and cabbage leaves extracts, and to characterise obtained nanoparticles. Furthermore, the effect of pH and temperature on the size and shape of nanoparticles as well as their antibacterial activity were evaluated.

MATERIAL AND METHODS

Preparation of plant extract

Commercially available fresh leafs of *Mentha piperita* and *Brassica oleracea* were purchased from a local market in Warsaw (Poland). Powder of $AgNO_3$ was obtained from Sigma (Saint Louis, USA). Leaves of *M. piperita* and *B. oleracea* were washed thoroughly four-fold with distilled water, dried and cut into small pieces. 2.5 g of mint leaves and 25 g of cabbage were flooded with 25 and 150 ml of distilled water respectively, and boiled for 10 min. Next, the plants extracts were filtered through filter paper round ø 125 mm FILTRAK 388.

Preparation of silver nanoparticles

We assumed that all amount of used/introduced silver nitrate was reduced to Ag-NP and the final concentration of the obtained Ag-NP corresponds the ratio of silver nitrate (μg) to the solution volume (ml). The assumption has been confirmed by transmission electron microscopy (TEM, JEOL, Japan). For the biosynthesis of Ag-NP using *M. piperita*, 2.5, 5 and 12.5 ml of filtered *M. piperita* extract solutions were added to the 50 ml of silver nitrate water solution (170 μg/ml) to obtain after synthesis: 162, 148 and 136 μg/ml concentrations of mint-Ag-NP. For the biosynthesis of cabbage-Ag-NP using white cabbage, 10 ml of filtered cabbage extract was added to 50 ml of 340 μg/ml silver nitrate water solution. The procedure was performed in triplicate to obtain tree separate mix plant extract – silver nitrate solutions. Next, each of $AgNO_3$ – plant extract mix solutions were heated under different temperatures: 30°C, 60°C and 90°C. After bioreduction the concentration of cabbage-Ag-NP was 283 μg/ml. Synthesised Ag-NP were sterilised in autoclave (Prestige Medical, UK) and sonicated for 15 min in ultrasonic bath (Bandelin Electronic, Germany) to avoid agglomeration. All Ag-NP characterisations were performed in triplicate, using TEM and zeta potential analyser (Malvern, UK) according to the procedures described by Sawosz et al. (2010).

Evaluation of the antibacterial effect of nanoparticles

Salmonella enterica subspecies *enterica* serovar Enteritidis (ATCC 13076), *Escherichia coli* (ATCC MP-26) and *Staphylococcus aureus* subspecies *aureus* (ATCC 12600) were obtained from LGC Standards (Łomianki, Poland). The bacteria were then grown on nutritive agar (2.8%) with the addition of NaCl

(Bio-Rad, Warsaw, Poland). Sterilisation of media was carried out at 121°C for 30 min (Tuttnauer 2450EL, Tuttnauer Ltd., Jerusalem, Israel). Next, bacterial cultures were prepared overnight. Volumetric flasks, which contained 10.75 ml of peptone water (Biocorp, Warsaw, Poland), were filled with 50 µl of night culture in six repetitions for each species of bacteria. Subsequently, mint-Ag-NP and cabbage-Ag-NP with the smallest diameter were added (solution 1 for mint and cabbage). The final concentrations in volumetric flasks were 1, 8 and 16 µg/ml for the mint-Ag-NP and 1, 14 and 28 µg/ml for the cabbage-Ag-NP. Each species of bacteria had its own control, which contained water instead of nanoparticles. During the night, the cultures were shaken up at 100 rpm and 37°C. Then, the cultures were seeded at 5×10^5 cells per well in a sterile 96-well plate. The assessment of antibacterial activity of Ag-NP against *E. coli*, *S. aureus* and *S. enterica* were evaluated by metabolic assays – PrestoBlue (Life Technologies, USA) and XTT test (Roche Protocol, Germany). PrestoBlue and XTT cell viability assays are based on the ability of metabolically active cells to reduce and form a coloured product (PrestoBlue – pink product, XTT – orange product). The reducing environment within viable cells converts PrestoBlue reagent into pink dye. Only living cells are capable to reduce tetrazolium salt (XTT) to formazan by trans-plasma membrane electron transport at the cell surface. Cell viability was expressed as the percentage (ODtest – ODblank) / (ODcontrol – ODblank), where "ODtest" is the optical density of cells exposed to Ag-NP, "ODcontrol" is the optical density of the control sample, and "ODblank" is the optical density of wells without bacterial cells.

Statistical analysis

Statistical significance was determined by one-way analysis of variance (ANOVA) with Tukey's post-test using Statgraphics Plus 4.1 (StatPoint Technologies, Warrenton, VA, USA). Differences at $P \leq 0.05$ were defined as statistically significant.

RESULTS AND DISCUSSION

Formation of Ag-NP was confirmed by the change in colour of solution (Fig. 1). The fresh suspensions of mint and cabbage extracts were yellowish-green and pale yellow respectively. After addition of $AgNO_3$ solution, the colours change was observed within 60 min. After 60 min of the incubation at room temperature, the mint solution had almost black (1 solution), dark (2 solution) and pale brown (3 solution) colour while cabbage solution had almost black (solution 1) and dark brown (solutions 2 and 3) colour.

The addition of 5 ml mint extract was most effective for the synthesis of nanoparticles. Menthol can enhance penetration of other agents and have great cooling taste and smell properties. The cooling sensation results from the ability to chemically activate the cold-sensitive transient receptor potential cation channel (TRPM8). Kamatou et al. (2013) reported that when 0.02% menthol solutions held in the mouth, solutions above 37°C seemed warmer than water without menthol of the same temperature (warmth enhancement). However, menthol solutions below 37°C seemed cooler than water of the same temperature (cold

FIGURE 1. Colour change after incubation for 20 and 60 min at room temperature: A – mint-nanosilver, B – cabbage-nanosilver

enhancement). Menthol treatments of silver nitrate solution resulted in the conversion of menthol and Ag^+ to menthone and Ag^0 (Kamatou et al. 2013).

The synthesis of cabbage-Ag-NP was most efficient at 30°C. Thermal treatments of cabbage at 30°C and 60°C resulted in the conversion of L-ascorbic acid (L-AA) and Ag^+ to dehydroascorbic acid (DHHA) and Ag^0 but treatments at 90°C retained vitamin C as L-AA. The conversion is made possible by ascorbic acid oxidase (AAO) – thermolabile enzyme (EC 1.10.3.3) (Munyaka et al. 2010).

Figure 2 shows representative TEM images of obtained Ag-NP. The shape of newly synthesised Ag-NP was regular and rounded, and hydrocolloids showed a high density of Ag-NP. The pictures showed Ag-NP but absence of crystals salts. Nanoparticles prepared using the mint extract had a smaller diameter (from 5 to 50 nm) than cabbage (from 10 to 100–150 nm). The nanoparticles of smaller diameter can easily pass through the membrane channel of the bacteria (Kuppusamy et al. 2015). The mean zeta-potential for the most stable samples of mint-Ag-NP was –11.5 mV and cabbage-Ag-NP was –18.6 mV. Results showed that use of biological methods allows obtaining stable nanoparticles without stabiliser.

During green synthesis, pH is an important factor for regulating the size and shape of Ag-NP. The pH induces the reactivity of plant extract with silver ions (Vanaja et al. 2013). Fayaz et al. (2009) reported that at low pH nanoparticles of large size were formed. At alkaline pH,

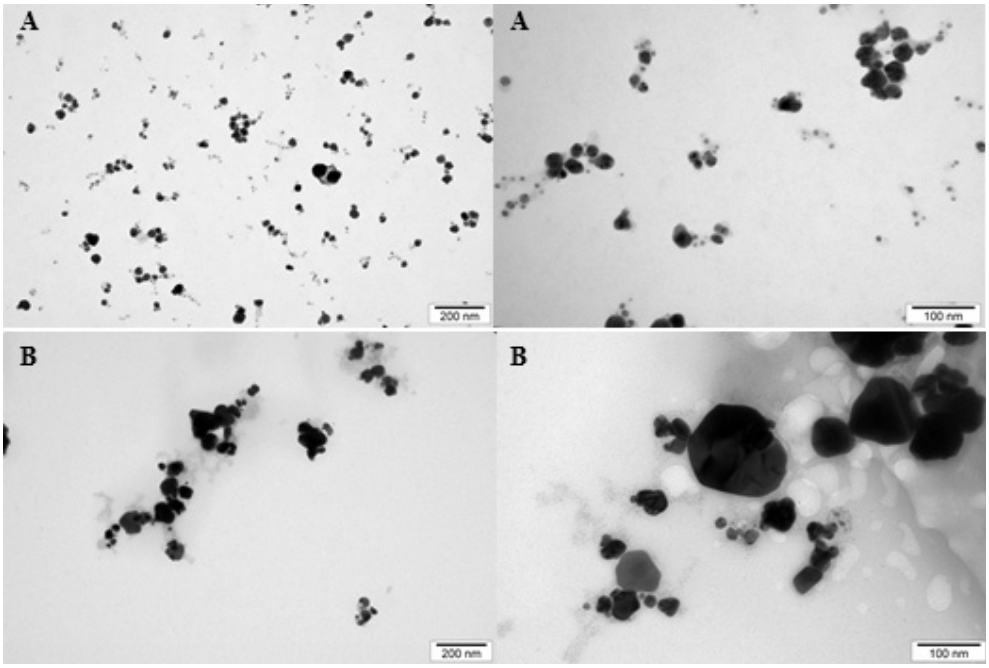

FIGURE 2. Transmission electron microscopy (TEM) images of silver nanoparticles. Bar scale 200 and 100 nm. AgNPs obtained using the aqueous plant extracts: A – mint, B – cabbage

a large number of Ag-NP with the small surface area are present due to the bioavailability of functional groups (Fayaz et al. 2009). Similarly, Vanaja et al. (2013) reported that alkaline pH is suitable for synthesis of Ag-NP.

The influence of mint-Ag-NP and cabbage-Ag-NP on the growth of *E. coli*, *S. aureus* and *S. enterica* is presented in Figures 3–5. The results showed that bacterial cells treated with Ag-NP had decreased metabolic activity compared to the control cells. Experiments demonstrated that the nanoparticles prepared using the mint extract had higher antimicrobial activity than cabbage-Ag-NP. The smaller diameter may be a reason of a high antibacterial activity of nanoparticles obtained from mint. The cabbage-Ag-NP did not show significant antibacterial activity. According to the PrestoBlue assay, the lowest bacterial viability was observed for *S. enterica* cells, irrespective of the type of nanoparticles (mint-Ag-NP: 5–8%, cabbage--Ag-NP: 35–36%). XTT assay showed a lower viability of both used species of Gram-negative than Gram-positive bacteria, probably due to the thin peptidoglycan layer in the cell wall and presence of beta barrel proteins called porins (Shukla and Vankar 2012). The XTT is more sensitive than PrestoBlue test because the reduction process occurs mainly on the cell surface or plasma membrane with the transmembrane electron transport chain (on the cell membrane of bacteria). Only living cells (possessing an intact cell membrane, active dehydrogenase) are capable to reduce XTT to formazan.

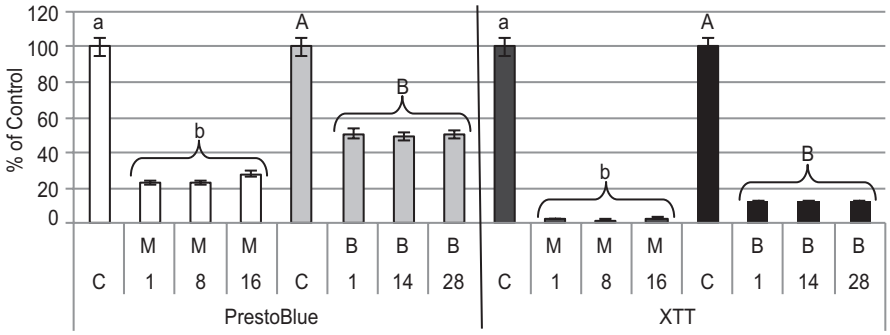

FIGURE 3. Mean viability of *Escherichia coli* cells evaluated for two tests, i.e. PrestoBlue and XTT, after treatment with: C – control (no treatment Ag-NP), M – *Mentha piperita* (1, 8, 16 µg/ml of mint-Ag-NP), B – *Brassica oleracea* (1, 14, 28 µg/ml of cabbage-Ag-NP). The differences between the treated group Ag-NP and non-treated control group were statistical significance ($P \leq 0.05$). Different letters (a, b and A, B) indicate significant differences between the groups (different colours show groups). ANOVA, Tukey post-test

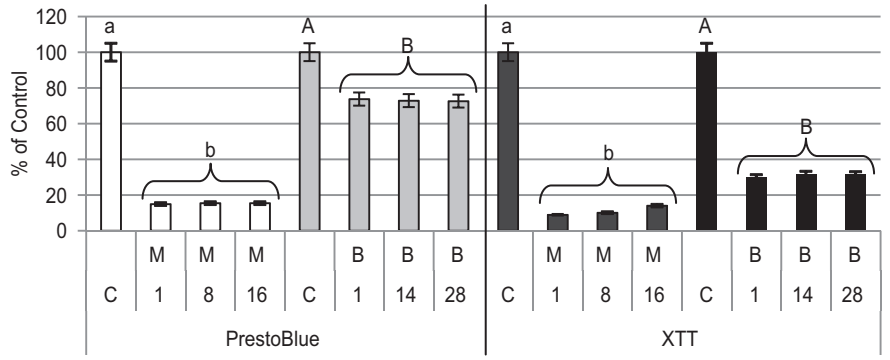

FIGURE 4. Mean viability of *Staphylococcus aureus* cells evaluated for two tests, i.e. PrestoBlue and XTT, after treatment with: C – control (no treatment Ag-NP), M – *Mentha piperita* (1, 8, 16 µg/ml of mint-Ag-NP), B – *Brassica oleracea* (1, 14, 28 µg/ml of cabbage-Ag-NP). The differences between the treated group Ag-NP and non-treated control group were statistical significance ($P \leq 0.05$). Different letters (a, b and A, B) indicate significant differences between the groups (different colours show groups). ANOVA, Tukey post-test

Therefore, the concentration of the dye is proportional to the number of metabolically active cells. Consequently, antibacterial activity of Ag-NP is bacteria species dependent.

Most studies used disc diffusion method and measured zone of inhibition. Tamileswari et al. (2015) synthesised of Ag-NP using cabbage extract. Their results showed good antibacterial and antifungal activity against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pneumocystis* sp. (Kappusamy 2015, Tamileswari et al. 2015). Some researchers reported that the mint-Ag-NP have

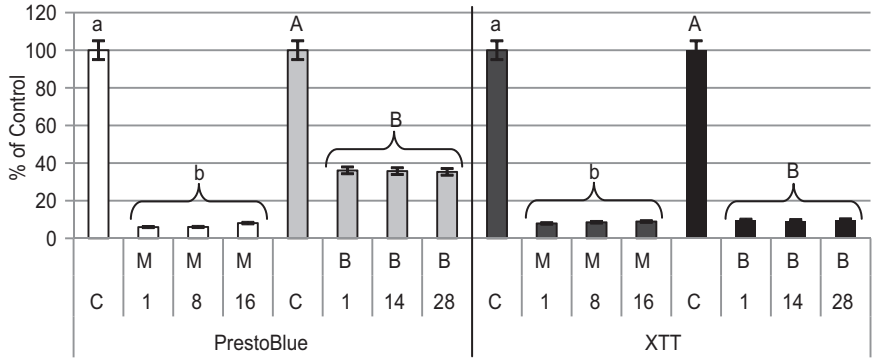

FIGURE 5. Mean viability of *Salmonella enterica* cells evaluated for two tests, i.e. PrestoBlue and XTT, after treatment with: C – control (no treatment Ag-NP), M – *Mentha piperita* (1, 8, 16 µg/ml of mint-Ag-NP), B – *Brassica oleracea* (1, 14, 28 µg/ml of cabbage-Ag-NP). The differences between the treated group Ag-NP and non-treated control group were statistical significance ($P \leq 0.05$). Different letters (a, b and A, B) indicate significant differences between the groups (different colours show groups). ANOVA, Tukey post-test

antimicrobial effect against *E. coli* (the maximum zone of inhibition), *S. aureus* and additionally *Pseudomonas aeruginosa* and *Bacillus subtilis* (MubarakAli et al. 2011, Sarkar and Paul 2017). Saikia et al. (2015) reported that antibacterial activity of Ag-NP is plant extract dependent.

Antibacterial activity of Ag-NP can have several causes e.g. nanoparticles penetration inside and binding DNA, hampering the normal replication, loss of cell viability by modulating tyrosine phosphorylation, attack on the respiratory chain and finally resulting in cell death (Sarkar and Paul 2017).

The aqueous extract of *M. piperita* and *B. oleracea* showed the presence of carbohydrates, amino acids, tannins, flavonoids, terpenoids, quinones, phenols, proteins and coumarins (Satya Prasad et al. 2015, Patil et al. 2016). Secondary metabolites act as interfering protein synthesis agents (tannins) and inhibitors of the extracellular enzymes (required for microbial growth and oxidative phosphorylation) (Satya Prasad et al. 2015).

Al-Sum et al. (2013) reported that aqueous *Mentha* species extract was active against six pathogenic bacteria, i.e.: *Bacillus fastidiosus* (the highest inhibitory effect), *Proteus mirabilis*, *P. vulgaris*, *Salmonella choleraesuis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Serratia odorifera* except for *Staphylococcus aureus*. According to Patil et al. (2016), the aqueous mint extract showed inhibitory effect against *Proteus vulgaris* and *Staphylococcus aureus* but *Bacillus cereus* and *Salmonella typhimurium* did not show a zone of inhibition. Extracts from *Brassica oleracea* exhibit distinct zones of inhibition towards bacterial strains like *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermis*, *Salmonella typhimurium* and *S. paratyphi* (Satya Prasad et al. 2015). Ethanol extract has a greater activity and protein content than the other

extracts. This extract is the most effective solvent for extracting a broad spectrum of antibacterial compounds from plant origin (Satya Prasad et al. 2015). Antibacterial nature of plants and their secondary metabolites could be useful to improve the efficiency of nanoparticles.

CONCLUSION

Silver nanoparticles were successfully synthesised from silver nitrate solution using mint and cabbage extracts. Green synthesis using mint and cabbage leaves extracts provides an eco-friendly, stable, simple, cheap and efficient route of Ag--NP synthesis. The pH and temperature play a major role in size control of the Ag-NP. Mint with silver nanoparticles complex had a smaller diameter than cabbage with silver nanoparticles complex probably due to the higher pH and lower temperature of mint extract used in nanoparticle synthesis. The obtained nanoparticles showed powerful antibacterial activity against human pathogens, i.e. *E. coli*, *S. aureus* and *S. enterica*, indicating that Ag-NP are good candidates for their usage as antibacterial agents. These nanoparticles had higher antibacterial activity against Gram-negative than Gram-positive bacteria. In the future nanoparticles synthesised from plants may be a better alternative for elimination of multidrug resistance microorganism than commercial antibiotics.

Acknowledgement

This work was supported by Warsaw University of Life Sciences – SGGW, statutory activity of the Department of Animal Nutrition and Biotechnology.

REFERENCES

Al-SUM B.A., Al-ARFAJ A.A., 2013: Antimicrobial activity of the aqueous extract of mint plant. Sci. J. Clin. Med. 2: 110–113.

CHUNG I.M., PARK I., SEUNG-HYUN K., THIRUVENGADAM M., RAJAKUMAR G., 2016: Plant-mediated synthesis of silver nanoparticles: their characteristic properties and therapeutic applications. Nanoscale Res. Lett. 11: 1–14.

FAYAZ A.M., BALAJI K., KALAICHELVAN P.T., VENKATESAN R., 2009: Fungal based synthesis of silver nanoparticles – an effect of temperature on the size of particles. Colloids Surf. B. 74: 123–126.

JEYARAJ M., REJESH M., ARUN R., MUBARAKALI D., SATHISHKUMAR G., SIVANANDHAN G., DEVA G.K., MANICKAVASAGAMA M., PREMKUMAR K., THAJUDDINC N., GANAPATHI A., 2013: An investigation on the cytotoxicity and caspase-mediated apoptotic effect of biologically synthesized silver nanoparticles using *Podyphyllum hexandrum* on human cervical carcinoma cells. Colloids Surf. B. 102: 708–717.

KALISHWARALAL K., DEEPAK V., RAMKUMARPANDIAN S., NELLAIAH H., SANGILIYANDI G., 2008: Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. Mater. Lett. 62: 4411–4413.

KAMATOU G.P.P., VERMAAK I., VILIOEN A.M., LAWRENCE B.M., 2013: Menthol: A simple monoterpene with remarkable biological properties. Phytochem. 96: 15–25.

KAPPUSAMY P., ICHWAN S.J.A., REDDY PARINE N.R., YUSOFF M.M., MANIAN G.P., GOVINDAN N., 2015: Intracellular biosynthesis of Au and Ag nanoparticles using ethanolic extract of *Brassica oleracea* L. and studies on their physicochemical and biological properties. J. Environ. Sci. 29: 151–157.

KUPPUSAMY P., YUSOFF M.M., MANIAM G.P., GOVINDAN N., 2016: Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report. Saudi Pharm. J. 24: 473–484.

MUBARAKALI D., THAJUDDIN N., JEGANATHAN K., GUNASEKARAN M., 2011:

Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf. B. 85: 360–365.

MUNYAKA A.W., MAKULE E.E., OEY I., Van LOEY A., HENDRICKX M., 2010: Thermal stability of L-ascorbic acid and ascorbic acid oxidase in broccoli (*Brassica oleracea* var. *italic*). J. Food Sci. 75: 336–340.

PATIL S.R., PATIL R.S., GODGHATE A.G., 2016: *Mentha piperita* Linn: Phytochemical, antibacterial and dipterian adulticidal approach. Int. J. Pharm. Pharm. Sci. 8: 352–355.

PRABHU S., POULOSE M.K., 2012: Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int. Nano Lett. 2: 1–10.

SAIKIA D., GOGOI P.K., PHUKAN P., BHUYAN N., BORCHETIA S., SAIKIA J., 2015: Green synthesis of silver nanoparticles using Asiatic pannywort and *Bryophyllum* leaves extract and their antimicrobial activity. Adv. Mater. Lett. 6: 260–264.

SARKAR D., PAUL G., 2017: Green synthesis of silver nanoparticles using *Mentha asiatica* (mint) extract and evaluation of their antimicrobial potential. Int. J. Curr. Res. Biosci. Plant Biol. 4: 77–82.

SATYA PRASAD M., SUMAN JOSHI D.S.D., NARENDA K., NADIYA S.K., MASTHANI S.K., PADMAJA PHANI N., KRISHNA SATYA A., 2015: A comparative study of phytochemical analysis and in vitro antimicrobial activity of three important vegetables from *Brassicaceae* family. Int. J. Res. Ayurveda Pharm. 6: 767–772.

SAWOSZ E., CHWALIBOG A., SZELIGA J., SAWOSZ F., GRODZIK M., RUPIEWICZ M., NIEMIEC T., KACPRZYK K., 2010: Visualization of gold and platinum nanoparticles interacting with *Salmonella* Enteritidis and *Listeria monocytogenes*. Int. J. Nanomedicine 5: 631–637.

SHUKLA D., VANKAR P.S., 2012: Synthesis of plant parts mediated gold nanoparticles. Int. J. Green Nanotechnol. 4: 277–288.

TAMILESWARI R., HANIFF NISHA M., JESURANI Sr.S., 2015: Green synthesis of silver nanoparticles using *Brassica oleracea* (cauliflower) and *Brassica oleracea capitata* (cabbage) and the analysis of antimicrobial activity. IJERT 4: 1071–1074.

VANAJA M., RAJESHKUMAR S., PAULKUMAR K., GNANAJOBITHA G., MALARKODI C., ANNADURAJ G., 2013: Kinetic study on green synthesis of silver nanoparticles using *Coleus aromaticus* leaf extract. Adv. Appl. Sci. Res. 4: 50–55.

Streszczenie: *Zielona synteza nanocząstek srebra przy użyciu wodnych ekstraktów z mięty (*Mentha piperita*) i kapusty (*Brassica oleracea *var.* capitata*) oraz ich aktywność przeciwbakteryjna.* Celem pracy była synteza nanocząstek srebra (Ag-NP) przy użyciu ekstraktów z liści mięty i kapusty jako czynników redukujących i stabilizujących. Obecność nanocząstek była początkowo stwierdzona przez zmianę koloru i następnie przez transmisyjną mikroskopię elektronową (TEM). Analiza TEM uzyskanych Ag-NP wykazała, że ich rozmiary były w przedziale wielkości 5–50 nm dla mięty i 10–150 nm dla kapusty. Aktywność przeciwbakteryjną nanocząstek przeciwko patogennym szczepom *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* oceniano przez oszacowanie aktywności metabolicznej z użyciem testu PrestoBlue i XTT. Większą inhibicję żywotności bakteryjnej obserwowano przeciwko Gram-ujemnym (*E. coli*, *S. enterica*) niż Gram-dodatnim (*S. aureus*) bakteriom.

Słowa kluczowe: nanocząstki srebra, zielona synteza, bioredukcja, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*

MS received 24.03.2017
MS accepted 26.04.2017

Authors' address:
Malwina Sosnowska
Zakład Nanobiotechnologii
Katedra Żywienia Zwierząt i Biotechnologii
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8, 02-785 Warszawa
Poland
e-mail: malwina.ewa.sosnowska@gmail.com

Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 56 (1), 2017: 147–157
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 56 (1), 2017)
DOI 10.22630/AAS.2017.56.1.17

Growth performance parameters and selected quality traits of meat and femoral bone of broiler chickens fed diet supplemented with amorphous diatomaceous earth

MAŁGORZATA WIEWIÓRA[1], MONIKA ŁUKASIEWICZ[2]
[1] Faculty of Animal Sciences, Aves Scientific Circle
[2] Department of Animal Breeding and Production, Warsaw University of Life Sciences – SGGW

Abstract: *Growth performance parameters and selected quality traits of meat and femoral bone of broiler chickens fed diet supplemented with amorphous diatomaceous earth.* This study was aimed at analyzing the effect of the addition of amorphous diatomaceous earth to feed on growth performance parameters and selected quality traits of meat and femoral bone of broiler chickens. The study was conducted with 60 fast-growing Ross 308 broiler chickens, reared until 42nd day of age and divided into a control group (C) and two experimental groups (D2 and D4) (20 birds each). The diatomaceous earth (diatomite) was administered to the birds' feed from the groups D2 – 2%, D4 – 4%. Individual body weight, feed intake and mortality of chickens were controlled. On day 42 of rearing, six males were selected from each group for slaughter followed by dissection. Dressing percentage, content of muscles and giblets (gizzard, liver and heart), were calculated, and in samples of breast and leg muscles the chemical and physicochemical properties were analyzed. Resistance of the femoral bone to fractures was determined. The addition of diatomite did not affect the health status of chickens. Significantly higher body weight at 42nd day of rearing was noted in the group C vs D4 ($P \leq 0.05$). Significantly higher ($P \leq 0.01$) content of pectoral muscles and lower ($P \leq 0.05$) fat in the carcass of group D2 vs C were noted. The addition of diatomaceous earth did not affect the chemical composition of the breast muscles. The fat content in leg muscles was significantly reduced ($P \leq 0.05$) and water content was increased ($P \leq 0.05$) in group D2 vs C. Femoral bones of D4 birds were significantly more resistant ($P \leq 0.05$) to breaking than in C. Direct relationship between the amount of diatomite and the strength of the femur was found. The most optimal supplementation was considered as 2%.

Key words: diatomaceous earth (diatomite), broiler chickens, growth performance, quality of meat and femoral bone

INTRODUCTION

Apart from pork, poultry meat is one of the most frequently consumed types of meat by an average Pole. In the intensive production of broiler chickens, being the main source of poultry meat in Poland, used is made of crosses with a high genetically-determined productive potential. Consumers of poultry meat are becoming increasingly interested in the welfare of animals as well as in the quality and safety of food products of animal origin. Many of them pay attention to maintenance conditions and rearing system. From the point of view of both, the producer and the consumer it is important to keep the high growth performance of chickens, but, simultaneously, to

ensure their good health status and body condition. This may be achieved by appropriate feeding. For this reason, it is desirable to support bird feeding with additives of natural origin that would positively affect those two traits. Considering the necessity and willingness to satisfy customer demands, producers of broiler chickens use a variety of preparations and feed additives in their diets that may positively influence bird condition and meat quality. In addition, they are still searching for novel solutions. The proposed substance that may be applied in broiler chicken feeding is diatomaceous earth. It is a completely natural preparation, unprocessed nor fortified with any chemical substances, which is significant from the point of view of both, consumers and producers. The use of diatomaceous earth in animal feeding and its effect on the body are, however, relatively poorly recognized, especially in Poland, and available study results are full of ambiguities and inaccuracies.

Diatomaceous earth (diatomite) is a natural sedimentary rock, formed mainly by fossilized frustules of diatoms – single-celled algae. These organisms had lived many millions years ago in the aquatic environment, and then – as a result of successive drying out of water bodies – they have formed deposits of diatomaceous earth (Round et al. 1990). As reported by Fields (2000), the frustules of diatoms in diatomite are built mainly of silica (SiO_2) and trace amounts of other mineral compounds, like: aluminum, iron oxide, lime, magnesium or sodium.

The mechanism of action of diatomaceous earth in an animal body may be discussed from two perspectives. The diatomaceous earth administered to animals with feed passages through the gastrointestinal tract and is absorbed, in a small amount, to the bloodstream in the form of orthosilicic acid (H_4SiO_4). This acid is synthesized through the reaction of silica with two molecules of water and in nature is the biologically-active form of silica and silicon. Diatoms may, therefore, constitute the source of bioavailable silicon (Abraham 2005). In addition, diatomaceous earth is applied as an anti-parasitic agent (Quarles 1992, Fields 2000, Dawson 2004, Maurer et al. 2009, Wiewióra et al. 2015). By passing through the gastrointestinal tract, frustules of diatoms destroy the encountered parasitic organisms and their forms, and then are excreted. These events are mechanical in character, the rough surface of frustules rubs against forms of parasites, causing their damage, which leads to their death or incapability of further reproduction. In addition, the cylindrical frustules of diatoms have pores that absorb water from the extrinsic environment, which contributes to dehydration of pathogen bodies and leads to their death as a result of dehydration (Haney 2016). According to Haney (2016), diatoms are not identical in all deposits of diatomaceous earth. The saltwater deposits contain a mixture of different types of diatoms having various shapes, and their fossilized frustules are relatively fragile. In turn, the freshwater deposits are characterized by a stable content of diatoms with a uniformly-shaped (cylindrical) and resistant frustules. The stable structure and unchangeable composition of diatomaceous earth are important for the effectiveness of its action. A significant aspect in this case is also the amorphous (non-crystalline, namely unordered) form

of the applied diatomaceous earth. The amorphous silica is delicate, it smoothly rubs against the gastrointestinal tract, causing no damages. In turn, the crystalline silica has very sharp, hard and relatively thick particles that may be hazardous to the walls of the gastrointestinal tract of animals – it is mainly applied in filtration systems. According to Norton (2015), owing to its adsorptive properties, diatomaceous earth may be used to eliminate from the body toxins secreted by bacteria, fungi or other infections, including yeast-like Candida. In addition, the administration of diatomite improves the generally understood body condition of animals and may also improve their production results (Carlisle 1986). Any negative impact of the amorphous diatomaceous earth on health or condition of animals has been document so far.

This study was aimed at analyzing the effect of the addition of amorphous diatomaceous earth to feed on production performance indices and selected quality traits of meat and femoral bone of broiler chickens.

MATERIAL AND METHODS

The experimental material included 60 fast-growing Ross 308 broiler chickens (males) kept until 42nd day of age at the experimental farm of Wilanów-Obory. One-day poults were vaccinated against Marek's diseases, Gumboro disease, and infectious bronchitis. Afterwards, they were weighed, tagged with individual tags, and randomly allocated to experimental groups. On the first day of experiment, the chicks were randomly divided into three groups of 20 birds (five birds in four replicates), i.e. control group (C) and two experimental groups in which diets were supplemented with diatomaceous earth: D2 (2%) and D4 (4%). The differentiating factor in the experiment was feeding the chickens from the experimental groups with a feed mixture supplemented with 2 and 4% of diatomaceous earth. The birds were fed *ad libitum*, in a three-stage system: starter (days 1–14), grower (days 15–35), finisher (days 36–42) – Table 1.

The diatomaceous earth used in the experiment originated from freshwater deposits, its dietary quality was suitable for men and animals and it was ground to the consistency of meal. Its composition was presented in Table 2.

Individual body weights, feed intake and mortality rate of the chickens were controlled throughout the study. On day 42 of production, six males with body weight close to the average body weight in a group were selected from each group. The selected birds were fasted for 12 h, with constant access to water, and then slaughtered. Carcasses were chilled with the air method at a temperature of 4°C for 24 h. Then, dissection was performed. Afterwards, a total of 18 carcasses (six from control group and six from each experimental group) were dissected following the methodology described by Ziołecki and Doruchowski (1989). Dressing percentage was calculated, i.e. content of muscles and content of giblets (gizzard, liver and heart), in respect of the body weight before slaughter. The collected breast and leg muscles were weighed, individually marked, protected, and left for further analyses.

The dissected breast and leg muscles were subjected to the chemical and physicochemical analysis. The proximate chemical composition of breast muscles

TABLE 1. Feed mixture composition and nutritional value

Specification	Feed mixture		
	starter (days 1–14)	grower (days 15–35)	finisher (days 36–42)
Component (%)			
Corn	10.00	11.40	10.00
Wheat	53.00	55.00	60.80
Soybean meal	30.60	27.40	21.60
Limestone	1.19	1.20	0.97
Sodium bicarbonate	0.20	0.14	0.16
NaCl	0.24	0.28	0.26
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 0.5%	0.50	0.50	0.50
Nutritional value			
ME (kcal)	2 990	3 047	3 217
Crude fat (%)	3.67	4.00	5.92
Crude protein (%)	21.99	20.78	18.51
Crude fiber (%)	3.60	2.55	2.41
Crude 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

TABLE 2. The composition of diatomaceous earth (MSDS diatomite 2016)

Chemical substance	Content (%)
SiO_2 amorphous	92.00
SiO_2 crystalline	<0.10
Al_2O_3	4.52
Fe_2O_3/FeO	1.16
P_2O_5	0.02
CaO	0.71
MgO	0.55
$Na_2O + K_2O$	0.28
S	0.01
Cl	0.02
MnO	0.02

and leg muscles was determined with standard methods: protein content – with the Kjeldahl's method using a conversion factor of 6.25 (PN-75/A-04018); and fat content – with the Soxhlet's method (AOAC 2005). Color parameters (L*, a*, b*) were measured in comminuted breast and leg muscles using a CR-410 colorimeter (Minolta) following producer's instructions. Each parameter was measured in five replications, and the mean value of five replications was assumed as measurement result. The parameter L* (lightness) may attain values from 0 to 100. Parameters a* and b* are trichromaticity coordinates and may attain positive and negative values: +a* denotes red color, –a* denotes green color, +b* denotes yellow color, and –b* denotes blue color.

Tests of femoral bone resistance to fractures were performed in a testing machine Zwick Roell 25.0 equipped in a head with the maximum force of 1 kN. Speed of the loading element was 50 mm/min.

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

RESULTS AND DISCUSSION

Results of analyses of growth performance and slaughter analysis of the broiler chickens were presented in Tables 3 and 4. On 42nd day of rearing, significantly ($P \leq 0.05$) highest body weight was noted in the group of control birds (3,249 g). Feed conversion ratio in group D2 (1.57 kg/kg of body weight gain) was the lowest, compared to the other groups (C – 1.59, D4 – 1.64 kg/kg of body weight gain). No deaths of birds were recorded in any of the groups. It may be indicative of very good microclimatic conditions and welfare of the chickens maintained throughout the rearing period, as well as of the lack of negative effects of diatomaceous earth administration on birds mortality rate. Worthy of notice is, however, that there are ample research reports and works concerning the positive effect of dietary inclusion of diatomite on production performance and generally understood health status of animals, including poultry (Carlisle 1972, 1976, Bennett et al. 2011). Although some differences were noted between groups in growth performance parameters, the effects achieved should be found satisfactory and comparable with these reported by other authors.

TABLE 3. Growth performance of broilers – average body weight (BW), feed conversion ratio (FCR), mortality

Group	BW (g), day 42	FCR (-), days 1–42	Mortality (%)
C	3 249^{a}	1.588	0
D2	3 132^{ab}	1.571	0
D4	3 072^{b}	1.638	0
SEM	43.88	0.02	–

Means within a column with different superscripts are significantly different at $P \leq 0.05$.

TABLE 4. Average results of slaughter analysis of broiler chickens (%)

Group	Dressing percentage	Muscles		Giblets			Abdominal fat
		breast	legs	gizzard	liver	heart	
C	73.54	23.36^{B}	15.03	0.60	1.80	0.45	0.51^{ab}
D2	76.05	26.56^{A}	14.78	0.55	1.90	0.45	0.48^{b}
D4	74.70	24.46^{AB}	15.26	0.58	1.83	0.45	0.77^{a}
SEM	0.85	0.70	0.33	0.03	0.11	0.01	0.08

Means within a column with different superscripts are significantly different: A, B at $P \leq 0.01$, a, b at $P \leq 0.05$.

In reference to the analyzed parameters, no significant differences were demonstrated in dressing percentage – its highest value (at the significance boundary of $P = 0.056$) was noted in group D2 (76.05%), whereas the lowest one in the control group – 73.54% (Table 4). The application of diatomaceous earth in diet for broiler chickens reared until 42nd day of age had a significant effect on the contribution of leg muscles and giblets in carcasses (Table 4). Analyses demonstrated a significant ($P \leq 0.01$) increase in the content of breast muscles and a lower fat content ($P \leq 0.05$) after diet supplementation with 2% of diatomite, which may be of great significant from the consumer's perspective. According to research reports, today, consumers of poultry meat purchase mainly those parts of carcasses that may be easily and quickly prepared for consumption – next to thigh muscles, these parts include breast muscles (Nowak and Trziszka 2010). In addition, consumers pay great attention to the nutritive value of food products, and poultry meat is commonly perceived as dietetic meat, hence a low fat content is desirable in this product (Nowak and Trziszka 2010, Zdanowska-Sąsiadek et al. 2013). The production of chickens with a higher content of breast muscles and a lower content of fat in carcass is, therefore, economically beneficial to the producer.

Results obtained in this study point to the feasibility of applying diatomite in feed mixtures for broiler chickens – though not necessarily in the large-scale commercial production owing to the possibility of reduced body weight, but definitely in the free-range, bio, and household systems – and indicate that it would potentially yield some economical benefits and would not deteriorate the growth performance of chickens. In case of the broiler chickens from group D2, even improvement was observed in the production effectiveness manifested in a reduced feed conversion ratio, compared to the control group, which ensures another economic benefit to the producer. Results of this study confirm findings reported by other authors. Research reports on the effects of diatomaceous earth demonstrate, e.g., the mechanism of agglutination of food particles in the gastrointestinal tract and separation of these particles from one to another (Ewuola et al. 2014). By this means, it is feasible to enlarge the available surface of digestion for enzymes and to retard digesta passage through the gastrointestinal tract, which results in more efficient absorption of nutrients from feed mixtures and in enhanced effectiveness of digestion. In addition, diatomaceous earth affects body detoxification and bowel purgation (also from residues of veterinary pharmaceuticals), which increases feed intake by animals. What is more, according to other researchers, the intake of fine-grain feed additives may improve absorption of nutrients in poultry (Quisenberry 1967, van der Meulen et al. 2008).

Poultry feeding affects not only the basic indices of post-slaughter yield and nutritive value of meat, but also significantly models the aroma and taste of meat. Inappropriate choice of feed components may cause some deviations towards deterioration of palatability, but through feeding it is also possible to regulate the level of indispensable components in meat, being the basis of

its nutritive value. Meat plays a key role in man's diet, especially in developed countries (Speedy 2003). It is affected by many factors like, e.g., social status, wealth, the size of animal productions, and socio-economical status of the country, which explains the higher consumption of meat in Western populations (Speedy 2003).

A typical muscle tissue consists of about 75% of water, 20% of protein, 3% of fat, and 2% of solubles non-protein substances. Results of chicken meat quality analysis were presented in Tables 5 and 6. In most cases, the addition of diatomaceous earth to the feed mixture had no significant effect on the proximate chemical composition of breast muscles. The chemical composition of leg muscles (Table 5) was, generally, similar in all groups, however significantly the highest content of crude fat was determined in leg muscles of the chickens from control group (7.34%), and the lowest one – in these of the chickens from group D2 (5.89%). A similar tendency was observed in breast muscles. Generally, leg muscles contain more fat than breast muscles (Castellini et al. 2002), however, it needs to be emphasized that fat has a positive impact on taste, juiciness, and tenderness of meat (Aberle et al. 2001). As reported by Niewiarowicz (1993) the increasing content of fat is accompanied by a decreasing content of protein or by an increasing dry matter content of mus-

TABLE 5. Chemical composition of chicken meat (%)

Group	Breast muscles				Leg muscles			
	water	protein	fat	collagen	water	protein	fat	collagen
C	74.06	22.10	3.71	1.17	72.33^{b}	19.44	7.34^{a}	1.16
D2	74.39	21.80	3.33	1.11	73.78^{a}	19.68	5.89^{b}	1.22
D4	73.69	22.32	3.54	0.96	72.79	19.49	6.91	1.15
SEM	0.28	0.22	0.25	0.09	0.38	0.18	0.45	0.09

Means within a column with different superscripts are significantly different at $P \leq 0.05$.

TABLE 6. Color parameters of breast and leg muscles

Group	Breast muscles			Leg muscles		
	L*	a*	b*	L*	a*	b*
C	50.99	6.44^{a}	3.45	53.76	9.07	6.76^{a}
D2	51.81	4.32^{b}	4.13^{a}	53.13	8.91	5.67
D4	50.58	4.65	2.79^{b}	53.59	8.41	5.44^{b}
SEM	1.09	0.62	0.40	0.83	0.46	0.41

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

Means within a column with different superscripts are significantly different at $P \leq 0.05$.

cles, which was confirmed in this study (Table 5). Simultaneously, as mentioned earlier, the lower content of fat in poultry meat may be desirable by consumers.

Breast muscles of broiler chickens contain ca. 25% of protein (Lonergan et al. 2003). A higher content of protein was determined in the breast than in the leg muscles. The highest content of protein in breast muscles was assayed in group D4 (22.32%), and the lowest one in group D2 (21.80%). In the case of leg muscles, the highest protein content was found in group D2 (19.68%), and the lowest one in the control group (19.44%). Nevertheless that above results are consistent with findings of other authors, according to whom the mean content of protein in breast muscles of chickens ranged from 17.8 to 20.2% (Gawęcki and Gornowicz 2000). In case of leg muscles, analyses showed a significantly ($P \leq 0.05$) higher content of water in group D2 than in the control group (73.78 and 72.33%, respectively). Results obtained by Grabowski (1993) demonstrated an inversely proportional dependency between water content and fat content, which was confirmed in our study in case of significant differences. The content of water decreases along with bird age, which is linked with increasing adiposity and processes ongoing in muscles.

Color sensation is one of the key criteria taken into account during the choice and purchase of a food product by consumers. Based on the visual assessment of this trait, a customer is concluding about the freshness or even quality of meat. The statistical analysis of results of color measurements demonstrated that diet supplementation with diatomaceous earth had no effect on the values of L* parameter in breast muscles of birds. Observations confirmed no effect of various dietary doses of diatomite on better muscle coloration compared to the control group (Table 6). The color of meat is determined by, among other things, concentration of heme pigments and pH value. The highest values of saturation with red color (parameter a*) were found in breast and leg muscles of the control birds, however these values were confirmed statistically only in case of breast muscles. The contribution of yellow and blue color is indicated by values of parameter b*. Results obtained regarding this color parameter demonstrated breast and leg muscles saturation with yellow color. The highest value of saturation with yellow color (parameter +b*) was determined in breast muscles of birds from group D2 (4.13) and in leg muscles of birds from the control group (6.76), whereas the lowest values of this parameter were noted in both breast and leg muscles of birds from group D4. According to Kirkpinar et al. (2001), the more beneficial coloration of carcasses of broiler chickens is indicated by lower values of L* parameter and by higher values of a* and b* parameters. Therefore, diet supplementation with diatomaceous earth had no explicit positive or negative effect on color parameters of chicken meat.

Current conditions of rearing and breeding as well as feeding and genetic modification of domestic fowl are supposed to ensure, most of all, a high growth rate. The fast increase of body weight but also no balance between muscle mass gain and bone mass may increase the risk of deformations or

fractures of different bones. The selection of broiler chickens for the highest possible muscle mass has led to disruption of body homeostasis which results from the non-uniform development of the whole organism (Wnuk et al. 2013). The greatest problems faced by producers are these linked with skeleton incapability to lift such a heavy body of chickens, especially in the last weeks of the production cycle. Considering bone resistance expressed by means of the breaking force (Table 7), dietary inclusion of diatomaceous earth was demonstrated to have a significant effect on the value of this trait. Femoral bones of birds from group D4 were characterized by a significantly greater resistance (395.83 N) to the breaking force than these of the control birds (369.66 N). In addition, a growing tendency was observed in bone resistance along with an increasing diatomite dose in the feed mixture. There is growing need for understanding the bone resistance in poultry because bone fractures and resultant infections often contribute to deterioration of dressing percentage and quality of carcass. One of the aims of diatomite addition to feed mixtures for chickens is to supplement their bodies with silicon. Negative effects of a silicon-poor diet were demonstrated by Carlisle (1972) – deficiency of this element caused skeleton deformation and incorrect joint development in chickens. The effect of diatomaceous earth on the improvement of skeleton resistance and condition was also confirmed in studies conducted by Calomme et al. (2002) and López-Álvarez et al. (2009). Feed supplementation with silicon may, additionally, significantly affect the appropriate development of young organisms, which was proved by Carlisle (1972) – one-day cockerels fed a diet based on amino acids were later characterized by inhibited growth and development, whereas those administered a feed mixture supplemented with silicon were characterized by a 50% higher growth rate and normal development. Another study by Carlisle (1976) demonstrated the presence of silicon at the active sites of calcium attachment in bones of young hens. In turn, our previous studies (Wiewióra 2016 – unpublished data) confirmed the effect of diatomite on skeleton strengthening in hens, i.e. femoral bones of laying hens receiving a feed mixture with the addition of diatomaceous earth were significantly more resistant to fractures.

TABLE 7. The strength of the femur bone

Group	Strength (N)
C	369.7^{b}
D2	371.3^{ab}
D4	395.8^{a}
SEM	8.17

Means within a column with different superscripts are significantly different at $P \leq 0.05$.

CONCLUSION

Results obtained in the study enable presenting the following summary and conclusions:

1. The application of diatomite in the feed mixture had no effect on deterioration of the health status nor on mortality rate of broiler chickens, and ensured other production effects at a comparable level.
2. The amorphous diatomaceous earth may be applied in broiler chicken feeding as an additive safe to the environ-

ment and to bird welfare, which provides a source of biologically-active silicon to birds and has a potentially positive effect on their organisms.

3. Diatomaceous earth may be applied in broiler chicken feeding as an additive improving the resistance and condition of bones, which may be desirable from breeder's and producer's perspective, and most of all from the perspective of animal welfare.

Acknowledgement

The paper is a part of the MSc thesis.

REFERENCES

ABERLE E.D., FORREST J.C., GERRARD D.E., MILLS E.W., 2001: Principles of Meat. Sci. 4th edn. Kendall/Hut Publ., Dubuque, IA.

ABRAHAM G.E., 2005: The importance of bioactive silicates in human health. The Original Internist. Spring: 13–19.

AOAC, 2005: Official methods of analysis of AOAC international. Gaithersburg, Md. AOAC International, USA.

BENNETT D.C., YEE A., RHEE Y.J., CHENG K.M., 2011: Effect of diatomaceous earth on parasite load, egg production, and egg quality of free-range organic laying hens. Poult. Sci. 90: 1416–1426.

CALOMME M.R., SINDAMBIWE J.B., COS P., VYNCKE C., GEUSENS P., VANDEN BERGHE D.A., 2002: Effect of choline stabilized orthosilicic acid on bone density in chicks. Calcif. Tissue Int. 70: 292.

CARLISLE E.M., 1972: Silicon: an essential element for the chick. Science 178 (4061): 619–621.

CARLISLE E.M., 1976: *In vivo* requirement for silicon in articular cartilage and connective tissue formation in the chick. J. Nutr. 106 (4): 478–484.

CARLISLE E.M., 1986: Silicon as an essential trace element in animal nutrition. Ciba Foundation Symposium 121 – Silicon Biochemistry: 123–139.

CASTELLINI C., MUGNAI C., Dal BOSCO A., 2002: Effect of organic production system on broiler carcass and meat quality. Meat Sci. 60: 219–225.

DAWSON R.D., 2004: Efficacy of diatomaceous earth at reducing populations of nest-dwelling ectoparasites in tree swallows. J. Field Ornithol. 75: 232–238.

EWUOLA E.O., ADEBIYI O.A., SOKUNBI O.A., 2014: Haematological and some serum biochemical response of growing cockerels to dietary diatomaceous earth. J. Anim. Sci. Adv. 4 (1): 699–704.

FIELDS P.G., 2000: Diatomaceous earth: Advantages and limitations. In: Proc. VII International Working Conference on Stored-Product Protection. Sichuan Publishing House of Science and Technology, Chengdu, China 1: 781–784.

GAWĘCKI W., GORNOWICZ E., 2000: Ocena podstawowego składu chemicznego mięśni kurcząt brojlerów pochodzących z różnych hodowli zagranicznych. Gosp. Mięsna 52 (7): 42.

GRABOWSKI T., 1993: Surowiec do produkcji mięsa drobiowego. Cz. 2. Pol. Drob. 10: 8–15.

HANEY M., 2016: Perma Guard. Available at http://www.perma-guard.com.pl.

KIRKPINAR F., BOZKURT M., ERKER R., 2001: The effects of dietary dried alfalfa meal on skin pigmentation and performance of broiler. In: Proc. XV Europ. Symp. Quality Poultry Meat, 9–12 September, Turkey: 199–204.

LONERGAN S.M., DEEB N., FEDLER C.A., LAMONT S.J., 2003: Breast meat quality and composition in unique chicken populations. Poult. Sci. 82 (12): 1990–1994.

LÓPEZ-ÁLVAREZ M., SOLLA E.L., GONZÁLEZ P., SERRA J., LEÓN B., MARQUES A.P., REIS R.L., 2009: Silicon-hydroxyapatite bioactive coatings (Si-HA) from diatomaceous earth and silica. Study of adhesion and proliferation of osteoblast-like cells. J. Mater. Sci. Mater. Med. 20 (5): 1131–1136.

MAURER V., PERLER E., HECKENDOM F., 2009: *In vitro* efficacies of oils, silicas and plant preparations against the poultry red mite *Dermanyssus gallinae*. Exp. Appl. Acarol. 48 (1–2): 31–41.

NIEWIAROWICZ A., 1993: Struktura, skład chemiczny, zmiany poubojowe i smakowitość mięsa drobiowego. In: Technologia mięsa drobiowego. T. Garbowski (Ed.). WNT, Warszawa.

NORTON K., 2015: eHow Contributor. Available at http://www.ehow.com/facts_5862967_diatomaceous-earth-candida.html.
NOWAK M., TRZISZKA T., 2010: Zachowania konsumentów na rynku mięsa drobiowego. ZNTJ 1 (68): 114–120.
PN-75/A-04018. Produkty rolniczo-żywnościowe. Oznaczanie azotu metodą Kjeldahla i przeliczanie na białko.
QUARLES W., 1992: Diatomaceous earth for pest control. The IPM Practitioner. Monitoring the Field of Pest Management 14 (5–6): 1–11.
QUISENBERRY J.H., 1967: The use of a clay in poultry feed. Clays Clay Miner. 16: 267–270.
ROUND F.E., CRAWFORD R.M., MANN D.G., 1990: The diatoms: biology and morphology of the genera. Cambridge University Press, Cambridge.
SPEEDY A.W., 2003: Global production and consumption of animal source foods. J. Nutr. 133: 4048–4053.
Van Der MEULEN J., KWAKERNAAK C., KAN C.A., 2008: Sand intake by laying hens and its effect on egg production parameters. J. Anim. Physiol. Anim. Nutr. 92 (4): 426–431.
WIEWIÓRA M., ŁUKASIEWICZ M., BARTOSIK J., MAKARSKI M., NIEMIEC T., 2015: Diatomaceous earth in the prevention of worm infestation in purebred pigeons. Ann. Warsaw Univ. Life Sci. – SGGW, Anim. Sci. 54 (2): 161–166.
WNUK A., MROCZEK-SOSNOWSKA N., ADAMEK D., KAMASZEWSKI M., ŁUKASIEWICZ M., NIEMIEC J., 2013: Effect of rearing system and gender on histological profile of chicken breast and leg muscles in hybrid (Cobb × Zk). Ann. Warsaw Univ. Life Sci. – SGGW, Anim. Sci. 52: 211–218.
ZDANOWSKA-SĄSIADEK Ż., MICHALCZUK M., MARCINKOWSKA-LESIAK M., DAMAZIAK K., 2013: Czynniki kształtujące cechy sensoryczne mięsa drobiowego. Bromat. Chem. 46 (3): 344–353.
ZIOŁECKI J., DORUCHOWSKI W., 1989: Metody oceny wartości rzeźnej drobiu. COBRD Poznań.

Streszczenie: *Wskaźniki odchowu oraz wybrane cechy jakości mięsa i kości udowej kurcząt brojlerów żywionych paszą uzupełnioną amorficzną ziemią okrzemkową.* Celem badania była analiza wpływu dodatku amorficznej ziemi okrzemkowej do paszy na wyniki odchowu oraz wybrane cechy jakości mięsa i kości udowej kurcząt brojlerów. Badanie przeprowadzono na 60 szybkorosnących kurczętach Ross 308 utrzymywanych do 42. dnia życia i podzielonych na grupę kontrolną (C) i dwie grupy doświadczalne (D2 i D4), po 20 sztuk w każdej. Ziemię okrzemkową (diatomit) podawano z paszą ptakom z grupy D2 – 2%, D4 – 4%. W doświadczeniu kontrolowano masę ciała, spożycie paszy oraz śmiertelność kurcząt. W 42. dniu odchowu z każdej grupy ubito po sześć kogutów i wykonano dysekcję. Obliczono wydajność rzeźną, udział mięśni oraz podrobów jadalnych (żołądka mięśniowego, wątroby oraz serca), a w próbach z mięśni piersiowych i nóg wykonano analizy chemiczne i fizykochemiczne. Określono również wytrzymałość kości udowej na złamanie. Dodatek diatomitu nie wpłynął na status zdrowotny kurcząt. Większą masę ciała w 42. dniu odchowu odnotowano w grupie C względem D4 ($P \leq 0,05$). Stwierdzono większy ($P \leq 0,01$) udział mięśni piersiowych oraz mniejszy ($P \leq 0,05$) udział tłuszczu w tuszce w grupie D2 względem C. Dodatek ziemi okrzemkowej do paszy nie różnicował składu chemicznego mięśni piersiowych. W mięśniach nóg kogutów D2 stwierdzono zmniejszenie ($P \leq 0,05$) zawartości tłuszczu oraz zwiększenie ($P \leq 0,05$) zawartości wody w porównaniu do grupy C. Kości udowe ptaków z grupy D4 charakteryzowały się większą ($P \leq 0,05$) wytrzymałością na złamanie niż kości kurcząt z grupy C. Stwierdzono wprost proporcjonalną zależność między ilością zastosowanego diatomitu w paszy a wytrzymałością kości udowej. Za najbardziej optymalną uznano suplementację do paszy na poziomie 2%.

Słowa kluczowe: ziemia okrzemkowa (diatomit), kurczęta brojlery, jakość mięsa i kości udowej

MS received 15.03.2017
MS accepted 25.04.2017

Authors' address:
Monika Łukasiewicz
Katedra Szczegółowej Hodowli Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8, 02-786 Warszawa
Poland
e-mail: monika_lukasiewicz@sggw.pl

Agriculture
(Agricultural and Forest Engineering)
Animal Science
Forestry and Wood Technology
Horticulture and Landscape Architecture
Land Reclamation

Annals of Warsaw University of Life Sciences were originally published in 1957 as **Zeszyty Naukowe SGGW (Scientific Fascicles of SGGW)**. In 1980 the name was changed to **Annals of Warsaw University of Life Sciences**.

The **Annals** (5 subject series) are published once or twice a year and will carry previously unpublished papers that are mainly in English, but also in French, German or Russian, followed by a short summary in Polish. Manuscripts for publication should be typewritten and submitted to the Warsaw University of Life Sciences Press in two copies. Papers submitted for consideration by the Editorial board should not exceed 0.5 of a printed sheet (about 11 pages including illustrations, and should consist of the following elements: 1) name and surname of the author, 2) title of the paper, 3) abstract (about 20 lines), 4) text of the paper, 5) date when the paper was sent to the Warsaw University of Life Sciences Press and mailing address of the author, 6) summary (one page), 7) tables and figures with captions.