Number 1
2015
Volume 48
48 (1) 1–48
ISSN 1337-9984

Slovak Journal of Animal Science

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak Journal of Animal Science

Formerly
Journal of Farm
Animal Science

AIMS AND SCOPE

Slovak Journal of Animal Science (ISSN 1337-9984) is an international scientific journal that publishes original scientific papers, reviews, short communications, chronicles of important jubilees, reports of participation in important international conferences on animal science in English language.
Topic of the journal are problems of animal production, mainly in the sphere of genetics, breeding, nutrition and feeding, physiological processes of digestion, conservation and treatment of feeds, biotechnology, reproduction, ethology, ecologization of breeding, biology and breeding technology in farm animals, quality of meat, milk, wool, economy of breeding and production of farm animals, mainly: cattle, pigs, sheep, goats, horses, poultry, small farm animals and farm game. There are published also articles from the sphere of biochemistry, genetics, embryology, applied mathematical statistics as well as economy of animal production. There can be published also articles from the sphere of veterinary medicine concerning the themes of the journal.

SJAS is published quarterly.

Online version of the journal (ISSN 1338-0095) is at disposal at http://www.vuzv.sk/index.php/slovak-journal-of-animal-science.

This journal is comprised in: AGRIS/FAO database (the full texts, too); CAB Abstracts; Knovel.

Slovak Journal of Animal Science is published under the authorization and direction of the National Agricultural and Food Centre - Research Institute for Animal Production Nitra, Slovak Republic.

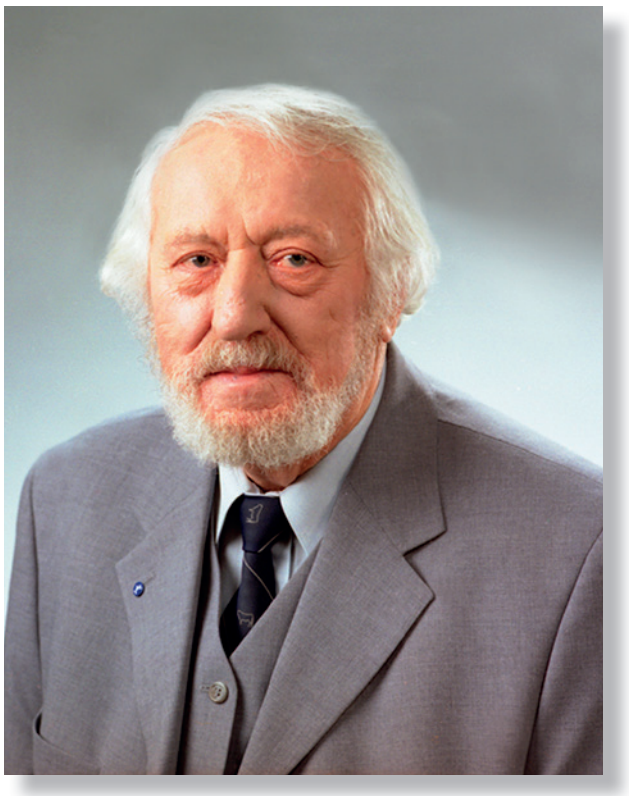
Editorial office, orders, subscription and distribution: NAFC - RIAP Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic. Phone +421 37 6546 249; E-mail: editor@vuzv; http://www.vuzv.sk/; www.nppc.sk/
Filed at the Ministry of Culture of the Slovak Republic: EV 3659/09.

Jubilee of Professor Ján Plesník

Significant Slovak scientist, teacher and humanist Prof. Ján Plesník, DSc., Dr.h.c., important representative of the Czech-Slovak and Slovak science, founder and long-time director of the Research Institute for Animal Production (RIAP) in Nitra and pedagogue at the Slovak University of Agriculture in Nitra, celebrates his 90th birthday on 23rd April 2015.

Professor Plesník was born in Krajné, in a family of farmers. As it was a family with traditional respect for the heritage of fathers and the culture of the nation, which esteemed education, so it allowed education also to the son. After finishing the two-year Agricultural School in Martin he went on to study at the four year Higher Economic School in Košice, where he passed the school-leaving exams. During the years 1945–1950 he studied at the University for Agriculture and Forest Engineering in Brno aimed at scientific-research work.

The first place of work of Prof. Plesník was the Research Institute for Animal Production in Vígľaš. The institute was replaced to Nitra in 1961. Prof. Plesník performed the function of director from 1959 to 1991. I take the liberty to cite the esteemed toastee: *"The goal of my life and working sense of my life was to create a modern reputable scientific institution, create a material and personal democratic atmosphere and conditions for free research work as well as realization of the workers' abilities."* He motivated and involved the whole personnel of the institute to fulfil this grandiose goal by his hard work, respect for the truth and personal enthusiasm. During this period defended more than 70 workers the title CSc. or PhD. and 12 workers the title DSc. in RIAP.



The bibliography of the toastee is also rich. It contains nearly two dozen of books and some hundred scientific, specialist and popular-professional publications, which draw from the results of his own scientific work. Ján Plesník devoted much time also to the Slovak Academy of Sciences; he was its member, correspondent and academician during the years 1969–1980. He was also member of the Czechoslovak Academy of Sciences, Russian Academy of Agricultural Sciences and other European scientific societies. After 1990 he became initiator of the foundation and in 1993 the first chairman of the Slovak Academy of Agricultural Sciences. Professor Plesník remains spiritual father of this learned society till nowadays.

Ján Plesník is a many-sided personality. As an art lover he gained recognition for creation of an exhibition hall in the Research

Institute for Animal Production, in which are regularly presented works of outstanding Slovak and foreign artists. He was also at the origin of the International Film Festival Agrofilm, which represents successfully Slovakia and our agro-sector in the world for 30 years.

The toastee is laureate of high state national and foreign distinctions. The personal qualities and performance results of Prof. Plesník evaluated a number of universities by awarding him the academic degree honorary doctor. He is honorary citizen of the town Nitra.

Multidimensional personality of Ján Plesník is expressed in his life and work, based on a deep, honest and humble analysis of phenomena that results in a characteristic philosophical manuscript. These values led him to a special and very exceptional position among the representatives of animal sciences. If there is anything typical for Prof. Plesník, it is his effort to hand out himself to colleagues without the need to retain something of his wealth for himself. Here are the roots of Plesník's school, which today represent dozens of scientific workers and teachers who express gratitude and respect for his lifework.

Ad multos annos Profesor Plesník

Jaroslav Slamečka
Director of the Research Institute
for Animal Production

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 48, 2015 (1): 1–7

ISSN 1337-9984

FIRST ESTIMATES OF LACTATION CURVES IN WHITE SHORTHAIRED GOATS IN SLOVAKIA

M. ORAVCOVÁ[1]*, M. MARGETÍN[1, 2]

[1]NAFC – Research Institute for Animal Production Nitra, Slovak Republic
[2]Slovak University of Agriculture in Nitra, Slovak Republic

ABSTRACT

The objective of this study was to compare Cubic, Ali-Schaeffer, Guo-Swalve (modified Khanderkar) and Wilmink models for fitting lactation curves of White Shorthaired goats in Slovakia. In total, 19 002 test day records of daily milk yield, 18 441 test day records of fat content and 18 757 test day records of protein content of 1 486 does collected between 1996 and 2011, were analyzed. Milk traits were grouped by the week of lactation, and hence weekly averages of milk yield, fat and protein content were used for modelling the first estimates of lactation curves. The goodness-of-fit of the models was of similar quality (R^2 slightly above 0.9 for milk yield, above 0.6 for fat content and above 0.7 for protein content); with the exception of Wilmink model which gave lower R^2 for the three traits (0.842, 0.600, 0.426). Lack of data in early and late lactation caused some difficulties in modelling the respective phases of lactation curves with chosen models. The greatest differences in lactation curves were found between Wilmink model and the remaining models. Research on individual days in milk is needed for better understanding of variation of milk traits during the course of lactation, since they may depend on such effects as number of kids, feeding, parity or individual doe.

Keywords: doe, White Shorthaired goat, test day, lactation, milk traits, weekly averages, regression coefficient

INTRODUCTION

The White Shorthaired goats are predominant among the goat population of Slovakia. Goats represent the livestock sector of minor importance, mainly farmed for milk. The production system is extensive with the only one kidding per year applied. The performance testing involves few flocks of the White Shorthaired goats; the numbers have been gradually decreasing since the beginning of the millennium. Selection is done under the umbrella of the Sheep and Goat Breeders' Association and the Breeding Services of the Slovak Republic. Breeding is aimed at improvement of milk, prolificacy and exterior. The animals are selected on the basis of 100-point scale (55 points for milk yield, 25 points for prolificacy and 20 points for exterior). Milk yield expressed as milk yield adjusted for 240-day standardized milking period is considered the most important trait among Slovak goats (milking period starts after the kids are weaned).

Although goat lactation curves and factors affecting variation of daily milk traits are commonly known worldwide (Akpa *et al*., 2001; Ciappesoni at al., 2004; Macciotta *et al*., 2005; Pala and Savas, 2005; Waheed and Khan, 2013), the only analysis done with goats in Slovakia is the study dealing with milking period yield (Margetín and Milerski, 2000). In small ruminants, daily milk traits were analyzed only in Slovak sheep and ewes' lactation curves were estimated (Oravcová *et al.*, 2006 and 2007).

The objective of this study was to study the variation of goat milk, fat and protein content throughout the lactation and to estimate average lactation curves for milk yield, fat and protein content in White Shorthaired goats in Slovakia.

***Correspondence:** e-mail: oravcova@vuzv.sk
Marta Oravcová, NAFC – Research Institute for Animal Production Nitra,
Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Phone.: +421 37 6546 360 Fax: +421 37 6546 401

Received: July 20, 2014
Accepted: February 11, 2015

MATERIAL AND METHODS

Test-day records of milk performance testing in a single (most numerous) flock of the purebred White Shorthaired goats collected by the Breeding Services of the Slovak Republic between years 1996 and 2011, were analyzed. The traits investigated were daily milk yield, fat and protein content. Milk samples were taken in accordance with A_4 or AC standard method (ICAR, 2003). In total, 19 002 test day records of daily milk yield, 18 441 test day records of fat content and 18 757 test day records of protein content of 1 486 does on their 1st to 10th parities (3 646 parities) were included in the analysis. The average number of test-day measurements per parity was 5.22 ± 0.88; almost 90 % of does had 5 or 6 test days per lactation. The measurements, taken between days 17 and 251 after parturition, were grouped by the week of lactation. Thus, week averages of the respective traits were used for modelling the first estimates of lactation curves in Slovak goats. The following models: Cubic, Ali-Schaeffer (1987), Guo-Swalve (1995) i.e. modified Khanderkar and Wilmink (1987) were applied (GLM procedure, SAS 9.2 software; 2009) for modelling of first estimates of lactation curves for milk yield, fat and protein content in goats of the White Shorthaired breed.

Cubic model:

$$y = b_0 + b_1 DIM + b_2 DIM^2 + b_a DIM^a + e \quad (1)$$

Ali-Schaeffer model:

$$y = b_0 + b_1\left(\frac{DIM}{240}\right) + b_2\left(\frac{DIM}{240}\right)^2 + b_a \ln\left(\frac{240}{DIM}\right) + b_4 \ln\left(\frac{240}{DIM}\right)^2 + e \quad (2)$$

Guo-Swalve model (modified Khanderkar):

$$y = b_0 + b_1 DIM + b_2 DIM^2 + b_a DIM^a + b_4 \ln(DIM^l) + e \quad (3)$$

Wilmink model:

$$y = b_0 + b_1 2.718281^{-0.05DIM} + b_2 DIM + e \quad (4)$$

where:
y is weekly averages of daily milk yield, fat and protein content; b_0 to b_4 is regression coefficients; ln is natural logarithm; 2.718281 is base of natural logarithm; 240 is length of standardized lactation period; *DIM* is days in milk; e is random error assumed to be normally distributed, N is $(0, \partial_e^z)$

The goodness-of-fit of the models was assessed by comparison of coefficients of determination and residual standard deviations.

RESULTS AND DISCUSSION

In the analyzed performance records of White Shorthaired goats in Slovakia, the average number of days in milk from parturition to the first test day was 60 ± 15.9. Most measurements (81.2 %) were taken in the middle of lactation (months 3 to 7), whereas in the beginning of lactation (week 3, 4 and 5) and the end of lactation (week 33, 34 and 35) minimum measurements were done (see Table 1 for distribution of test days by the week of lactation). Some monthly measurements lacked the information on milk composition (3 % for fat content and 1.3 % for protein content).

The estimated regression coefficients for Cubic, Ali-Schaeffer, Guo-Swalve (modified Khanderkar) and Wilmink model are given in Tables 2 (for milk yield), 3 (for fat content) and 4 (for protein content). The goodness-of-fit of the models is also given. The coefficients of determination (R^2) were almost identical or similar within the individual milk traits. The models explained from 84.2 % to 91.5 % of total variability for milk yield (Table 2), from 60.0 % to 69.9 % for fat content (Table 3) and from 42.6 % to 71.3 % for protein content (Table 4). Wilmink model explained the lowest amount of total variability for the three traits investigated: about 7 % lower variability than the remaining models for milk yield, about 10 % lower variability for fat content and about 30 % lower variability for protein content. The goodness-of-fit differed minimally among Cubic, Ali-Schaeffer and Guo-Swalve (modified Khanderkar) models: from 0.1 % to 0.4 % for milk yield, from 0.8 % to 2.9 % for fat content and from 0.1 % to 0.4 % for protein content. After fitting the models, standard deviations for milk traits diminished as follows: from 55.5 % to 66.4 % for milk yield (Table 2), from 34.5 % to 56.5 % for fat content (Table 3) and from 21.7 % to 45.4 % for protein content (Table 4).

The slightly higher coefficients of determination (above 92 %) were reported by Ángel Marín *et al.* (2009) when fitting four mathematical models (Wood, Brody, Wilmink, and Papayscik- Bodero) to milk lactation curves in Colombian hybrid goats with 865 daily milk yields measured. Comparable coefficients of determination (from 89.27 to 97.82 %) to those found in this study were reported by León *et al.* (2012) when fitting six mathematical models (Wood, modified Wood, Cobby-Le Du, Wilmink, Quadratic spline, and Legendre polynomials of order three) to milk lactation curves in Murciano-Granadina goats. The authors used the mean daily milk production in the individual day of lactation

Table 1: Weekly averages for milk yield, fat and protein content

Week	Days	N	Milk, l	N	Fat (%)	N	Protein (%)
			$x \pm s$		$x \pm s$		
3	15 to 21	3	0.843 ± 0.455	3	4.03 ± 0.41	3	3.12 ± 0.14
4	22 to 28	5	1.173 ± 0.499	5	4.72 ± 0.88	5	3.16 ± 0.31
5	29 to 35	7	1.464 ± 0.609	7	4.42 ± 0.58	7	3.14 ± 0.32
6	36 to 42	156	1.566 ± 0.481	139	3.64 ± 0.89	128	2.91 ± 0.32
7	43 to 49	645	1.527 ± 0.479	604	3.39 ± 0.67	616	2.84 ± 0.29
8	50 to 56	610	1.634 ± 0.507	509	3.38 ± 0.66	609	2.76 ± 0.37
9	57 to 63	983	1.725 ± 0.498	880	3.29 ± 0.79	980	2.68 ± 0.33
10	64 to 70	930	1.839 ± 0.504	901	3.42 ± 0.70	925	2.76 ± 0.29
11	71 to 77	709	1.724 ± 0.519	665	3.41 ± 0.71	667	2.83 ± 0.25
12	78 to 84	539	1.861 ± 0.661	512	3.12 ± 0.90	538	2.73 ± 0.29
13	85 to 91	1028	1.817 ± 0.532	983	3.17 ± 0.79	1028	2.68 ± 0.38
14	92 to 98	927	1.894 ± 0.612	914	3.23 ± 0.67	921	2.73 ± 0.26
15	99 to 105	620	1.952 ± 0.673	620	3.34 ± 0.63	620	2.77 ± 0.28
16	106 to 112	702	1.800 ± 0.609	701	3.29 ± 0.65	701	2.81 ± 0.24
17	113 to 119	866	1.794 ± 0.536	864	3.33 ± 0.66	865	2.76 ± 0.23
18	120 to 126	877	1.816 ± 0.507	875	3.43 ± 0.64	875	2.74 ± 0.24
19	127 to 133	744	1.965 ± 0.5617	742	3.27 ± 0.61	742	2.75 ± 0.19
20	134 to 140	710	1.839 ± 0.561	699	3.28 ± 0.56	699	2.72 ± 0.23
21	141 to 147	602	1.761 ± 0.515	583	3.44 ± 0.57	583	2.80 ± 0.22
22	148 to 154	814	1.794 ± 0.477	805	3.58 ± 0.56	805	2.77 ± 0.20
23	155 to 161	760	1.883 ± 0.538	753	3.35 ± 0.59	753	2.75 ± 0.20
24	162 to 168	682	1.650 ± 0.506	679	3.35 ± 0.56	679	2.75 ± 0.24
25	169 to 175	563	1.591 ± 0.546	546	3.42 ± 0.53	546	2.75 ± 0.23
26	176 to 182	1011	1.594 ± 0.420	996	3.50 ± 0.56	996	2.77 ± 0.22
27	183 to 189	704	1.565 ± 0.419	698	3.44 ± 0.63	698	2.87 ± 0.29
28	190 to 196	659	1.498 ± 0.451	652	3.43 ± 0.58	652	2.90 ± 0.29
29	197 to 203	716	1.440 ± 0.446	704	3.47 ± 0.64	704	2.93 ± 0.28
30	204 to 210	618	1.378 ± 0.411	600	3.59 ± 0.72	600	2.90 ± 0.29
31	211 to 217	369	1.446 ± 0.397	361	3.67 ± 0.58	361	3.00 ± 0.33
32	218 to 224	199	1.494 ± 0.390	198	3.67 ± 0.62	198	3.19 ± 0.64
33	225 to 231	66	1.177 ± 0.538	66	4.20 ± 1.41	66	4.36 ± 2.02
34	232 to 238	109	1.466 ± 0.353	108	3.96 ± 0.96	118	3.48 ± 0.90
35	239 to 244	65	1.459± 0.374	65	4.37 ± 0.83	65	3.48 ± 1.14
36	245 to 251	4	1.667 ± 0.456	4	4.90 ± 0.95	4	3.86 ± 2.40

N – number of observations, s – standard deviation

Table 2: Regression coefficients of lactation curves and goodness-of-fit for milk yield

Regression coefficients	Cubic	Ali-Schaeffer	Guo-Swalve	Wilmink
b_0	0.33685	11.92651	-0.79539	2.16785
b_1	0.03601	-14.28572	0.01855	-0.00320
b_2	-0.00025	3.71046	-0.00017	-3.4936
b_3	0.0000005	-6.18178	0.0000003	
b_4		0.89233	0.47026	
Statistical characteristics		Goodness-of-fit of the model		
R^2	0. 911	0.912	0. 915	0.842
RSD	0.0843	0.0859	0.0833	0.1102
Mean		1.590		
SD		0.2478		

R^2 – coefficient of determination, RSD – residual standard deviation, SD – standard deviation

Table 3: Regression coefficients of lactation curves and goodness-of-fit for fat content

Regression coefficients	Cubic	Ali-Schaeffer	Guo-Swalve	Wilmink
b_0	4.90121	-8.36509	8.41672	2.84866
b_1	-0.03359	15.07721	0.02064	4.42390
b_2	0.00019	-2.67597	-0.00007	0.00412
b_3	-0.0000003	7.61460	0.0000002	
b_4		-1.20193	-1.46013	
Statistical characteristics		Goodness-of-fit of the model		
R^2	0.670	0.699	0.691	0.600
RSD	0.2457	0.2385	0.2416	0.3594
Mean		3.605		
SD		0.5485		

R^2 – coefficient of determination, RSD – residual standard deviation, SD – standard deviation

Table 4: Regression coefficients of lactation curves and goodness-of-fit for protein content

Regression coefficients	Cubic	Ali-Schaeffer	Guo-Swalve	Wilmink
b_0	3.11615	18.04985	6.51895	2.24659
b_1	-0.00203	-25.41579	0.05453	2.57739
b_2	0.00005	11.39490	-0.00031	0.00450
b_3	0.0000003	-9.04542	0.0000008	
b_4		1.55180	-1.41332	
Statistical characteristics		Goodness-of-fit of the model		
R^2	0.713	0.709	0.710	0.426
RSD	0.1952	0.2062	0.2062	0.2797
Mean	2.940			
SD	0.3574			

R^2 – coefficient of determination, RSD – residual standard deviation, SD – standard deviation

as input data of all the models (in total, 518 557 test-day records were taken).

Lactation curves for milk yield, fat and protein content in goats of the White Shorthaired breed in Slovakia plotted on the base of the regression coefficients estimated by the considered models are given in Fig. 1, 2 and 3. The pattern of lactation curves was in accordance to the general pattern of goats (Ciappesoni *et al.*, 2004; Macciotta *et al.*, 2005; Waheed and Khan, 2013) and ewes (Cadavez *et al.*, 2006; Oravcová *et*

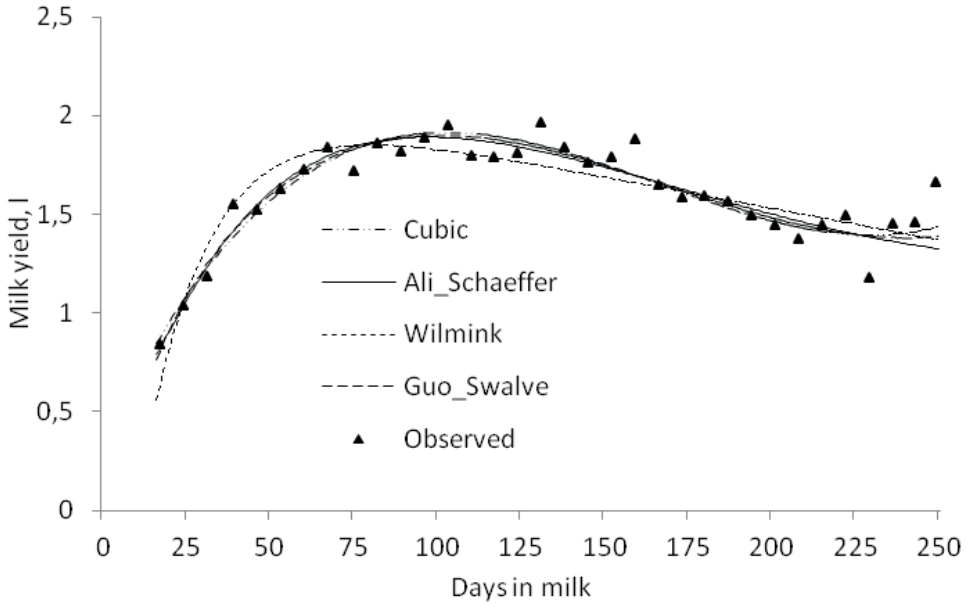


Fig. 1: Lactation curves for milk yield

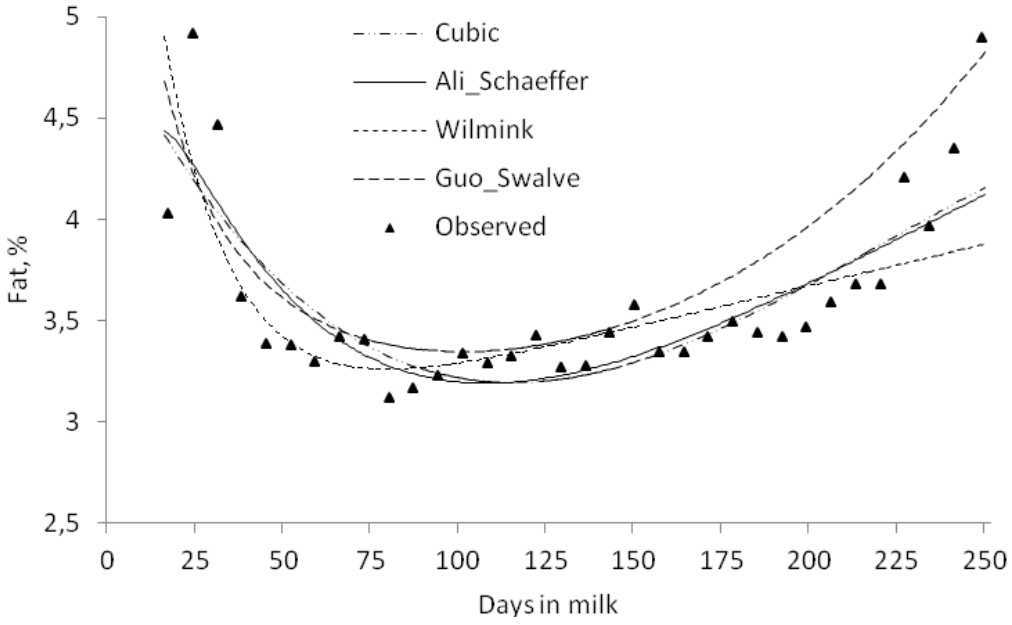


Fig. 2: Lactation curves for fat content

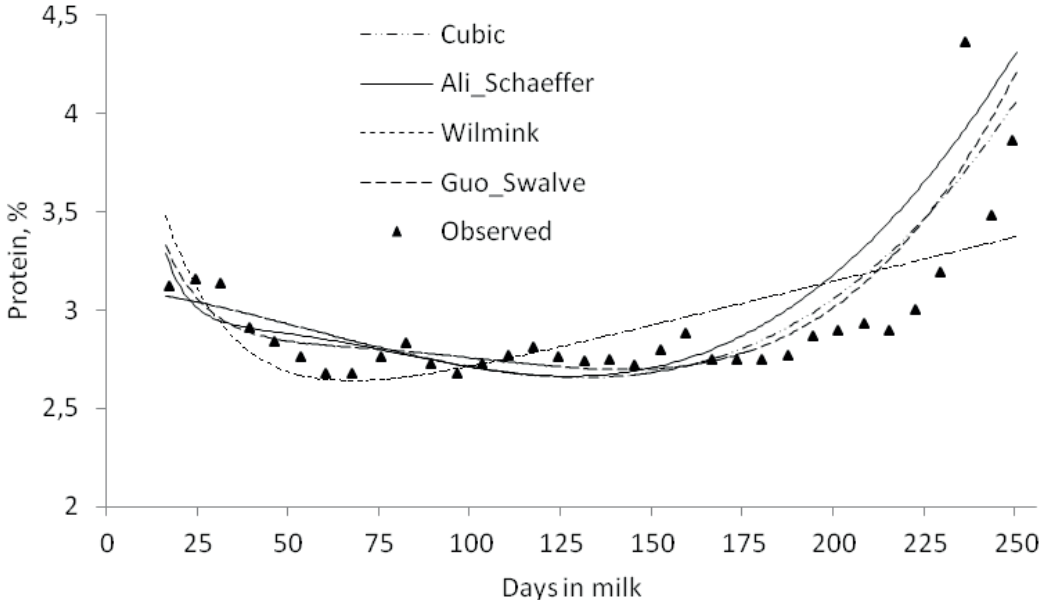


Fig. 3: Lactation curves for protein content

al., 2006 and 2007; Komprej *et al.*, 2012). Milk yield showed an increasing trend from 100 to 130 days in milk and decreased afterwards. At the end of lactation, only Cubic model showed sensitivity to the increase of milk yield (day 240 after parturition), the remaining models followed the decreasing trend of lactation curve (Fig. 1).When Quadratic model was fitted (not shown here), it was similar to Cubic model as far as the coefficient of determination and the residual standard deviation is concerned. The only difference was its insensitivity to the increase of milk yield in day 240 after parturition, the same as was shown by Ali-Schaeffer, Guo-Swalve (modified Khanderkar) and Wilmink models. Lactation curves for fat and protein content (Fig. 2 and 3) showed the opposite trend. These decreased from days 100 to 130 after parturition and increased afterwards. Cubic, Ali-Schaeffer and Guo-Swalve (modified Khanderkar) models were more similar to each other than to Wilmink model.

CONCLUSIONS

This study was the first attempt to estimate lactation curves for milk yield, fat and protein content in White Shorthaired goats in Slovakia. The course of lactation curves followed the pattern which is generally known for ruminants. No remarkable differences in the goodness-of-fit of the models were found (except for Wilmink model), therefore, Cubic, Ali-Schaeffer and Guo-Swalve (modified Khanderkar) models may support the belief that they seem to be useful for modelling of lactation curves in Slovak goats. Further research based on investigations of influence of individual days in milk during the course of lactation and study of effects such as parity, number of kids born, nutrition as well as rearing conditions in individual year and month in which monthly measurements were taken, is needed for better understanding of variation of milk traits in goats. The effect of individual doe is also not negligible.

ACKNOWLEDGEMENT

Thanks are due to the Breeding Services of the Slovak Republic for kind support in making the data available, and the Sheep and Goat Breeders' Association for collaboration. The research supported by the Ministry of Agriculture and Regional Development of the Slovak Republic (RUVV No. 0910503-17-6040001) is gratefully acknowledged.

REFERENCES

AKPA, G.N. – ASIRIBO, E.O. – ONI, O.O. – ALAWA, J.P. 2001. The influence of non-genetic factors on the shape of lactation curves in Red Sokoto goats. *Animal Science*, 2001, 72, p. 233–239.

ALI, T.E. – SCHAEFFER, L.R. 1987. Accounting for covariances among test day milk yields in dairy cows.

Canadian Journal of Animal Science, 1987, 67, p. 637–644.

ÁNGEL MARÍN, P. A. – AGUDELO GÓMEZ, D. A. – RESTREPO, L.F. – CÁNAS ALVAREZ, J.J. – CERÓN-MUNOZ, M. F. 2009. Curvas de lactancia de cabras mestizas utilizando modelos matemáticos no lineáles. [Lactation curves in hybrid goats by the use of nonlinear mathematical models]. Revista lasallista de investigación, 2009, 6, p. 43–49 (in Spanish).

CADAVEZ, V.A. – SILVA, A. – MALOVRH, Š. – KOVAČ, M. 2006. Modelling lactation curves in dairy sheep rose under extensive production system. Proceeding of the 43rd Croatian and 3rd International Symposium on Agriculture, 2006, Opatija, p. 845–848.

CIAPPESONI, G. – PŘIBYL, J. – MILERSKI, M. – MAREŠ, V. 2004. Factors affecting goat milk yield and its composition. *Czech Journal of Animal Science*, 2004, 49, p. 465–473.

ICAR. 2003. International agreement of recording practices, 298 p.

GUO, Z. – SWALVE, H.H. 1995. Modelling of the lactation curves as a sub-model in the evaluation of test day records. Proceedings of the Interbull Annual Meeting, Prague, Czech Republic, September. 7–8. 1995, 4 p.

KOMPREJ, A. – GORJANC, G. – KOMPAN, D. – KOVAČ, M. 2012. Lactation curves for milk yield, fat and protein content in Slovenian dairy breeds. *Czech Journal of Animal Science*, 2012, 57, p. 231–239.

LEÓN, J.M. – MACCIOTTA, N.P.P. – GAMA, L.T. – BARBA, C. – DELGADO, J.V. 2012. Characterization of the lactation curve in Murciano-Granadina dairy goats. *Small Ruminant Research*, 2012, 107 (2), p. 76–84.

MACCIOTTA, N. – FRESI, P. – USAI, G. – CAPPIO-BORLINO, A. 2005. Lactation curves of Sarda breed goats estimated with test day models. *Journal of Dairy Research*, 2005, 72, p. 470–475.

MARGETÍN, M. – MILERSKI, M. 2000. The effect of nongenetic factors on milk yield and composition in goats of Shorthaired breed. *Czech Journal of Animal Science*, 2000, 45, p. 501–509.

ORAVCOVÁ,M.–MARGETÍN,M.–PEŠKOVIČOVÁ, D. – DAŇO, J. – MILERSKI, M. – HETÉNYI, L. – POLÁK, P. 2006. Factors affecting milk yield and ewe's lactation curves estimated with test-day models. *Czech Journal of Animal Science*, 2006, 51, p. 483–490.

ORAVCOVÁ,M.–MARGETÍN,M.–PEŠKOVIČOVÁ, D. – DAŇO, J. – MILERSKI, M. – HETÉNYI, L. – POLÁK, P. 2007. Factors affecting ewe's milk fat and protein content and relationships between milk yield and milk components. *Czech Journal of Animal Science*, 2007, 52, p. 189–198.

PALA, A. – SAVAS, T. 2005. Persistency within and between lactations in morning, evening and daily test day milk yield in dairy goats. Archiv Tierzucht, 2005, 48, p. 396–403.

SAS Institute Inc. *SAS/STAT ® 9.2User's Guide, Second Edition*, 2009. Cary, NC: SAS Institute Inc.

WAHEED, A. – KHAN, M. S. 2013. Lactation curve of Beetal goats in Pakistan. *Archiv Tierzucht*, 2013, 56. DOI: 10.7482/0003-9438-56-089.

WILMINK, J.B.M. 1987. Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. *Livestock Production Science*, 1987, 16, p. 335–348.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 48, 2015 (1): 8-15

ISSN 1337-9984

EFFECT OF *IN OVO* ADMINISTRATION OF BUTYRIC ACID INTO BROILER BREEDER EGGS ON CHICKEN SMALL INTESTINE PH AND MORPHOLOGY

A. SALAHI[1]*, S. G. ADABI[2], M. M. KHABISIP[3], A. ANISSIAN[4], R. G. COOPER[5]

[1]Raam Toyour Company, Tehran, Iran
Tel.: (+98) 09131434146. Fax: (+98) 02166942030. E-mail: ahmad.salahi2010@gmail.com
[2]Faculty of Agriculture, Department of Animal Science, Ankara University, Ankara, Turkey
Email: adabishahram@gmail.com
[3]Department of Animal Science, Islamic Azad University, Kahnooj Branch, Kahnooj, Kerman , Iran
E-mail: m_sheflera@yahoo.com
[4]Department of Veterinary, Islamic Azad University, Abhar Branch, Abhar, Zanjan, Iran
E-mail: Dr_anissian@yahoo.com
[5]Birmingham City University, Birmingham, UK. E-mail: rgcooperuk@yahoo.com

ABSTRACT

This experiment was conducted to evaluate effects of *in ovo* butyric acid (BA) administration into broiler eggs on chicken small intestine pH and morphology. 480 fertile eggs were obtained from Ross broiler breeder (45 wk) and divided into 3 treatments with 4 replicate and 160 eggs per treatment. On the 18th day of incubation, 1 ml of *in ovo* solution was injected into amniotic fluid. Treatments were including 0.3 % BA solution, 0.9 % NaCl solution and control group. For pH and intestinal morphometric examination, 4 chicks per replicate were euthanized. The results showed that effect of BA injection on jejunum ($p < 0.01$) and ileum pH ($p < 0.05$) on hatch day was significant. Jejunum villi height increased ($p < 0.05$) on the 7th day compared with the control group. The highest ileum villi was observed following the BA injection ($p < 0.01$). It can be concluded that BA injection affects small intestine morphology and increases body weight of chicks.

Key words: *in ovo* injection, butyric acid, pH, small intestine morphology

INTRODUCTION

Butyric acid (CH3CH2CH2COOH; BA) is a medium-chain fatty acid that is absorbed from first section of gastrointestinal.

Pryde *et al*. (2002) reported that BA can be used as an energy source for growth and development of intestine epithelial cells in human. There is not adequate production of BA in the intestine of chick and it is related to the incomplete establishment of gastrointestinal (GI) microbes in the early post-hatch (Hosseini Mansoub *et al.*, 2011). Also the level of short-chain fatty acids in chicken caecum and intestine is very low at early period of life. Dietary supplementation of BA had positive effects on the regulation of intestinal bacterial populations (Bolton and Dewar, 1965) and improved broiler performance and carcass parameters (Leeson *et al*., 2005). It has been shown that BA injection had a significant differences in chick length on hatch day ($P < 0.05$); body weight was increased significantly at the 10th day post hatch by injection of the 0.3 % BA glyceride solution ($P < 0.05$) (Salahi *et al*., 2011). Application of BA (3 g.kg^{-1}) increased chicken small intestine length compared with the control group (Mahdavi and Torki, 2009). BA consumption in chickens had stimulating effects on the intestinal villi (Hosseini Mansoub *et al*., 2011). Addition of 0.2 % mixed triglyceride of BA (mono, two and triglycerides) decreased villi length, increased microvilli length and crypt depth of jejunum (Antongiovanni *et al*., 2007). Application of organic acid in broiler diets decreased intestinal pH. Intestinal pH reduction had beneficial effect on the decreasing of the harmful intestinal bacteria hereby decreasing bacterial fermentation and improving protein and energy digestibility (Adil *et al*., 2011). When pH of digesta was reduced, the pancreatic secretion was increased, and had trophic effects on the mucosa of gastro-intestinal tract. Short chain fatty acids can accelerate gut epithelial cell proliferation, thereby increasing intestinal tissue weight,

***Correspondence:** e-mail: ahmad.salahi2010@gmail.com
Ahmad Salahi, Raam Toyour Company, Tehran, Iran
Tel.: (+98) 09131434146, Fax: (+98) 02166942030

Received: December 13, 2013
Accepted: February 13, 2015
Amended: May 19, 2017

which will result in changes of mucosal morphology and the gastro-intestinal cell proliferation. Based on beneficial effects of BA on broilers' performance we thought that *in ovo* administration of BA could improve the nutritional status during last period of embryonic development and first week after hatch. Therefore, the objective of the study was to determine the effects of dietary administration of BA (HYDRO C4-30) (SILOSPA, Italy) on intestine morphology and pH during last days of incubation and early phase of chicken growth.

MATERIAL AND METHODS

1. Experimental groups

480 fertile eggs were obtained from a 45 wk breeder flock (Ross 308) and then allocated to three treatment groups. These groups included as follows: I (control, non-injected), II (*in ovo* injection of Nacl 0.9 % solution, positive control) and III (*in ovo* injection of 0.3 % BA glyceride solution in 0.9 % saline). Experiments were performed in 4 replicate (40 eggs per replicate) so that totally 160 eggs (66.5 ± 0.5 g) per treatment were used.

2. Butyric acid solution

BA solution used in the experiment was Monobutyrin-Hydro C4-30 (HYDRO C4-30) liquid, water soluble product. The Hydro C4 composition assigned by manufacturer was 47–53 % BA glycerides, 42–46 % free glycerol, 28–32 % BA and 0.5–1 % moisture. pH and osmolarity were 6–7 and 0.017–0.028 mol L^{-1} (0.2–0.33 % solution), respectively and the recommended Hydro C4 in broiler drinking water were 0.2 % to 0.33 % from 0 to 21 days of age (Uni and Ferket, 2003).

3. Incubation and hatching

After 6 hours of injection, the eggs were transferred to hatcher and were set in hatching trays horizontally. Trays were covered by mesh wire to avoid mixing of chicks between adjacent compartments. The hatcher (*Petersime* model 192, analog) temperature and relative humidity were 37.5 °C and 50 %, respectively (Salahi *et al.*, 2011). Chicks were taken-off from hatcher at 508 hours of incubation when 5 % of chicks were still damp at the back of the neck (Kawalilak *et al.*, 2010).

4. *In ovo* injection procedure

Prior to injection, all eggs were candled for examination of embryo position, amniotic fluid and embryo movement, also a Coumassie blue dye solution was injected into the 480 eggs to ensure that the solution is administrated into amniotic fluid. After examination the eggs were *in ovo* injected using 22 needle-gauge at 453 h (18.8 d) of incubation. Solution volume for each injection was 1 ml and needle depth was 2.54 cm (1 inch) (Zhai *et al.*, 2008). After injection, the site of injection was sanitized with ethanol 70 % (Ohta *et al.*, 1999) and sealed with a liquid adhesive.

5. Birds housing

Hatched chicks were raised on floor pens until 10 days. Birds of each pen had *ad libitum* access to similar diet and water. Chicks were raised under similar environmental conditions according to broiler breeder recommendation (Aviagen, 2009) and allocated to pens at 10 m^2 stocking density. Light intensity was maintained 30–40 Lux and 23L: 1D h photo schedule was applied throughout the experiment. No vaccine or drug antibiotics were used during 10 days rearing period.

6. Collection of samples

Upon hatch, 4 chicks were randomly selected and euthanized by CO_2 asphyxiation (Salahi *et al.*, 2011). In this study all chicks were sampled on hatch day (DOH), the 3rd day (D3) and the 7th day post-hatch. The gut samples were taken from the following 3 sites: duodenum (from gizzard until the end of duodenal loop), jejunum (from the end of duodenal loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to ileo-ceacal junction) (Aviagen, 2009).

7. Processing of Samples and Intestinal Morphology Examination

After opening the abdominal area, the central segment of each sample was extracted and flushed with 0.85 % normal saline (sterile water) to remove the intestinal content (Wang and Peng, 2008); tissue samples (approximately 3 and 2 cm of jejunum and ileum) were obtained from the midpoints of these segments. Furthermore, tissue samples were separated and post fixed in 10 % neutral buffered formalin for histological evaluation (Buchanan *et al.*, 2010). Samples were fixed for 72 h (Kawalilak *et al.*, 2010) and stored in a 70 % alcohol solution until further processing. Each sample was dehydrated, cleared and embedded into paraffin (Wang and Peng, 2008); one 5 μm section of each sample was placed on a glass slide and stained with hematoxylin-eosin and periodic acid-Schiff (PAS) stain. These sections were deparaffinized in xylene and rehydrated in a graded alcohol series (Wang and Peng, 2008). Eight samples obtained for each intestinal tissue and 2 cross-sections were prepared for evaluation. Examination and assays were done under a light microscope (Micros MCX300), photographs were taken of the transverse sections and analyzed using *Ivemp* Image Tool software. Measurement by the software was done on 7 villi per sampled chick (Aptekmann *et al.*, 2001). First, we looked for the highest villi (Kawalilak *et al.*, 2010) and then, selected neighbor of this villi from left side. Pictures of small intestine

villi were acquired under a light microscope with 10× magnification. Villus height was measured from the tip of the villi to the crypt area mouth (Buchanan *et al.*, 2010; Smulikowska *et al.*, 2009) villus-crypt junction (Wang and Peng, 2008) or the top of the *lamina propria* (Aptekmann *et al.*, 2001). Another morphometric variables were included; villus width was measured at half height in the middle of its length) Buchanan *et al.*, 2010; Smulikowska *et al.*, 2009). The crypt depth was defined as from the base upwards to the region of transition between the crypt and villus (from crypt mouth to base) (Aptekmann *et al.*, 2001; Smulikowska *et al.*, 2009). Villus apical width at the villus tip, basally to the crypt-villus junction, was measured, then villus surface area was estimated by the following mathematical formula; [((villus tip + villus base/ 2)× villus height [((Incharoen and Yamauchi, 2009). Finally, villus height: crypt depth ratio and crypt: villus height ratio were calculated.

8. pH Measurement

pH was measured in jejunum and ileum segments of the gastrointestinal tract (GIT) of broiler chickens at different ages including the 3rd and 7th day of the post-hatch .In this study we measured pH using Ex-situ protocol (inserting the pH probe). Then intestinal contents collected and transferred into 3 ml of distilled water (Chaveerach *et al.*, 2004). pH measurement was done by PH/°C meter (A∂WA AD 110, Romania) and sensor was placed for 20 second into the solution.

9. Statistical Analysis

The experiment had a completely randomized design with 3 treatments; each of them applied to the same number of units. The data were subjected to statistical analysis based on 4 replications per treatment. One-way ANOVA was performed to examine differences among groups by using the SAS software (SAS Institute Inc, 2004). The means of variables were compared using Duncan's multiple range test.

RESULTS

Results of this study showed that effect of *in ovo* injection of different treatments on the pH of jejunum was significant on hatch day (p <0.01) so the lowest pH was observed by injection of 0.3 % BA .Jejunum pH at the 3rd and 7th days after the hatch was not affected by different treatments. Also ileum pH at the hatch day was affected by different treatments (p <0.05) and the lowest pH was observed in ileum by injection of 0.3 % BA (Table 1). Jejunum villi height was affected at the hatch day (p <0.001) and the maximum jejunum villi height was observed on the control of the group. Also jejunum villi height on the 7th day of the post-hatch was affected by different treatments (p <0.05), so the maximum height was observed in the Nacl and BA injection groups, respectively. Jejunum villi width on the 3rd and 7th days of the post-hatch on BA injection treatment was higher than in other groups, although this effect was not statistically significant. Jejunum crypt depth at hatch day and the 3rd day after the hatch on BA injection group were larger than in other groups, although this effect was not significant (Table 2) too. The ileum villi height on the hatch day was the impact of different treatments (p <0.01) and the maximum height was observed in the BA treatment. Ileum villi width on the 7th day of the post-hatch in BA injection group was more than in other groups, also this difference was not significant too. However, ileum crypt depth on the 3rd and 7th days of the post-hatch in the BA treatment were larger than in other groups, differences were observed between treatments, but this effect was

Table 1: Effects of BA injection on small intestine pH

Treatment	Control	NaCl 0.9 %	BA 0.3 %	SEM	*p*-value
			Jejunum pH		
Hatch day	6.9[a]	6.8[b]	6.7[c]	0.04	0.01
3 Day	5.3	4.9	4.7	0.26	0.28
7 Day	5.67	5.58	5.8	0.21	0.59
			Ileum pH		
Hatch day	7.06[ab]	7.2[a]	6.8[b]	0.09	0.05
3 Day	5.91	5.96	5.93	0.13	0.67
7 Day	5.85	5.79	6.02	0. 15	0.68

[a–c] within main effects, values followed by different letters within columns are significantly different (P <0.05*); SEM – Standard Error of Mean

Table 2: Effects of BA injection on jejunum morphology

Treatment	Control	NaCl 0.9 %	BA 0.3 %	SEM	*p*-value
Jejunum Villus height (µm)					
Hatch day	473[a]	460[b]	458[c]	8	0.001
3 Day	963	906	888	76	0.73
7 Day	1 231[b]	1617[a]	1426[ab]	83	0. 02
Jejunum villus width (µm)					
Hatch day	130	114	96	12.1	0.18
3 Day	167	153	168	20.5	0.72
7 Day	188	211	237	19	0.46
Jejunum Crypt depth (µm)					
Hatch day	147	161	158	16.4	0.81
3 Day	228	248	257	17.1	0.64
7 Day	383	324	344	26.4	0.13

[a–c] within main effects, values followed by different letters within columns are significantly different ($P < 0.05$*); d – day; J – Jejunum

Table 3: Effects of BA injection on ileum morphology

Treatment	Control	NaCl 0.9 %	BA 0.3 %	SEM	*p*-value
Ileum villus height (µm)					
Hatch day	378[c]	409[b]	414[a]	6	0. 01
3 Day	597	600	602	33	0. 84
7 Day	955	937	1 064	76	0. 53
Ileum villus width (µm)					
Hatch day	109	100	101	7.6	0.34
3 Day	159	144	156	13.7	0.38
7 Day	214	200	238	24	0.55
Ileum Crypt depth (µm)					
Hatch day	153	171	176	12.1	0.65
3 Day	222	210	233	21. 8	0.74
7 Day	267	291	297	22.6	0.46

[a–c] within main effects, values followed by different letters within columns are significantly different ($P < 0.05$*)

not statistically significant (Table 3). Jejunum villi surface area was not affected by different treatments. Ileum villi area on the hatch day and the 7th day of the post-hatch on BA injection treatment were larger than in other groups, but there was no significant difference between treatments. Crypt to villi ratio in the jejunum on the 7th day of the post-hatch was influenced by different treatments ($p < 0.05$) and in control, and BA groups were more than Nacl group, but this was not significant. Crypt to villi ratio in ileum on the hatch, 3rd and 7th days of the post hatch were not affected by different treatments (Table 4).

Table 4. Effects of BA injection on Villus Area and Crypth to villi ratio

Treatment	Control	NaCl 0.9 %	BA 0.3 %	SEM	*p*-value
Jejunum Villus Surface Area (μm^2)					
Hatch day	25 833	23 953	22 561	3 800	0. 84
3 Day	51 223	50 832	49 787	8 100	0. 98
7 Day	82 369	112 162	102 796	12 600	0. 74
Ileum Villus Surface Area (μm^2)					
Hatch day	14 177	14 318	15 934	943	0.44
3 Day	37 291	36 781	34 092	636	0.74
7 Day	70 640	72 230	79 520	515	0.62
Jejunum Crypt to villi ratio					
Hatch day	0.29	0.33	0.42	0.05	0.21
3 Day	0.24	0.27	0.29	0.02	0.35
7 Day	0.31^a	0.21^b	0.24^{ab}	0.02	0.03
Ileum Crypt to villi ratio					
Hatch day	0.41	0.43	0.42	0.06	0.65
3 Day	0.38	0.33	0.38	0.04	0.61
7 Day	0.28	0.32	0.28	0.03	0.46

$^{a–b}$ within main effects, values followed by different letters within columns are significantly different (P <0.05*)

DISCUSSION

1. pH of intestine

Our findings showed that injection of BA affected the pH of jejunum and ileum on the hatch day with a decrease in the pH of the jejunum. Many studies showed that organic acids have effects on the pH of gastro-intestinal tract (GIT). These studies reported the inclusion of organic acids in the diets of broiler chickens diminished pH of the GIT (Adil *et al.*, 2008) and crop, but no effect on the caeca (Bolton and Dewar, 1965). pH variability between birds was high, but standard deviation between segments was low (Angel *et al.*, 2010). These observations were consistent with Paul *et al.* (2007). This is resulted from strong buffering action of the GI tract in poultry. In the present study, we found no significant effects of BA on pH at the 3rd and 7th days. Therefore, age may not have this significant effect on pH of the ileum and jejunum at the 3rd and 7th day in broilers. The finding is in agreement with Angel *et al.* (2010), who suggested that age had no effects on pH at days 5, 18, 46. They reported the flowing pH for different segments of the GIT: duodenum (5.99, 5.87), jejunum (6.07, 5.97), ileum pH (7.12, 7.15), crop (5.32, 5.85), gizzard (2.37, 2.64, 2.04) and proventriculus (2.14, 2.50, 2.36). Gastric reducing pH increases activity of pepsin, and pepsin proteolysis may cause an increase in peptides. This results in the release of hormones including gastrin and cholecystokinin, (CCK) and these hormones can regulate protein digestion and absorption physiological regulators of feeding, feeding behavior and emotional responses in the central nervous system by affecting gallbladder contractions, exocrine pancreatic secretions and gastric acid secretions in the GIT (Noble and Roques, 1999). Also CCK modulated gastro-duodenal and intestinal motility in chickens (Martinez *et al.*, 1995). Another advantage of reducing pH was direct antimicrobial effects on intestinal bacteria by two methods: penetrating the bacteria cell wall and disrupting the normal physiology of certain types of bacteria when it was used in non-dissociated (non-ionized, more lipophilic) form and reducing bacterial competition with the host for available nutrients and diminution in the level of toxic bacterial metabolites as a result of lessened bacterial fermentation which results in the improvement of protein and energy digestibility, thereby improving the weight gain and performance of broiler (Adil *et al.*, 2011). These effects will ultimately result in a better performance.

2. Jejunum Morphology

Jejunum villi height was affected at the hatch (P <0.001) and different treatments affected (P <0.05) the

height at the 7th day of the post-hatch. This finding is an agreement with other experimental studies. Addition of 1 % of organic acid mixture significantly increased villus height, compared with 0 and 0.5 % addition of organic acid mixture at the 21st day of age (P <0.05) (Saki *et al.,* 2011). Dietary supplementation of organic acids significantly (P <0.05) increased the villus height in the duodenum and jejunum and the highest duodenal and jejunal villus heights were observed in bird fed diets supplemented with 3 % BA and 3 % fumaric acid (Smulikowska *et al.*, 2010). Jejunal villus height was higher (P <0.05) in formate + propionate supplemented group. This result revealed that combination of supplemental acidifier improved or maintained similar villus height when compared with antibiotic supplementation. The increase in villus height of different segments of the small intestine may be attributed to the fact that the intestinal epithelium acts as a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen (Paul *et al.*, 2007). The villus height in the BMD (bacitracin methylene disalicylate) group of birds was higher than the rest of the treatment group in the jejunum where maximum digestion and absorption takes place as there is a large luminal site, and more mature enterocytes are present. The higher villus height coupled with a higher Lactobacillus count caused a better body weight and FCR in the organic acid blend dietary groups. In the present study, jejunum villi width between the 3rd and 7th days of the post-hatch in butyric acid injection treatment was higher than in other groups, although this effect was not statistically significant. These effects, especially the observed increase in duodenal villous length, are potentiated when the probiotics are used and antibiotics induce enlargement of villous length and width (Markovic *et al.*, 2007). We also observed that jejunum crypt depth on the day of hatch and the 3rd day after the hatch in BA injection group was higher than in other groups, although this effect was not significant. Crypt depth was increased by addition of 0.5 and 1 % of organic acid on the 21st and 42nd days of age (P < 0.05) (Saki *et al.*, 2011). Effects of organic acid on the crypt depth in the duodenum and jejunum did not differ among different treatment groups (Smulikowska *et al.*, 2010). The crypt depth in the duodenum and jejunum was not affected among different treatment groups (Adil *et al.*, 2010). The crypt can be regarded as the villus factory and a large crypt indicates rapid tissue turnover and a high demand for new tissue (Choct, 2009). *In ovo* injection of BA proved that jejunum villi surface area was not affected by different treatments. Crypt to villi ratio in the jejunum on the 7th day of the post-hatch was influenced by different treatments (p <0.05) and in control and butyric acid injection group more than Nacl injected group, but this was not significant. This finding is in contrast with Saki *et al*. (2011) who found that villus surface was significantly increased by addition of 1 % of organic acid mixture compared with 0 % level on the 21st day of age. These histological changes probably had increased the intestinal surface area, facilitating the nutrient absorption to a greater extent and boosted the growth promoting effect of the organic acid supplementation (Smulikowska *et al.*, 2010). It is well known that the presence of increased villus height suggests an increased surface area capable of greater absorption of nutrients (Caspary, 1992).

3. Ileum Morphology

The ileum villi height on the hatch day was affected by different treatments (P <0.01) and the maximum height was observed in the treatment with BA injection. Ileum villi width on the 7th day of the post-hatch in butyric acid injection group was higher than in other groups and this difference was not significant. In other studies, dietary supplementation of organic acids increased the villus height in the ileum but the values were significant (P <0.05) (Smulikowska *et al.*, 2010). Addition of organic acids increased villus height in the small intestine but the differences were not significant in case of the ileum (Adil *et al.*, 2010). The highest ileal villus heights were recorded in the bird fed diets supplemented with 2 % fumaric acid (Smulikowska *et al.*, 2010). More effects on ileum villi height were observed in organic acid levels especially at 1 %, which resulted in an increase in lactic acid bacteria and decrease in Enterobacteriaceae counts in the ileum of broiler chicken (P <0.05) (Saki *et al.*, 2011). We found that ileum crypt depth on the 3rd and 7th days of the post-hatch in the BA treatment was higher than in other groups but differences between treatments were not significant. Using of the organic acid did not result in any effect on the crypt depth in the ileum among different treatment groups (Smulikowska *et al*., 2010). The crypt depth in the ileum was not affected among different treatment groups (Adil *et al*., 2010). In the current study, ileum villi area on the hatch day and the 7th day of the post-hatch on butyric acid injection treatment was higher than in other groups, but there was no significant difference. Dunham *et al.* (1993) presented similar conclusions in domestic fowl fed a diet supplemented with L. Reuteri (probiotics) which had longer villi and lower crypts in ileum compared to the controls. The ileal crypt depth was decreased by synbiotic supplementation (117 ± 2 μm) compared with control (128 ± 2 μm); the addition of synbiotic increased (P <0.001) the villus height/ crypt depth ratio and villus height in ileum compared with control (Awad *et al.*, 2008). Our findings showed that crypt to villi ratio in ileum on the hatch day, 3rd and 7th days of the post-hatch was not affected by different treatments. It can be concluded that BA injection during embryonic period via egg can increase the absorption

area by improving the small intestine morphology, so that it may have effect on the increase in body weight. Also pH of jejunum and ileum at hatch day was affected by *in ovo* injection and the lowest pH was observed in ileum and Jejunum by injection of 0.3 % BA.

REFERENCES

ADIL, S.H. – TUFAIL, B. – GULAM, A.B. – MASOOD, S.M. – MANZOOR, R. 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology and serum biochemistry of broiler chicken. *Veterinary Medicine International*, vol. 1 (1), 2010, p. 479–485.

ADIL, S.H. – TUFAIL, B. – GULAM, A.B. – QURESHI, S.D. – WANI, S.A. 2011. Effect of supplemental organic acids on growth performance and gut microbial population of broiler chicken. *Livestock Research Rural Development*, vol. 23 (6), 2011.

ANGEL, R. – HUMPHREY, B. – SAYLOR, W.W. 2010. Age changes in gastrointestinal pH in broilers. *Poultry Science*. 2010. http://www.jtmtg.org/2010/abstracts/0650.pdf

ANTONGIOVANNI, M. – BUCCIONI, A. – PETACCHI, F. – LEESON, S. – MINIERI, S. – MARTINI, A. – CECCHI, R. 2007. Butyric acid glycerides in the diet of broiler chickens: effects on gut histology and carcass composition. *Italian Journal of Animal Science*, vol. 6, 2007, p. 19–25.

APTEKMANN, K.P. – BARALDI ARTON, S.M. – STEFANINI, M.A. – ORSI, M.A. 2001. Morphometric analysis of the intestine of domestic quails (*Coturnix coturnix japonica*) treated with different levels of dietary calcium. *Anatomy Histology Embryology,* vol. 30, 2001, p. 277–280.

AVIAGEN. 2009. Ross broiler management manual. Scotland, UK. 2009; 1, 2009, p. 1–114.

AWAD, W.A. – GHAREEB, K. – BÖHM, J. 2008. Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing Enterococcus faecium and oligosaccharides. *International Journal of Molecular Science*, 2008, vol. 9, 2008, p. 2205–2216.

BOLTON, W. – DEWAR, W.A. 1965. The digestibility of acetic, propionic and butyric acids by the fowl. *British Poultry Science*, vol. 6, 1965, p. 103–105.

BUCHANAN, N.P. – LILLY, K.G.S. – MORITZ, J.S. 2010. The effects of diet formulation, manufacturing technique, and antibiotic inclusion on broiler performance and intestinal morphology. *Applied Poultry Research Journal*, vol. 19 (2), 2010, p. 121–131.

CASPARY, W.F. 1992. Physiology and pathophysiology of intestinal absorption. *American Journal Clinical Nutrition*, vol. 55, 1992, p. 299–308.

CHAVEERACH, P. – KEUZENKAMP, D.A. – LIPMAN, L.J. – VAN KNAPEN, F. 2004. Effect of organic acids in drinking water for young broilers on campylobacter infection, volatile fatty acid production, gut microflora and histological cell changes. *Poultry Science*, vol. 83, 2004, p. 330–334.

CHOCT, M. 2009. Managing gut health through nutrition. British Poultry Science Science, vol. 7, 2009, p. 9–15.

DUNHAM, H.J. – WILLIAMS, C. – EDENS, F.W. – CASAS, I.A. – DOBROGOSY, W.J. 1993. Lactobacillus reuteri immunomodulation of stressor-associated diseases in newly hatched chickens and turkeys. *Poultry Science,* vol. 103 (Suppl. 2), 1993.

HOSSEINI MANSOUB, N. – RAHIMPOUR, K. – MAJEDI, ASLL. – MOHAMMAD, M. – NEZHADY, A. – ZABIHI, S.L. – MOHAMMADI KALHORI, M. 2011. Effect of different level of butyric acid glycerides on performance and serum composition of broiler chickens. *Journal of World Zoology*, vol. 6 (2), 2011, p. 179–182.

INCHAROEN, T. – YAMAUCHI, K. 2009. Production performance, egg quality and intestinal histology in laying hens fed dietary dried fermented ginger. *International Journal of Poultry Science*, vol. 8 (11), 2009, p.1078–1085.

KAWALILAK, L.T. – ULMER FRANCO, A.M. – FASENKO, G.M. 2010. Impaired intestinal villi growth in broiler chicks with unhealed navels. *Poultry Science*, vol. 89, 2010, p. 82–87.

LEESON, S. – NAMKUNG, H. – ANTONGIOVANNI, M. – LEE, E.H .2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. *Poultry Science*, vol. 84, 2005, p. 11418–1422.

MAHDAVI, R. – TORKI, M. 2009. Study on usage period of dietary protected butyric acid on performance, carcass characteristics, serum metabolite levels and humoral immune response of broiler chickens. *Journal of Animal Veterinary Advance*, vol. 8 (9), 2009, p. 1702–1709.

MARKOVIC, R. – ŠEFER, D. – KRSTIC, M. – PETRUJKIC, B. 2009. Effect of different growth promoters on broiler performance and gut morphology. *Archive Medicine of Veterinary*, vol. 41, 2009, p. 163–169.

MARTINEZ, V. – JIMENEZ, M. – GONALONS, E. – VERGARA, P. 1995. Modulation of the migrating myoelectric complexes by cholecystokinin and gastrin in the gastrointestinal tract of chickens. *Poultry Science*, vol. 74, 1999, p. 563–576.

NOBLE, F. – ROQUES, B.P. 1999. CCK-B receptor Chemistry, molecular biology, biochemistry and pharmacology. *Progress in Neurobiology*, vol. 58, 1999, p. 349–379.

OHTA, Y. – TSUSHIMA, N. – KOIDE, K. – KIDD, M.T. – ISHIBASHI, T. 1999. Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. *Poultry Science*, vol. 78, 1999, p. 1493–1498.

PAUL,S.K.–SAMANTA,G.–HALDER,G.–BISWAS, P. 2007. Effect of a combination of organic acid salts as antibiotic replacer on the performance and gut health of broiler chickens. *Livestock Research Rural Development*, vol. 19 (11), 2007.

PRYDE, S.E. – DUNCAN, S.H. – HOLD, G.L. – STEWART, C.S. – FLINT, H.J. 2002. The microbiology of butyrate formation in the human colon. *FEMS Microbiology Letter*, (217), p.133–139.

SAKI, A.A. – EFTEKHARI, S. M. – ZAMANI, P. – ALIARABI, H. – ABBASINEZHAD, M. 2011. Effects of an organic acid mixture and methionine supplements on intestinal morphology, protein and nucleic acids content, microbial population and performance of broiler chickens. *Animal Production Science*, vol. 51 (11), 2011, p.1025–1033.

SALAHI, A. – MOUSAVI, S.N. – FOROUDI, F. – MOOSANEZHAD KHABISI, M. – NOROZI, M. 2011. Effects of *in ovo* injection of butyric acid in broiler breeder eggs on hatching parameters, chick quality and performance. *Global Veterinaria*, vol. 7 (5), 2011, p. 468–477.

SAS Institute Inc.2004. SAS/STAT User's Guide, Release 9.1 ed. Cary, NC. 2004.

SMULIKOWSKA, S. – CZERWIŃSKI, J. – MIECZKOWSKA, A. – JANKOWIAK, J. 2009. The effect of fat-coated organic acid salts and a feed enzyme on growth performance, nutrient utilization, microflora activity and morphology of the small intestine in broiler chickens. *Journal of Animal and Feed Science,* vol. 18, 2009, p. 478–489.

SMULIKOWSKA, S. – CZERWIŃSKI, J. – MIECZKOWSKA, A. 2010. Effect of an organic acid blend and phytase added to a rapeseed cake-containing diet on performance, intestinal morphology, caecal microflora activity and thyroid status of broiler chickens. *Journal of Animal Physiology and Animal Nutrition*, vol. 94 (1), 2010, p. 15–23.

UNI, Z. – FERKET, P.R. 2003. Enhancement of development of oviparous species by *in ovo* feeding. North Carolina State Univ., Raleigh and Yissum Res. Dev. Co. of the Hebrew Univ. Jerusalem, Israel. *US Regular Patent US* 6, 592, 878, vol. 1, 2003, p. 1–12.

WANG, J.X. – PENG, K. 2008. Developmental morphology of the small intestine of African ostrich chicks. *Poultry Science*, vol. 87, 2008, p. 2629–2635.

ZHAI, W. – NEUMAN, S. – LATOUR, M.A. – HESTER, P.Y. 2008. The effect of *in ovo* injection of L-Carnitine on hatchability of white leghorns. *Poultry Science*, vol. 87, 2008, p. 569–572.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 48, 2015 (1): 16–22

ISSN 1337-9984

GROWTH PERFORMANCE, CARCASS YIELD AND ORGAN WEIGHT OF GROWING PIGS FED DIFFERENT LEVELS OF FEED

C. P. NJOKU*, O. A. ADEYEMI, O. M. SOGUNLE, A. B. J. AINA

University of Agriculture, Department of Animal Production and Health, Abeokuta, Nigeria

ABSTRACT

A total of forty eight Large White weaner male pigs of 8 weeks old with average initial weight of 9.67±0.26 were used in a 150-day trial to determine the effect of feed quantity offered (1.5, 2.0 or 2.5 kg) on performance, carcass yield and organ weights of growing pigs. Final weight, daily feed intake and daily weight gain increased (P <0.05) significantly with increase in feed quantity offered. Feed conversion ratio, daily water intake and frequency of faecal excretion decreased significantly (P <0.05) with increasing feed quantity offered. Carcass weight and dressing percentage were significantly (P <0.05) influenced by feed quantity offered. The backfat depth increased significantly (P <0.05) with increase in feed quantity offered. Pigs fed 2.5 kg feed daily had higher (P <0.05) head, ham and shoulder compared to the values recorded for pigs fed 1.5 kg feed daily. Liver weights of pigs fed 2.0 and 2.5 kg feed daily had comparably similar values which differed significantly from those fed 1.5 kg feed daily. These results showed that quantity of feed offered greatly influenced feed intake, weight, carcass yield, liver and heart of growing pigs, hence, it could be used as a management tool to improve performance and carcass yield of pigs.

Keywords: feed quantity; performance; carcass yield; organs; growing pigs

INTRODUCTION

Profitability of pig enterprise depends on efficient use of feed for lean tissue growth and the rate of growth. Growth rate and nutritional requirement of pigs are two essential factors necessary for maximum pork productivity. An ideal nutritional programme should provide adequate nutrients to maximize pig productivity while minimizing excreted nutrients and feed costs. Since 75 % of total feed used in a farrow-finish operation is consumed in the grower-finisher phase (Edwards, 2010), nutritional accuracy in this phase has a substantial economic impact. Insufficient feed intake (quantitative and qualitative) has negative impact on the performance of pigs, hence, leading to higher maintenance cost. Fat deposition occurs when feed intake exceeds the rate at which maximum lean growth is achieved.

The meat industry requires animals to be as lean as possible since pork with low fat content reduces human caloric intake and intramuscular fat is related to lower sensory quality traits (Fernandez *et al.*, 1999). High level of carcass fat is therefore unacceptable because of the associated health problems. Under tropical conditions, it is therefore logical to adopt a system of feeding that promotes feed intake and lean tissue growth. High temperature leads to decrease in voluntary feed intake, and hence a reduction in growth rate. The *ad libitum* feeding, particularly, if involves feeds of high energy density, tends to promote synthesis of body fat which is inefficient in terms of feed conversion. Restricted feed allowance reduces back fat thickness and intramuscular fat content (Gondret and Lebret, 2002) resulting to acceptable carcass grading. Although progress has been made in swine nutrition in the last 30 years, there is still a need for more information relative to the various methods of feed management practices in swine production. The present study therefore seeks to completely define the levels of feeding necessary for

***Correspondence:** e-mail: ncfred0859@yahoo.co.uk
Chiemeka Promise Njoku (Ph.D), University of Agriculture, Department of Animal Production and Health, Abeokuta, Nigeria
phone: +23480 34 64 77 41, +23480 23 74 11 45
Received: July 18, 2014
Accepted: February 13, 2015

lean meat productivity and for minimizing feed waste especially in a humid tropical environment.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Piggery Unit of the Teaching and Research Farms Directorate (TREFAD), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The farm lies within latitude 7° 10′ N, longitude 3° 2′ E and altitude 76 mm. It is located in the derived savannah zone of South-Western Nigeria. It has a humid climate with mean annual rainfall of about 1037 mm and temperature of about 34.7 °C. The relative humidity ranges from 63 to 96 % in the rainy season (late March to October) and from 55 to 82 % in the dry season (November to early March) with an annual average of 82 %. The seasonal distribution of annual rainfall is approximately 44.96 mm in the late dry season (January–March); 212.4 mm in the early wet season (April–June); 259.3 mm in the late wet season (July–September) and 48.1 mm in the early dry season (October–December) as documented by Federal University of Agriculture, Abeokuta Meteorological Station, respectively.

Experimental Animals and Their Management

Forty eight weaner Large White male pigs of eight weeks old with mean body weight of 9.67 ± 0.26 kg were randomly assigned to three experimental groups in a completely randomized design. The pigs were grouped based on weight equalization to three groups (group 1 consisting of pigs fed 2.5 kg feed daily, while pigs in groups 2 and 3 were offered 2.0 and 1.5 kg feed daily, respectively) of sixteen pigs each. Each group was replicated four times with 4 pigs per replicate. Each replicate consisting of four pigs were fed and housed together in naturally ventilated pen with floor size dimension of 3 × 2 m. Fresh and clean water was supplied *ad libitum* throughout the duration of the experiment.

Experimental Design

The pigs were randomly assigned to 3 experimental groups in a Completely Randomized Design of 16 pigs per group. The experimental groups consisted of the daily amount of dietary portion fed to each of the pigs. Pigs were offered 2.5, 2.0 or 1.5 kg feed daily for duration of 150 days. Each pig received halves of their daily ration at 08:00 hr and the remaining portion at 14:00 hr. Diets were formulated to meet the body requirements of growing pigs. The ration contained 18 % crude protein and metabolizable energy of 12.16 MJ kg^{-1} as depicted in Table 1.

Table 1: Composition of Experimental Diet (%)

Ingredients	Grower ration
Maize	45.00
Groundnut cake	20.00
Wheat offal	20.00
Palm kernel cake	12.50
Bone meal	2.00
Premix*	0.35
Common salt	0.30
Lysine	0.05
Methionine	0.05
Total	**100.00**
Calculated Analysis	
Crude protein (%)	18.06
Crude fibre (%)	5.84
Calcium (%)	0.72
Phosphorus (%)	0.34
ME (MJ kg^{-1})	12.16

*Composition of diet per kg: Vit. A 12 600 IU; Vit. D_3 2 800 IU; Vit. E 49 IU; Vit.K_3 2.8 mg; Vit. B_1 1.4 mg; Vit. B_2 5.6 mg; Vit. B_6 1.4 mg; Vit. B_{12} 0.014 mcg; Niacin 21 mg; Pantothenic Acid 14 mg; Folic Acid 1.4 mg; Biotin 0.028 mcg; Choline Chloride 70 mg; Manganese 70 mg; Zinc 140 mg; Iron 140 mg; Copper 140 mg; Iodine 1.4 mg; Selenium 0.28 mg; Cobalt 0.7 mg; Antioxidant 168 mg

Data Collection

Feed and water intakes were determined daily by subtracting the feed and water left-over from those supplied. Initial body weight of weaner pigs were taken using weighing scale with a 0.05 g precision and documented when the pigs arrived at the experimental site and weekly records of change in body weight were subsequently taken and documented. The feed conversion ratio was calculated as ratio of feed/gain. Quantity of faecal excreted by pig was measured with the aid of measuring scale while the frequency of faecal excretion was determined by observing the number of times a pig defecates daily.

Carcass Characteristics

Twenty four pigs consisting of 8 pigs per treatment were randomly selected, slaughtered and analyzed for carcass yield, cut-up parts and fat composition at the end of the experiment. The pigs were weighed and fasted for 16 hours, and the fasted weight of each pig meant for slaughtering was taken before they were stunned by

percussion method and bled by incision using a sharp knife cutting through the jugular vein between the skull and the atlas. Complete bleeding and dehairing were done. The stomach of the pigs was opened along the greater curvature and emptied. After the removal of the visceral organs, the remaining part was measured as carcass weight and later expressed as percentage of the live weight to get the dressing percentage. The head was removed by section at the occipito-atlas joint and the feet by sawing through the hock joint at a right angle to the long axis of the leg. The carcass was divided longitudinally. The left half of the carcass was dissected as described by Barca *et al.* (2006). Ham was separated by locating the division between the 2nd and 3rd sacral vertebrae and saw perpendicularly along axis of the ham. Shoulder of the pig was separated from the loin and belly by a straight cut between the second and third ribs and a straight cut 2.5 centimetre ventral to the ventral edge of the scapula. The parts were weighed and recorded. Back-fat depth was taken at the last rib using vernier calliper. The fat-free index was estimated using the formulae postulated by National Pork Producers Council (1994).

Fat-free index = 50.767 + (0.035 × hot carcass weight in kilogram) – (8.979 × last rib midline back-fat on hot carcass in centimetre).

Statistical Analysis

Data were processed by one-way analysis of variance using Statistical Analyst Software (SAS Institute, 2000) package. Significantly (P <0.05) different means among variables were separated using New Duncan's Multiple Range Test as contained in the same package.

RESULTS AND DISCUSSION

The response of pigs to quantity of feed offered is shown in Table 2. There was no significant (P >0.05) difference in the initial body weights of pigs fed at different levels. This implied that the experimental pigs were equalized before the commencement of the experiment. The final body weight, daily weight gain and daily feed intake increased significantly (P <0.05) with increase in feed quantity offered. Pigs fed 2.5 kg feed daily had higher final body weight of 53.67 kg compared to 48.42 and 45.50 kg obtained for those fed 2.0 and 1.5 kg feed daily, respectively. Daily weight gain followed the same trend with the highest mean value of 296.13 g found among pigs on 2.5 kg feed daily while the least value of 238.33 g was recorded for pigs fed 1.5 kg feed daily. Pigs fed 2.5 kg feed daily had highest feed intake (1.05 kg), followed by those on 2.0 and then 1.5 kg feed daily. These significant differences attested to the fact that pigs on higher nutritional plane (2.5 kg feed) obtained adequate intake of nutrients required to sustain rapid growth and development (Njoku *et al.*, 2013). Sufficient offering of feed to pigs is vital in optimizing overall growth performance. Garcia-Valverde *et al.* (2008) reported that pigs on high level of nutrition deposited both lean and fat at a faster rate than those fed moderate level of nutrition on both age- and weight-constant bases. This is in line with the observations noted in this study where weight gain and feed intake were significantly influenced by feed quantity offered. Quantity of feed offered significantly (P <0.05) influenced feed conversion ratio of growing pigs. Feed conversion ratio decreased significantly (P <0.05) with increase in feed quantity offered. The pigs on daily ration of 2.5 kg feed required less feed (3.01 kg) to gain one kg weight.

Table 2: Effect of feed quantity offered on performance of growing pigs

	Quantity offered			
Parameters/Treatments (kg)	1.5	2.0	2.5	SEM
Initial weight (kg)	9.75	10.00	9.25	0.58
Final weight (kg)	45.50^{b}	48.42^{ab}	53.67^{a}	2.10
Daily weight gain (g)	238.33^{b}	256.13^{ab}	296.13^{a}	12.05
Daily feed intake (kg)	0.81^{c}	0.95^{b}	1.05^{a}	0.12
Feed conversion ratio	4.13^{a}	3.56^{ab}	3.01^{b}	0.27
Daily water intake (l)	4.80^{a}	3.97^{b}	3.59^{b}	0.34
Excreted faecal weight (g/day)	665.17	591.98	575.27	29.55
Frequency of faecal excretion (times)	3.18^{a}	2.89^{b}	2.96^{b}	0.11

^{abc} – means within rows followed by different superscripts are significantly different (P <0.05)

The increase in growth rate with increasing feed offered indicates that protein deposition had larger effect on growth rate than fat deposition. The extra feed consumed by the pigs on 2.5 kg feeding regimen could have resulted to increase in protein deposition which mainly determines the growth rate of growing pigs. The extra gain in growth rate could be hypothesized to be proportionately higher than the increase in feed intake resulting in a reduced and therefore improved feed conversion ratio. Affentranger *et al.* (1996) reported better feed intake and feed efficiency in pigs fed under different feeding regimes. Feeding level, feed composition and feeding patterns have been used as tools to manipulate growth rate, weight gain, fat deposition and pork quality (Wood *et al.*, 2004). So, feeding level have been applied to increase/decrease growth rate and thereby decrease/increase age at slaughter at a given body weight (Garcia-Valverde *et al.*, 2008; Lebret, 2008a). Daily water intake of pigs fed 2.0 and 2.5 kg feed daily were statistically similar (3.97 and 3.59 litres, respectively) but differed significantly (P <0.05) from those on 1.5 kg feed daily (4.80 litres). The water intake decreased significantly with increase in feed quantity offered. The decrease in water intake with increasing feed quantity offered could be linked to level of satiety attained among the pigs on higher nutritional plane, since they had more access to feed than those on limited nutritional plane. Pigs fed limited amount of feed might have increased their water in order to compensate for low abdominal fill. This corroborates the findings of Silanikove and Brosh (1989) that reported 6-fold increase in water intake of pigs whose total daily ration was halved or completely withheld for a period. Under limited-feeding conditions, pigs consume excessive and highly variable quantities of water (Yang *et al.*, 1981). Excess water intake often referred to as hunger-induced polydypsia could be reduced by *ad libitum* feeding (Shaw *et al.*, 2006). There was no significant (P >0.05) difference in excreted faecal weight, although numerical differences were recorded among treatments. Pigs on 1.5 kg feed quantity offered had the highest faecal weight (665.17 g day^{-1}) while those on 2.5 kg feed daily had the least value (575.27 g day^{-1}). The numerical differences could be attributed to the level of water intake which was higher among the pigs fed 1.5 kg feed daily. Hosseini-Assal and Hosseini (2000) reported that faecal weights of human subjects depend on amount of water intake and fibre content of diet. Pigs on 1.5 kg feed offered drank more water in order to compensate for low abdominal fill; this must have increased the level of unabsorbed water in the large intestine, leading to increase in faecal weight. Frequency of faecal excretion is defined as the number of times an animal passes out faeces per day. The significant difference noted in the frequency of faecal excretion could be associated with the rate of water intake. Water aids digestion and promotes the rate of passage of digesta in the gastrointestinal tract. The quicker the rate of passage through the gastrointestinal tract, the earlier it reaches the rectum and therefore excretion occurs more frequently.

Carcass yield, cut-up parts and organ weight of growing pigs fed different quantity of feed is shown in Table 3. The fasted weight, bled weight, carcass weight and dressed percentage of the experimental pigs increased significantly (P <0.05) with increasing level of feed offered. The values for fasted weight ranged from 42.17 kg (pigs fed 1.5 kg feed daily) to 51.83 kg (pigs fed 2.5 kg daily). Bled weights of pigs on 1.5 and 2.0 kg daily ration were statistically similar (37.53 and 41.00 kg) which differed significantly (P <0.05) from 48.33 kg recorded for those on 2.5 kg daily ration. Pigs on 2.5 kg daily ration had the highest carcass weight (36.83 kg) and dressed percentage (81.86 %), while those on 1.5 kg daily ration had the least values (26.17 kg and 72.09 %, respectively). The significant differences observed in these parameters could be attributed to the level of feed offered to the individual pigs in each treatment group. The pigs on higher nutritional plane must have obtained sufficient amount of nutrients from the dry matter intake to compensate the energy requirement for body maintenance and tissue growth. Adequate quantity of energy intake is critical to optimize lean growth rate and efficiency (Augenstein *et al.*, 1997). The feeding level, pattern and protein: energy ratio of the diet, together with the genetic growth potential of pigs determine the growth rate and composition of weight gain at both whole-body and muscle level (Lebret, 2008a; Merck, 2008). There was a reduction in the rate and efficiency of gain as limited-feeding intensifies, though a better carcass quality was obtained. Many research findings had shown that the level of feed offered greatly influenced the back fat deposition of pigs. Feed restriction affects more fat tissue than lean tissue deposition when applied during the finishing stage of pigs. Therefore, restricted feeding leads to leaner carcasses compared with *ad libitum* feeding (Lebret *et al.*, 2001). Decrease in back fat thickness, adipocyte volume and lipogenic capacity in pigs are some of the effect of restricted feeding (Gondret and Lebret, 2002). The least back fat deposition as observed from the pigs on 1.5 kg per day feeding regime must have resulted from restriction of feed intake. Hence, the importance of feed restriction on production indices over the growth period and meat quality cannot be over-emphasized and this depends very much on feeding pattern, degree and duration (Critser *et al.*, 1995). The amount of feed offered per day played vital role in the growth performance which therefore had direct bearing on the quality of carcass produced. Limited-feeding leads to depletion of apparent rate of glycogen as measured by muscle acidity (McPhee and Trout, 1995) resulting to

Table 3: Effect of feed quantity offered on carcass yield, cut-up parts and organ weight of growing pigs

	Quantity offered			
Parameters/treatments	1.5	2.0	2.5	SEM
	Carcass yield			
Fasted weight (kg)	42.17^{c}	47.67^{b}	51.83^{a}	2.93
Bled weight (kg)	37.53^{b}	41.00^{b}	48.33^{a}	2.29
Carcass weight (kg)	26.17^{c}	30.33^{b}	36.83^{a}	2.23
Dressed percentage (%)	72.09^{c}	74.43^{b}	81.86^{a}	2.47
Backfat depth (cm)	0.56^{ab}	0.62^{b}	0.71^{a}	0.09
Fat free index	49.20	49.10	48.83	0.30
	Cut-up parts[1]			
Head weight	10.17^{b}	10.97^{a}	10.84^{a}	0.35
Ham weight	13.47^{c}	13.95^{b}	14.80^{a}	0.54
Shoulder weight	12.07^{b}	11.94^{b}	12.60^{a}	0.38
Feet weight	2.51^{a}	2.45^{ab}	2.37^{b}	0.05
Tail weight	0.31	0.23	0.25	0.01
	Organ weight[2]			
Empty Stomach weight	2.06	1.80	2.06	0.07
Lung weight	1.00	1.03	0.93	0.03
Liver weight	2.21b	2.36a	2.42a	0.04
Spleen weight	0.40	0.31	0.31	0.01
Heart weight	0.43c	0.52b	0.62a	0.02

^{abc} – means within rows followed by different superscripts are significantly different (P <0.05); $^{1, 2}$ – values are expressed as percentage of fasted weight

reduction in back fat depth and increase in rate of lean growth (McPhee *et al.*, 1988). Pigs raised on restricted feeding was reported by Nguyen and Cam (2001) to have high growth rate, low back fat and high lean percentage in the carcass of their descendants. Hence, the advantage of restricted feeding transcend generations.

The head, ham and shoulder weights increased significantly with increase in feed quantity offered. Head weight of the pigs on 2.0 and 2.5 kg feed daily had statistically similar values (10.97 and 10.84 %) but differed (P <0.05) from that of pigs on 1.5 kg daily feed. The ham weights (13.47, 13.95 and 14.80 %) increased significantly (P <0.05) with increase in feed quantity offered. The shoulder weight of pigs on 1.5 and 2.0 kg feed daily had comparable values which differed significantly (P <0.05) from those fed 2.5 kg feed daily (12.60 %). Feet weight decreased significantly (P <0.05) with increase in feed quantity offered. The significant differences noted in these parameters might have resulted from better conformation of pigs' carcass in relation to the final body weight (body mass) of the pigs' slaughtered. Pigs with larger body weight had higher head, ham, shoulder and feet weights. This is in line with the findings of Latorre *et al.* (2008) who observed that the weight of ham, shoulder and loin increased with weight at slaughter, which is also close to the result of Lo Fiego *et al.* (2005). However, the observation contradicted the findings of Virgili *et al.* (2003) who suggested that primal cut proportion decreases with increasing body weight because the growth rate of primal cuts was lower with age than the growth rate of the whole body. Liver was also observed to increase with increasing feed quantity. This might have resulted from the numerous chemical changes taking place in the liver whenever feed is consumed. De Lange *et al.* (2003) reported that feed intake stimulates visceral organ growth and it also alters the distribution of whole-body protein. Liver weight of pigs on 2.0 and

2.5 kg feeding regime had comparable mean values (2.36 and 2.42 %) which were higher than the value (2.21 %) obtained among pigs on 1.5 kg feeding regime. Heart weight mean values (0.43, 0.52 and 0.62 %) increased significantly (P <0.05) with increase in feed quantity offered. Variability in carcass weight may have contributed to variation in some visceral organ mass (liver and heart). Some factors known to influence visceral organ size are body weight, feeding level, diet composition and pig genotype (Nyachoti, 1998). The relationship between visceral organ mass and body weight appears to reflect both changes in feed intake and maintenance energy requirements with increasing body weight (van Milgen and Noblet, 2003). Lebret (2008b) reported that feeding level and pattern of feeding are tools used to manipulate growth rate, composition of weight gain and intramuscular fat deposition.

CONCLUSION

Growth indices (final body weight, daily weight gain, daily feed intake), carcass cut parts (head, ham, shoulder) and some visceral organs (liver and heart) were influenced by higher feed quantity offered. Hence, 2.0 kg daily feed offered can be used as management tool in order to improve the performance, carcass parameters and some visceral organs of growing pigs.

REFERENCES

AFFENTRANGER, P. – GERWIG, C. – SEEWER, G. J. F. – SCHWORER, D. – KUNZI, N. 1996. Growth and carcass characteristics as well as meat and fat quality of three types of pigs under different feeding regimes. *Livestock Production Science*, vol. 45, 1996, p. 187–196.

AUGENSTEIN, M.L. – JOHNSTON, L.J. – SHURSON, G.C. – HAWTON, J.D. – PETTIGREW, J.E. 1997. Formulating farm-specific swine diets. Minnessota Extension Publication FO-5639. Minnessota Extension Service, St. Paul, MN, 1997.

BARCA, R. – NIETO, R. – LARA, L. – GARCIA, M.A. – VILCHEZ, M.A. – AQUILERA, J.F. 2006. Effects of dietary protein content and feeding level on carcass characteristics and organ weights of Iberian pigs growing between 50 and 100 kg liveweight. *Animal Science*, vol. 82, 2006, p. 405–413.

CRITSER, D.J. – MILLER, P.S. – LEWIS, A.J. 1995. The effects of dietary protein concentration on compensatory growth in barrows and gilts. *Journal of Animal Science*, vol. 73, 1995, p. 3376–3383.

DE LANGE, C.F.M. – FAN, M.Z. – SWIDERSKY, A. 2003. Optimum dietary levels for grower finisher pigs. Proceedings of the Centralia Swine Research Update, January 29, 2003, p. 25–27.

EDWARDS, S. 2010. Practical approaches to improving the efficiency of finishing pigs. Proceedings of the 1st Nigerian International Pig Summit, Institute of Agricultural Research and Training, Moor Plantation, Ibadan, 22nd – 25th November, 2010, p 69–77.

FERNANDEZ, X. – MONIN, G. – TALMANT, A. – MOUROT, J. – LEBRET, B. 1999. Influence of intramuscular fat content on the quality of pig meat. *Meat Science,* vol. 53, 1999, p. 59–72.

GARCIA-VALVERDE, R. – BAREA, R. – LARA, L. – NIETO, R. – AGUILERA, J.F. 2008. The effects of feeding level upon protein and fat deposition in Iberian heavy pigs. *Livestock Science*, vol. 114, 2008, p. 263–273.

GONDRET, F. – LEBRET, B. 2002. Feeding intensity and dietary protein level affect adipocyte cellularity and lipogenic capacity of muscle homogenates in growing pigs, without modification of the expression of sterol regulatory element binding protein. *Journal of Animal Science*, vol. 80, 2002, p. 3184–3193.

HOSSEINI-ASSAL, S.M.K. – HOSSEINI, S.D. 2000. Determination of the mean daily stool weight, frequency of defecation and bowel transit time: Assessment of 1000 healthy subjects. *Archives of Iranian Medicine*, vol. 3 (4), 2000, p. 1–5.

LATORRE, M.A. – GARCÍA-BELENGUER, E. – ARIÑO, L. 2008. The effects of gender and slaughter weight on growth performance and carcass traits of pigs intended for dry red ham from Teruel (Spain). *Journal of Animal Science*, vol. 86, 2008, p. 1933–1942.

LEBRET, B. – JUIN, H. – NOBLET, J. – BONNEAU M. 2001. The effects of two methods of increasing age at slaughter weight on carcass and muscle traits and meat sensory quality in pigs. *Animal Science*, vol. 72, 2001, p. 87–94.

LEBRET, B. 2008a. The response of various muscle types to a restriction realimentation feeding strategy in growing pigs. *Journal of Animal Science*, vol. 2, 2008, p. 849–857.

LEBRET, B. 2008b. Effects of feeding and rearing systems on growth, carcass traits and meat quality in pigs. *Journal of Animal Science*, vol. 2, 2008, p. 1548–1558.

LO FIEGO, D.P. – SANTERO, P. – MACCHIONI, P. – DE LEONIBUS, E. 2005. Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Science*, vol. 69, 2005, p. 107–114.

McPHEE, C.P. – TROUT, G.R. 1995. The effects of selection for lean growth and the halothane allele on carcass and meat quality of pigs transported long and

short distances to slaughter. *Livestock Production Science*, vol. 42 (1), 1995, p. 55–62.

McPHEE, C.P. – RATHMELL, G.A. – DANIELS, L.J. – CAMERON N.D. 1988. Selection for pigs for increased lean growth rate on a time-based feeding scale. *Animal Production*, vol. 47, 1988, p. 149–156.

MERCK & CO., INC. 2008. Whitehouse Station, NJ USA. Published in educational partnership with Merial Ltd.

NATIONAL PORK PRODUCERS COUNCIL. 1994. *"Procedures to evaluate market hogs."* National Pork Producers Council, Des Moines, Iowa.

NJOKU, C.P. – AINA, A.B.J. – SOGUNLE, O.M. – ODUGUWA, O.O. – ADEYEMI, O.A. – EKUNSEITAN, D.A. 2013. Influence of feed quantity offered on linear body measurements, nutrient digestibility and backfat composition of finishing pigs. *The Pacific Journal of Science and Technology,* vol. 14 (1), 2013, p. 387–396.

NGUYEN, H.N. – CAM, P.M. 2001: Selection for efficient lean growth on restricted feeding in large white pigs. Breeding and feeding pigs in Australia and Vietnam – ACIAR 9423.

NYACHOTI, C.M. 1998. Significance of endogenous gut protein losses in growing pigs. Ph.D. thesis. Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada.

SAS. 2000. SAS/STAT® User's guide (Version 8, 4th edition), SAS Institute Inc. Cary NC.

SHAW, M.I. – BEAULIEU, A.D. – PATIENCE, J.F. 2006. Effect of diet composition on water consumption in growing pigs. *Journal of Animal Science*, vol. 84, 2006, p. 3123–3132.

SILANIKOVE, N. – BROSH, A. 1989. Lignocellulose degradation and subsequent metabolism of lignin fermentation products by the desert black Bedouin goat fed on wheat straw as a single-component diet. *British Journal of Nutrition*, vol. 62, 1989, p. 509–520.

VAN MILGEN, J. – NOBLET, J. 2003. Partitioning of energy intake to heat, protein, and fat in growing pigs. *Journal of Animal Science*, vol. 81 (2), 2003, p. 86–93.

VIRGILI, R. – DEGNI, M. – SCHIVAZAPANA, C. – FAETI, V. – POLETTI, E. – MARCHETTO, G. – CCHIOLI, M.T. – MORDENTI, A. 2003. Effect of age at slaughter on carcass traits and meat quality of Italian heavy pigs. *Journal of Animal Science*, vol. 81, 2003, p. 2448–2456.

WOOD, J.D. – NUTE, G.R. – RICHARDSON, R.I. – WHITTINGTON, F.M. – SOUTHWOOD, O. – PLASTOW, G. – MANSBRIDGE, R. – DA COSTA, N. – CHANG, K.C. 2004. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science*, vol. 67, 2004, p. 651–667.

YANG, T.S. – HOWARD, B.E. – MCFARLANE, W.V. 1981. Effects of food on drinking behaviour of growing pigs. *Applied Animal Ethnology*, vol. 7, 1981, p. 256–270.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 48, 2015 (1): 23–28

ISSN 1337-9984

CARCASS, MEAT AND FAT QUALITY CHARACTERISTICS OF UKRAINIAN RED WHITE BELTED PIGS COMPARED TO OTHER COMMERCIAL BREEDS

I. BANKOVSKA[1], J. SALES[2],*

[1]Pig Breeding Institute and Agro-industrial Production of the National Academy of Agricultural Sciences of Ukraine, Poltava, Ukraine
[2]Czech University of Life Sciences, Faculty of Tropical AgriSciences, Department of Animal Science and Food Processing, Prague, Czech Republic

ABSTRACT

Carcass composition, and pH and electrical conductivity, were compared among Red White Belted (n = 10), Landrace (n = 9) and Large White (n = 10) pigs commercially reared and slaughtered at a live weight between 108 and 118 kg. Carcass yield was lower (P <0.05) in Red White Belted pigs (70.97 %) compared to Landrace (72.72 %) or Large White (72.83 %). At both the withers and between the 6th and 7th thoracic vertebrae, Red White Belted pigs presented greater (P <0.05) backfat measurements (45.80 and 33.70 mm, respectively) compared to Landrace (35.11 and 22.89 mm, respectively) or Large White (37.20 and 23.90 mm, respectively). Greater (P <0.05) pH values were measured at 24 hours *post-mortem* in the *musculus longissimus thoracis et lumborum* of Red White Belted pigs compared to Landrace. No differences (P >0.05) were detected in proximate composition (moisture, protein, fat, ash) of pork or in fat characteristics (moisture, melting point, refraction index) among pig breeds (five pigs per breed analysed).

Keywords: Red White Belted pigs; carcass characteristics; meat quality; fat traits

INTRODUCTION

Consumer demands for high quality pork in the absence of imports of pig breeds from outside Ukraine have resulted in the development of the Ukrainian Red White Belted pig. This synthetic breed was established from 1976 to 2007 at the Institute of Pig Breeding and Agro-industrial Production of the National Academy of Agricultural Sciences (Poltava, Ukraine) through complex crossing methods, which comprised Duroc (43.75 %), Poltava Meat (21.88 %), Hampshire (21.87 %), Landrace (6.25 %) and Large White (6.25 %) pigs (Rybalko *et al.*, 2011a). Red White Belted pigs:

1. are red-coloured with a narrow white strip on the chest behind the shoulder blades;
2. have a strong skeletal structure with a light head;
3. reach a live weight of 100 kg in 185 days;
4. have a high reproduction rate of 10 piglets in a litter;
5. produce a carcass with a carcass lean content of 62 % and backfat thickness of 26 mm (Rybalko *et al.*, 2011b).

Quality characteristics of pig carcasses and pork are largely affected by pig breed. Breed is often included as a variable while meat quality is an important consideration (Mörlein *et al.*, 2007), partly to help optimising the genetic choice of animals (Edwards *et al.*, 1992). With few published information on the meat producing quality of pig breeds developed in Ukraine, the objective of the current study was to compare carcass traits and meat and fat quality characteristics obtained

***Correspondence:** e-mail: James_Sales_1@hotmail.com
James Sales, Czech University of Life Sciences, Faculty of Tropical AgriSciences, Department of Animal Science and Food Processing, Kamycka 129, 165 21 Prague, Czech Republic
Phone: + 420 723 657 765

Received: April 3, 2014
Accepted: October 28, 2014

with Red White Belted to those from Landrace and Large White pigs. Information obtained could be utilized in countries outside of Ukraine with interest to introduce the Red White Belted pig into their local breeding programmes.

MATERIAL AND METHODS

Animals

Fifty eight barrows and 62 gilts which originated from three commercially available breeds (Red White Belted, Landrace, Large White) were reared and slaughtered at the facilities of the commercial company Freedom Farm Bacon, Ukraine. Landrace and Large White pigs were imported from Northern Ireland in 2003 and 2005, and their Ukrainian-bred offspring (sows) were inseminated in 2008 with semen from boars from the United States to improve feed efficiency and meat quality.

Pigs were housed by breed in pens of 30–40 animals during the weaner period and 25–30 animals during the grower and finisher periods. All animals were fed *ad libitum* on standard complete commercial pig diets. During growth from 30 to 60 kg, diets contained (per dry matter) 12.9 MJ kg^{-1} net energy, 19.1 % crude protein and 1.1 % lysine. These quantities were decreased to 12.8 MJ kg^{-1} net energy, 18.0 % crude protein and 1.0 % lysine during the phase from 60 to 90 kg live weight, and to 12.6 MJ kg^{-1} net energy, 17.1 % crude protein and 0.8 % lysine during 90 to 120 kg, respectively. All pigs were slaughtered at a live weight between 108 and 118 kg at 6–6.5 months of age. Nine to 10 animals per breed were selected for evaluation of carcass and meat (pH, electrical conductivity) quality, whereas proximate composition of pork and fat quality were measured in five animals per breed.

Carcass measurements

Carcass weight was calculated with skins intact, but without heads, feet, viscera and internal fat. Heads were separated cross-section perpendicular to the spine before the 1st cervical vertebra. The front feet were removed at the wrist joint, and rear feet at the hock joint. Carcass yield was calculated as the percentage of hot carcass weight divided by live weight. Carcass length was measured in the hanging position, and defined as the distance from the front surface of the 1st cervical vertebra (atlas) to the front perimeter of the pubic symphysis bones. Length of the bacon side was measured from the middle of the 1st rib to the front perimeter of the pubic symphysis bones.

Backfat thickness (together with skin) was measured in:

1. the thickest part of the withers;
2. over the 6–7 thoracic vertebrae;
3. in the loin.

Minimum thickness of visible fat (including rind) was determined on the midline of the split carcass which is covering the lumbar muscle (*gluteus medius*; *F*), whereas visual thickness of the lumbar muscle was measured as the shortest distance between the front (cranial) end of the lumbar muscle and the upper (dorsal) edge of the vertebral canal (*M*). From these two measurements, the percentage carcass lean ($CL = 58.10122 - (0.56495 \times F) + (0.13199 \times M)$) was calculated according to the 'Zwei-Punkt-Messverfahren' method used in Germany for pig carcasses weighing between 50 and 120 kg (EU, 2011).

Meat and fat quality characteristics

The pH and electrical conductivity (EC) values were recorded with a universal (multipurpose) portable digital LF-Meter "LF-Star CPU-Pistole" (Ing.-Büro & Klassifizierungsservice Rudolf Matthäus, Klausa, Germany) at 1, 5 and 24 hours *post-mortem*. Measurements were made on seven points in the carcass which were the most easily accessible on the slaughterhouse conveyor:

1. musculus semimembranosus (SM);
2. *musculus longissimus thoracis et lumborum* between the 4th and 5th lumbar vertebra (LTL1);
3. *musculus longissimus thoracis et lumborum* between the 10th and 12th thoracic vertebra (LTL2);
4. *musculus longissimus thoracis et lumborum* between the 2nd and 3rd thoracic vertebra (LTL3);
5. *musculus rectus thoracis* (RTH);
6. *musculus intercostales externus* between the 6th and 7th ribs (INEX);
7. *musculus rectus abdominis* (REAB). Temperature was adapted for by use of a digital thermometer 'AMA-digit ad 14th' (Amarell GmbH & Co. KG, Germany).

Ultimate pH (pH_{48}) was measured, as described above, after cooling of carcasses for 48 hours in the LTL2 of five pigs, and muscle and backfat samples for chemical and physical analyses, respectively, were sampled from this position on the right sides of carcasses. Moisture content of muscle samples was determined by drying of a sample at 103 °C to constant weight, ashing was performed at 550 °C in a muffle furnace, crude protein by the Kjeldahl method (nitrogen × 6.25), and ether-extractable intramuscular fat by Soxhlet solvent (petroleum ether) extraction (AOAC, 1990).

Fat analyses of the backfat samples were done according to methods described in the Methodical Recommendations of Agricultural Sciences (Misik, 1978). Moisture content of fat was measured by heating of a 0.5 g sample for 2.5 hours at 105 °C to a constant weight. Melting point temperature of fat was determined by the rising melting point (open capillary) method, and the refractive index by refractometry (IRF-454 B2M, Kazan Optical and Mechanical Plant, Russia) at 40 °C.

Statistical analysis

Preliminary statistical analyses showed that there were no differences between genders, probably due to small sample size. Therefore data for barrows and gilts were pooled. Differences among breeds in carcass, meat and fat quality characteristics were detected by one-way analysis of variance (ANOVA) followed by Tukey's test at the 0.05 level of significance. The General Linear Model procedure (GLM) of SAS version 9.2 (SAS Institute Inc, Cary, NC) was used as statistical package. With muscles originating from the same carcass dependent onto each other, breed was the only independent variable that could be evaluated for meat quality characteristics.

RESULTS AND DISCUSSION

Carcass characteristics

Notwithstanding comparable (P >0.05) live and hot carcass weights, carcass yield and length of the bacon side were lower (P <0.05) in Red White Belted pigs compared to Landrace and Large White (Table 1). Furthermore, backfat thickness measured at both the withers and between the 6th and 7th thoracic vertebrae were greater (P <0.05) in Red White Belted pigs. Differences in backfat thickness were also illustrated by computing the degree of evenness of backfat, determined by the difference in the thickness of backfat on the withers (at the thickest part) and loin (at thinnest part), among pig breeds. This measurement (in mm) presented a greater (P <0.05) value for Red White Belted (15.50 ± 2.099) compared to Landrace (8.22 ± 1.935) or Large White (6.50 ± 1.384). The greater backfat thickness in Red White Belted pigs could be attributed to the large proportion of Duroc genes used in the development of this breed. Duroc pigs are characterised by a greater backfat thickness compared to other breeds (Edwards *et al.*, 1992). A lower muscle growth potential was stated as the reason for a greater backfat thickness and lower carcass lean content in Creole pigs compared to Large White (Renaudeau and Mourot, 2007). However, carcass lean content did not present any differences among breeds in the current study (Table 1).

Meat and fat quality

Table 2 shows that breed had no effects on pH measured at 1 hour *post-mortem*, whereas differences in the LTL3 and READ at 5 hours *post-mortem* were greater (P <0.05) in Red White Belted pigs compared to Landrace. However, at 24 hours *post-mortem*, values obtained in all parts of the LTL were greater (P <0.05) in Red White Belted pigs compared to Landrace, and in the SM and READ compared to both Landrace and Large White. Greater (P <0.05) values in Red White Belted pigs (5.52 ± 0.045) compared to Landrace (5.39 ± 0.015) were also found by measuring pH at 48 hours *post-mortem* in the LTL2, with Large White presenting intermediate (5.44 ± 0.017) values.

Greater pH values in muscles from Red White Belted pigs could have resulted from the Duroc proportion used in their development. Duroc pigs present the greatest ultimate pH in the LTL, followed by Hampshire, Large White and Landrace (Barton-Gade, 1988). Whereas muscle metabolic activity (mainly ATPase activity) at slaughter will determine the speed of pH decline, the magnitude of pH decline depends mainly on muscle glycogen reserves (Hambrecht *et al.*, 2005). A low *post-mortem* pH could reduce the acceptability and shelf-life of meat, and its suitability for the manufacture of cured meat products (Ramírez and Cava, 2007).

Table 1: Effects of breed on carcass characteristics (mean ± standard error)

Characteristic	Red White Belted (n = 10)	Landrace (n = 9)	Large White (n = 10)
Live weight (kg)	118.60 ± 4.782	116.22 ± 3.205	108.50 ± 2.566
Hot carcass weight (kg)	84.22 ± 3.580	84.93 ± 2.536	79.04 ± 2.013
Carcass yield (%)	70.97 ± 0.491^{b}	72.71 ± 0.367^{a}	72.83 ± 0.483^{a}
Carcass length (cm)	99.38 ± 1.850	101.00 ± 0.816	101.55 ± 0.677
Length of bacon side (cm)	65.15 ± 1.145^{b}	68.89 ± 0.978^{a}	69.00 ± 0.394^{a}
Backfat thickness at withers (mm)	45.80 ± 2.133^{a}	35.11 ± 1.852^{b}	37.20 ± 1.504^{b}
Backfat thickness between the 6th and 7th thoracic vertebrae (mm)	33.70± 2.082^{a}	22.89 ± 1.728^{b}	23.90 ± 1.847^{b}
Backfat thickness at loin (mm)	30.30 ± 1.633	26.89 ± 2.003	30.70 ± 1.033
Carcass lean content (%)	56.61 ± 0.860	57.60 ± 1.478	59.24 ± 1.014

means in the same row with different subscripts are significantly different (P <0.05); n – number of pigs

Table 2: Effects of breed on pH (mean ± standard error) measured at different time periods in different pig muscles

Muscle	pH_1			pH_5			pH_{24}		
	Red White Belted (n = 10)	Landrace (n = 9)	Large White (n = 10)	Red White Belted (n = 10)	Landrace (n = 9)	Large White (n = 10)	Red White Belted (n = 10)	Landrace (n = 9)	Large White (n = 10)
SM	6.12 ± 0.121	6.10 ± 0.053	6.16 ± 0.052	5.71 ± 0.044	5.67 ± 0.076	5.62 ± 0.069	5.81 ± 0.027^{a}	5.64 ± 0.046^{b}	5.59 ± 0.026^{b}
LTL1	6.13 ± 0.065	6.15 ± 0.051	6.21 ± 0.047	5.75 ± 0.027	5.79 ± 0.066	5.78 ± 0.052	5.73 ± 0.022^{a}	5.56 ± 0.049^{b}	5.62 ± 0.032^{ab}
LTL2	6.18 ± 0.058	5.93 ± 0.078	6.20 ± 0.106	5.74 ± 0.053	5.62 ± 0.042	5.72 ± 0.041	5.73 ± 0.026^{a}	5.60 ± 0.022^{b}	5.65 ± 0.025^{ab}
LTL3	6.31 ± 0.047	6.19 ± 0.038	6.33 ± 0.049	5.95 ± 0.015^{a}	5.80 ± 0.030b	5.92 ± 0.031^{a}	5.97 ± 0.043^{a}	5.73 ± 0.103^{b}	5.81 ± 0.047^{ab}
RTH	6.22 ± 0.062	6.23 ± 0.046	6.20 ± 0.050	5.90 ± 0.048	5.76 ± 0.040	5.88 ± 0.036	5.90 ± 0.063^{a}	5.77 ± 0.039^{ab}	5.71 ± 0.028^{b}
INEX	6.15 ± 0.044	6.16 ± 0.061	6.08 ± 0.043	5.84 ± 0.022	5.80 ± 0.054	5.89 ± 0.041	6.03 ± 0.106	5.88 ± 0.057	5.89 ± 0.042
REAB	6.19 ± 0.058	6.09 ± 0.061	6.09 ± 0.036	5.89 ± 0.038^{a}	5.68 ± 0.031^{b}	5.82 ± 0.069^{ab}	5.87 ± 0.047^{a}	5.71 ± 0.022^{b}	5.69 ± 0.047^{b}

means in the same row within pH classification (pH_1, pH_5, pH_{24}) with different subscripts are significantly different (P <0.05); n – number of pigs; SM – *musculus semimembranosus*; LTL1 – *musculus longissimus thoracis et lumborum* between the 4th and 5th lumbar vertebra; LTL2 – *musculus longissimus thoracis et lumborum* between the 10th and 12th thoracic vertebra; LTL3 – *musculus longissimus thoracis et lumborum* between the 2nd and 3rd thoracic vertebra; RTH – *musculus rectus thoracis*; INEX – *musculus intercostales externus* between the 6th and 7th ribs; REAB – *musculus rectus abdominis*

Table 3: Effects of breed on electrical conductivity (EC; mean ± standard error) measured at different time periods in different pig muscles

Muscle	EC_1			EC_5			EC_{24}		
	Red White Belted (n = 10)	Landrace (n = 9)	Large White (n = 10)	Red White Belted (n= 10)	Landrace (n = 9)	Large White (n = 10)	Red White Belted (n= 10)	Landrace (n= 9)	Large White (n= 10)
SM	6.19 ± 0.464	5.61 ± 0.453	6.87 ± 0.413	12.26 ± 0.477	12.62 ± 0.463	12.74 ± 0.374	12.31 ± 0.222	12.21 ± 0.144	11.73 ± 0.277
LTL1	5.41 ± 0.332	4.61 ± 0.357	5.42 ± 0.227	8.61 ± 0.694	9.83 ± 0.566	8.05 ± 0.570	7.82 ± 0.511	8.09 ± 0.829	7.43 ± 0.831
LTL2	4.48 ± 0.242	4.49 ± 0.210	4.62 ± 0.196	5.52 ± 0.456	6.06 ± 0.485	6.40 ± 0.583	5.67 ± 0.672	5.71 ± 0.625	6.08 ± 0.416
LTL3	4.40 ± 0.321	4.80 ± 0.509	4.98 ± 0.214	4.46 ± 0.281	5.14 ± 0.853	4.94 ± 0.721	5.62 ± 0.777	5.87 ± 0.673	7.09 ± 0.749
RTH	4.57 ± 0.251	4.93 ± 0.362	5.01 ± 0.210	4.16 ± 0.346	4.11 ± 0.362	3.82 ± 0.280	5.18 ± 0.404	5.01 ± 0.291	4.39 ± 0.491
INEX	3.59 ± 0.412	3.02 ± 0.344	3.03 ± 0.289	2.45 ± 0.239	3.11 ± 0.475	2.64 ± 0.302	1.72 ± 0.138	2.02 ± 0.413	2.13 ± 0.280
REAB	4.33 ± 0.342	3.86 ± 0.334	3.63 ± 0.192	4.04 ± 0.296	3.29 ± 0.587	2.63 ± 0.300	5.67 ± 0.406^{a}	4.42 ± 0.478^{ab}	3.61 ± 0.227^{b}

means in the same row within EC classification (EC_1, EC_5, EC_{24}) with different subscripts are significantly different (P <0.05); n – number of pigs; SM – *musculus semimembranosus*; LTL1 – *musculus longissimus thoracis et lumborum* between the 4th and 5th lumbar vertebra; LTL2 – *musculus longissimus thoracis et lumborum* between the 10th and 12th thoracic vertebra; LTL3 – *musculus longissimus thoracis et lumborum* between the 2nd and 3rd thoracic vertebra; RTH – *musculus rectus thoracis*; INEX – *musculus intercostales externus* between the 6th and 7th ribs; REAB – *musculus rectus abdominis*

Table 4: Effects of breed on proximate composition of pork and characteristics of backfat (mean ± standard error)

Characteristic	Red White Belted (n = 5)	Landrace (n = 5)	Large White (n = 5)
Proximate composition (% wet weight)			
Moisture	73.70 ± 0.356	74.09 ± 0.198	73.98 ± 0.142
Protein	22.16 ± 0.348	22.64 ± 0.174	22.60 ± 0.138
Fat	1.98 ± 0.412	1.23 ± 0.149	1.32 ± 0.149
Ash	1.16 ± 0.012	1.15 ± 0.008	1.14 ± 0.013
Backfat			
Moisture (%)	9.48 ± 0.591	10.52 ± 0.773	9.80 ± 0.581
Melting point (°C)	27.74 ± 0.236	28.76 ± 0.604	28.88 ± 0.907
Refraction index	1.46 ± 0.000	1.46 ± 0.000	1.46 ± 0.000

n – number of pigs

It could be postulated that meat from Red White Belted pigs (with greater ultimate pH values) should provide better processing abilities into cured products compared to Landrace and Large White.

With muscle containing continuous electrolytes with relatively great EC values, this measurement could be applied for detection of exudative meat (Swatland, 2003). However, except for a greater (P <0.05) value in the REAB of Red White Belted pigs compared to Large White found at 24 hours *post-mortem*, no differences occurred among breeds in EC of the respective muscles (Table 3).

Proximate composition (moisture, protein, fat, ash) measured in the LTL2 did not differ (P >0.05) among the three pig breeds evaluated (Table 4). Although intramuscular fat content did not differ among breeds, it tended (P = 0.138) to be greater in Red White Belted pigs compared to Landrace and Large White. This could be attributed to the 44 % Duroc proportion, a breed from the United States that was introduced in Europe mainly due to its greater intramuscular fat content compared to other breeds (Barton-Gade, 1987). It was shown (Wood, 1993; NPPC, 1995) that Duroc pigs produce pork with a greater intramuscular fat content in comparison to the white European breeds, including the Large White and Landrace. According to De Vol *et al.* (1988), a threshold value of 2.5–3.0 % intramuscular fat in pork presented the most tender (lowest Warner-Bratzler values), with tougher meat obtained at lower levels of fat, and little effect of greater levels on tenderness. With Red White Belted pigs showing intramuscular fat levels near to this threshold value compared to other breeds, it could be assumed that they would have more tender meat than either Landrace or Large White.

Backfat characteristics were similar (P >0.05) among pig breeds (Table 4). With a decrease in melting point when unsaturation of fat increased (Wood *et al.*, 2004), the absence of any differences indicated that there would probably be no differences in the amount of saturation of backfat among breeds. Furthermore, no differences (P >0.05) among breeds were detected in the refractive index, which could be identified as the ratio of the speed of light in a vacuum to the speed of light in the fat.

CONCLUSIONS

It can be concluded from this study that Red White Belted pigs present comparable carcass lean contents to Landrace and Large White pigs, notwithstanding lower carcass yields and greater backfat thickness. However, differences among breeds in pH measured at 24 hours *post-mortem* suggested an evaluation of the rate of glycolysis in different muscles in future studies. Furthermore, the processing abilities of meat from Red White Belted pigs into cured products compared to other breeds should be evaluated.

ACKNOWLEDGEMENTS

This project was supported by the National Academy of Agricultural Sciences of Ukraine (Kiev, Ukraine, 0110U002534).

REFERENCES

AOAC. 1990. Official methods of analysis of the Association of Official Analytical Chemists, 15th edition. Washington, DC, USA: Association of Official Analytical Chemists, 1990.

BARTON-GADE, P. A. 1987. Meat and fat quality in boars, castrates and gilts. *Livestock Production Science*, vol. 16, 1987, p. 187–196.

BARTON-GADE, P. 1988. The effect of breed on meat quality characteristics in pigs. In: *Proc 34th International Congress of Meat Science and Technology,* 1998, Brisbane, Australia, p. 568–570.

De VOL, D. L. – MCKEITH, F. K. – BECHTEL, P. J. – NOVAKOFSKI, J. – SHANKS, R. D. – CARR, T. R. 1988. Variation in composition and palatability traits and relationships between muscle characteristics and palatability in a random sample of pork carcasses. *Journal of Animal Science*, vol. 66, 1987, p. 385–395.

EDWARDS, S. A. – WOOD, J. D. – MONCRIEFF, C. B. – PORTER, S. J. 1992. Comparison of the Duroc and Large White as terminal sire breeds and their effect on pig meat quality. *Animal Production*, vol. 54, 1992, p. 289–297.

EU. 2011. Commission implementing Decision 2011/258/EC of 27 April 2011 amending Decision 89/471/EEC authorising methods for grading pig carcasses in Germany (notified under document number C (2011) 2709). *Off. J. EU*, 2011, vol. L75, p. 24–25.

HAMBRECHT, E. – EISSEN, J. J. – NEWMAN, D. J. – SMITS, C. H. M. – DEN HARTOG, L. A. – VERSTEGEN, M. W. A. 2005. Negative effects of stress immediately before slaughter on pork quality are aggravated by suboptimal transport and lairage conditions. *Journal of Animal Science*, vol. 83, 2005, p. 440–448.

MISIK, A. T. 1978. Methodological guidelines for assay of quality of carcass, meat and subcutaneous fat of slaughtered pigs. Moscow, Russia: VASHNIL, 1978 (Russian).

MÖRLEIN, D. – LINK, G. – WERNER, C. – WICKE, M. 2007. Suitability of three commercially produced pig breeds in Germany for a meat quality program with emphasis on drip loss and eating quality. *Meat Science*, vol. 77, 2007, p. 504–511.

NPPC. 1995. National genetic evaluation program. Des Moines, IA, USA: National Pork Producers Council, 1995.

RAMÍREZ, R. – CAVA, R. 2007. Carcass composition and meat quality of three different Iberian·× Duroc genotype pigs. *Meat Science*, vol. 75, 2007, p. 388–396.

RENAUDEAUA, D. – MOUROT, J. 2007. A comparison of carcass and meat quality characteristics of Creole and Large White pigs slaughtered at 90 kg BW. *Meat Science*, vol. 76, 2007, p. 165–171.

RYBALKO, V. P. – GETYA, A. A. – GERASIMOV, V. I. 2011a. The gene pool of the national pig breeds of Ukraine, their creators and modern coordinators. Poltava, Ukraine: Poltava writer; 2011a, p. 103–121 (Russian).

RYBALKO, V. P. – LESNOY, V. A. – FESENKO, O. G. – NAGAEVICH, V. M. 2011b. Red White Belted breed of meat pigs and zootechnical requirements of manifestation of their productivity. Practical recommendations. Poltava, Ukraine, 2011b, p. 6–17 (Russian).

SWATLAND, H. J. 2003. On-line monitoring of meat quality. In: Kerry, J. P., Kerry, J. F., Ledward, D. A.: *Meat Processing. Improving Quality. Cambridge*, UK: Woodhead Publishing, 2003, p. 193–216.

WOOD, J. D. 1993. Production and processing practices to meet consumer needs. In: Batterham, E.: *Manipulating Pig Production IV.* Attwood, Victoria, Australia: Australasian Pig Science Association, 1993, p. 135–147.

WOOD, J. D. – RICHARDSON, R. I. – NUTE, G. R. – FISHER, A. V. – CAMPO, M. M. – KASAPIDOU, E. – SHEARD, P. R. – ENSER, M. 2004. Effects of fatty acids on meat quality: a review. *Meat Science*, vol. 66, 2004, p. 21–32.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 48, 2015 (1): 29–36

ISSN 1337-9984

EFFECT OF DIETARY COPPER ON PERFORMANCE, SERUM AND EGG YOLK CHOLESTEROL AND COPPER RESIDUES IN YOLK OF LAYING CHICKENS

A.V. JEGEDE,* A.O. OSO, A.O. FAFIOLU, R.A. SOBAYO, O.M.O IDOWU, O.O. ODUGUWA

*Federal University of Agriculture, Department of Animal Nutrition, Abeokuta, Nigeria

ABSTRACT

A 294-day study was conducted to determine the effects of organic and inorganic copper (Cu) sources on laying performance, blood and yolk cholesterol and Cu accumulation in the yolk of laying hens. 480 Kabir® laying hens (20 weeks old) reared for 294 days in three phases (early, mid and late lay) were used. They were randomly allocated to six dietary groups of 80 birds split into four replicates of 20 birds each The diets consisted of a basal diet (containing 37.24 mg kg^{-1} Cu) supplemented with organic Cu (Cu proteinate; Cu-P) or inorganic Cu (Cu sulphate pentahydrate; $CuSO_4$) fed at 3 dietary concentrations (50, 100 and 150 mg kg^{-1}). Data on laying performance, blood and yolk cholesterol and Cu accumulation in the yolk were collected and subjected to Completely Randomized Design of the Analysis of Variance, laid out in 2 × 3 factorial arrangements. $CuSO_4$ supplementation resulted in poor (P <0.05) feed conversion ratio (feed/dozen eggs and feed kg $eggs^{-1}$) and reduced (P <0.05) hen day egg production when compared to Cu-P. High concentration of Cu resulted in reduced hen day egg production. No significant (P >0.05) effect of Cu sources and concentration was observed for body weight, weight gain and daily feed intake. The blood cholesterol and triglyceride level were significantly (P <0.05) reduced in birds fed diets with Cu-P. The blood cholesterol level decreased as the Cu concentration increased. More Cu was accumulated in the yolk of birds fed Cu-P. The blood and yolk cholesterol levels were significantly lower (P <0.05) in birds fed Cu-P than $CuSO_4$. It was evident that Cu-P was more bioavailable than $CuSO_4$ owing to higher accumulation of Cu in the yolk of the birds fed Cu-P. Cu-P at 50 mg kg^{-1} is recommended in the diets for laying hen. A high level of Cu-P (150 mg kg^{-1}) in laying hens' diets was effective for cholesterol reduction in yolk and blood of experimental birds.

Keywords: blood; copper; chicken; cholesterol; performance; yolk

INTRODUCTION

Copper is a very important trace mineral required for proper functioning of the central nervous, immune and cardiovascular systems and in pigmentation of the skin (Close, 1998). It is also an essential component of several enzyme systems, such as cytochrome oxidase, lysyl oxidase, ceruloplasmin and superoxide dismutase (Klasing, 1998) and metalloenzymes which are important for cellular respiration.

The Cu requirement of laying hen is unknown (NRC, 1994). Dietary mineral supplementation in animal diets has traditionally being achieved through the use of inorganic sources such as sulphates, carbonates, chlorides and oxides. These salts are broken down in the digestive tract to form free ions which are absorbed. However, free ions are very reactive and can form complexes with other dietary molecules making them difficult to absorb or in some cases unavailable for absorption and, therefore, of little benefit to the animals (Close, 1998).

The experiments have shown that Cu regulates cholesterol biosynthesis by reducing hepatic glutathione concentration (Kim *et al.*, 1992). Feeding pharmacological level of Cu has been reported to play important roles in

***Correspondence:** e-mail: vinbjegede@yahoo.com
A.V. Jegede, Federal University of Agriculture, Department of Animal Nutrition, Abeokuta, Nigeria
phone: +2348 035 397 280

Received: May 30, 2014
Accepted: October 8, 2014

lipid metabolism (Bakalli *et al.*, 1995; Chowdhury *et al.*, 2004; Pesti and Bakalli, 1996, 1998). Metal-amino acid chelates have also been reported to be more efficiently absorbed from gut than those provided by inorganic salts (Wedekind *et al.*, 1994). Previous studies with broilers showed considerably higher utilization of copper from organic sources compared to copper sulphate (Jegede *et al.*, 2011). Copper proteinate was also reported to be more effective in reducing plasma cholesterol in growing pullets than copper sulphate (Jegede *et al.*, 2012), although the effect of supplemental Cu levels on egg-yolk cholesterol of laying birds was reported in previous experiments (Lien *et al.*, 2004). Literature sources reporting about effects of various forms of Cu (organic and inorganic) on egg-yolk cholesterol and Cu residues are scarce. This study, therefore, aims to investigate the bioavailability and utilization of organic and inorganic copper sources and their effects on laying performance, blood and yolk cholesterol and copper residue in egg yolk of laying hens.

MATERIALS AND METHODS

This study was carried out according to the research ethics and guidelines of the College of Animal Science and Livestock Production of the Federal University of Agriculture, Abeokuta, Nigeria.

The research work was carried out at the Poultry Unit of the Directorate of University Farms (DUFARM), Federal University of Agriculture, Alabata, Abeokuta, Nigeria (Latitude 7°13^{1}49.46″ N and Longitude 3°26^{1} 11.98″ E). This area lies in the tropical climate with an average rainfall of 1037 mm, mean ambient temperature of about 34 °C and yearly relative humidity of 82 % (Google Earth, 2010).

Table: 1 Gross composition of experimental diets (g kg^{-1})

Ingredients	Layers mash
Maize	440.00
Soybean meal	80.00
Groundnut cake	75.00
Fishmeal	20.00
Wheat offal	270.00
Bone meal	40.00
Oyster shell	71.00
Common Salt	2.50
[a]Premix (Cu free)	2.50
Methionine	2.00
Lysine	1.50
Total	1000.00
Determined Analysis	
Dry matter	898.2
Crude protein	171.0
Crude fibre	58.8
Ether extract	78.8
Ash	93.4
*Energy ME (Kcal kg^{-1})	2661.6
Basal Cu in the diet (mg kg^{-1})	37.24

Vitamins / mineral premix (Godoye) based on 2.5 kg per ton; vit. A: 6000000 IU, vit. D: 400000 IU, vit E: 40000 mg, vit k_3: 800 mg, vit B_1: 2000 mg, vit B_2: 6000 mg, vit. B_6: 5000 mg, vit B_{12}: 25 mg, Niacin: 80000 mg, Panthotenic Acid: 20000 mg, Folic Acid: 1000 mg, Biotin: 8 mg, Manganese: 300000 mg, Iron: 80000 mg, Zinc: 20000 mg, Copper: Nill, cobalt: 80 mg, Iodine: 400 mg, Selenium:40 mg, Choline: 2000 mg, BTH: 25,000 mg Anticaking agent: 6,000 mg; *ME (Kcal kg^{-1}) = 37 × % CP + 81 × % EE + 35 × % NFE (Pauzenga, 1985)

A basal diet was formulated to contain 37.24 mg kg^{-1} Cu. Six experimental diets were subsequently formulated in a 2 × 3 factorial arrangements of two sources of Cu salts ($CuSO_4$, Cu-P) supplemented at three levels of inclusion (50, 100 and 150 mg kg^{-1}) (Table 1). Supplemental Cu salts were included in the basal diet to formulate the experimental diets. Birds included into each group were assigned to the respective experimental diets. The diets were formulated to meet the nutrient requirements of layers according to NRC recommendations (NRC, 1994) and nutrient requirements of poultry in the tropics (Olomu, 1995).

A total of 480 twenty week-old Kabir® layers were randomly allotted to six treatment groups of 80 birds per group. Each treatment consisted of four replications with 10 cages (2 birds per 30.5 × 40.6 cm wire cage) and subjected to a photoperiod of 15 hours light and 9 hours darkness per day. Each group was randomly allotted to one of the six dietary treatments in a 2 × 3 factorial arrangement. The birds were fed layers mash for 294 days (Early, Mid and Late laying periods). Feed and water were supplied *ad libitum*.

The feed samples were dried at 65 °C for 36 h in an oven and milled to pass through 1.0 mm sieve and were analyzed for dry matter (DM), crude fibre (CF), ether extract (EE) and total ash (AOAC, 1995). The nitrogen content of feed samples was determined using the Kjeldahl method and crude protein (CP) was determined by multiplying the N value by 6.25. The metabolizable energy (ME) of feed samples was calculated using the prediction equation M. E. = 37 × % CP + 81.8 × % EE + 35.5 × % NFE (Pauzenga, 1985). The Cu content of the basal diet was determined by igniting the feed sample at 400 °C for 4 h in a muffle furnace. The ash was reconstituted using wet-ashing procedure (James, 1996). Analysis of Cu was done by using a Perkin-Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK). Data on body weight, weight gain and feed intake were collected weekly. Egg production was recorded daily.

Blood samples were obtained from the brachial veins of the birds at the end of early lay (0–14 weeks in lay), mid lay (15–28 weeks in lay) and late lay (29–42 weeks in lay). The blood at 2.5 ml was collected from 5 birds per replicate (20 birds per treatment) into a tube containing Ethylene-diamine-tetra-acetate (EDTA). Plasma was separated from the blood samples by centrifuging the whole blood samples at 3,000 rpm for 15 min into a test tube for plasma lipid analysis. The cholesterol and triglyceride assays of the blood plasma were done by enzymatic–colorimetric method using a RandoxR diagnostic cholesterol kit (BIOLAB with code 80106.2 × 100 ml cholesterol CHOD-PAPR) and a RandoxR diagnostic triglyceride reagent procedure (GPO-PAP Method Randox Laboratory Ltd., UK) respectively (Allain *et al.*, 1974).

Forty eggs per treatment (10 eggs per replicate), sampled at the end of each phase of the laying period, were weighed and hard cooked by immersion into boiling water for 10 minutes. The yolks were individually weighed, dried at 65 °C for 36 hrs, pooled, blended and digested using a modified wet-ashing procedure (James, 1996). Ash was reconstituted in 4 ml of 1N HCl solution and analyzed for copper via atomic absorption spectrophotometry (Perkin-Elmer, Optima 4300DV ICP spectrophotometer) at 324.7 nm (Chiou *et al.*, 1998). Egg total lipid was extracted with chloroform:methanol (2 : 1 v/v) from the dried yolk using the procedure described by Folch *et al.* (1957). Cholesterol determination was done using a commercial test kit for cholesterol analysis (Sigma Chemical Co., St Louise, MO USA). Cholesterol concentration was determined from absorbance read at 500 nm using a spectrophotometer. The data obtained were subjected to completely randomized design of the analysis of variance using the General Linear Models procedure of SAS (SAS Institute, 2002), The experiment was laid out in 2 × 3 factorial arrangement. Significant differences were determined using Duncan's multiple range test at the level of P <0.05 (Duncan, 1955). Each replicate was considered as an experimental unit. The Research Animal Ethic Committee approved this experimental protocol.

RESULTS AND DISCUSSION

The growth and production performance of laying birds fed experimental diet is shown in Table 2. The feed/kg egg and feed per dozen egg were significantly higher (P <0.05) in birds fed diets containing $CuSO_4$ compared to those fed Cu-P during the early lay period (0–14 weeks in lay). At the mid (15–28 weeks) and late (29–42 weeks) laying periods feed per dozen egg was significantly higher (P <0.05) in birds fed diets containing $CuSO_4$ compared to those fed Cu-P. The hen day egg production was significantly (P <0.05) improved by feeding Cu-P compared to $CuSO_4$ throughout the laying periods. There was no significant effect of Cu sources on the body weight, total weight gain and daily feed intake of the birds throughout the laying period. The levels of Cu concentration (Table 2) significantly (P <0.05) influenced hen-day egg production. Birds fed diets containing 50 mg kg^{-1} Cu concentration laid the highest (P <0.05) number of eggs during the early laying period. However, the birds fed diets containing 50 mg kg^{-1} and 100 mg kg^{-1} Cu concentration recorded higher number of eggs laid compared to those fed 150 mg kg^{-1} Cu concentration during the mid laying period. No significant effect (P >0.05) of Cu concentration on hen day egg production was noticed during the late lay period. The levels of Cu supplementation during 29–42

Table 2: Main effects of Cu sources and Cu levels on the performance of lay (0–42 Weeks)

Parameters	Copper Sources		*P*-value	SEM	Copper concentration (mg kg^{-1})^{z}			*P*-value	SEM	Source × concentration
	Cu-P	$CuSO_4$			50	100	150			
					(0–14 weeks in lay)					
Body weight (g)	2064.9	2012.1	0.087	26.4	2026.8	2036.0	2052.5	0.770	7.52	0.857
Total weight gain (g)	374.0	358.7	0.085	7.65	329.3	370.8	398.8	0.767	20.19	0.855
Daily feed intake (g)	110.9	110.1	0.403	0.40	109.7	110.9	110.9	0.528	0.40	0.653
Feed / kg egg	2.58^{b}	2.63^{a}	0.018	0.05	2.59	2.62	2.58	0.257	0.02	0.097
Feed / dozen egg	1.78^{b}	1.99^{a}	0.017	0.11	1.83	1.92	1.91	0.572	0.03	0.999
HDEP (%)	62.5^{a}	58.7^{b}	0.004	1.93	63.04^{a}	59.9^{b}	58.9^{b}	0.021	1.25	0.185
					(15–28 weeks in lay)					
Body weight (g)	2334.6	2295.2	0.089	19.70	2322.8	2302.6	2319.2	0.727	6.22	0.872
Total weight gain (g)	269.7	283.1	0.410	6.70	296.0	266.5	266.7	0.247	9.80	0.773
Daily feed intake (g)	119.3	117.8	0.182	0.75	118.1	119.5	118.1	0.521	0.47	0.473
Feed / kg egg	2.86	2.98	0.459	0.06	2.90	2.95	2.90	0.949	0.02	0.915
Feed / dozen egg	1.89^{b}	2.03^{a}	0.007	0.07	1.91	1.97	2.00	0.220	0.27	0.958
HDEP (%)	72.3^{a}	67.8^{b}	0.0007	2.23	70.2^{a}	72.9^{a}	67.1^{b}	0.002	1.66	0.178
					(29–42 weeks in lay)					
Body weight (g))	2630.9	2588.9	0.131	21.00	2612.8	2624.4	2600.0	0.701	7.05	0.897
Total weight gain (g)	296.3	293.70	0.824	1.30	290.0^{b}	321.8^{a}	280.8^{b}	0.015	12.42	0.148
Daily feed intake (g)	120.5	118.6	0.120	0.95	118.1	120.5	119.9	0.252	0.72	0.478
Feed / kg egg	3.60	4.00	0.005	0.40	3.70	3.80	3.80	0.600	0.03	0.963
Feed / dozen egg	2.80^{b}	3.20^{a}	0.0001	0.20	3.00	3.00	3.10	0.118	0.03	0.063
HDEP (%)	58.5^{a}	49.5^{b}	0.0001	4.50	55.0	54.8	52.2	0.294	0.90	0.809
N	*12*	*12*			*8*	*8*	*8*			

^{ab} – mean on the same row having different superscripts are significantly different (P <0.05); HDEP – hen day egg production; z – added dietary copper to basal copper concentration; N – number of observation per mean; SEM – Standard Error of Mean

Table 3: Main effects of Cu sources and Cu concentration on the Cu in yolk and cholesterol in yolk and blood of layers (0–42 weeks)

Parameters	Cu Sources		*P*-value	SEM	Cu concentrations (mg kg^{-1})^{z}			*P*-value	SEM	Source × concentration
	Cu-P	$CuSO_4$			50	100	150			
0–14 weeks in lay										
Cu in yolk (mg kg^{-1})	1.01^{a}	0.85^{b}	0.0039	0.08	0.77^{b}	0.99^{a}	1.04^{a}	0.0001	0.08	0.7703
Yolk Cholesterol (mg g^{-1})	12.2^{b}	14.2^{a}	0.0003	1.00	14.4^{a}	13.3^{a}	12.1^{b}	0.0002	0.67	0.4509
Blood Cholesterol (mg g^{-1})	90.1^{b}	99.8^{a}	0.0024	4.82	100.1^{a}	94.5^{ab}	90.3^{b}	0.0260	2.82	0.0249
Triglyceride (mg dl^{-1})	54.4^{b}	90.7^{a}	0.0001	18.16	78.7	75.00	64.0	0.0023	4.41	0.0005
15–28 weeks in lay										
Cu in yolk (mg kg^{-1})	1.15^{a}	0.94^{b}	0.0311	0.11	0.89^{b}	1.06^{a}	1.19^{a}	0.0068	0.09	0.8713
Yolk Cholesterol (mg g^{-1})	13.2^{b}	15.2^{a}	0.0003	1.00	15.4^{a}	14.3^{a}	13.1^{b}	0.0020	0.67	0.4509
Blood Cholesterol (mg g^{-1})	103.8^{b}	114.9^{a}	0.0419	5.64	115.7	108.9	103.4	0.0557	3.56	0.8233
Triglyceride (mg dl^{-1})	55.8^{b}	91.7^{a}	0.0001	17.99	73.6^{a}	80.7^{a}	66.9^{b}	0.0192	3.99	0.2061
15–28 weeks in lay										
Cu in yolk (mg kg^{-1})	1.68^{a}	1.56^{b}	0.0311	0.06	1.51^{b}	1.61^{b}	1.75^{a}	0.0068	0.07	0.8713
Yolk Cholesterol (mg g^{-1})	15.5^{b}	17.5^{a}	0.0003	0.50	17.6^{a}	16.6^{a}	15.2^{b}	0.0016	0.68	0.4101
Blood Cholesterol (mg g^{-1})	120.8	127.8	0.0516	3.53	129.5^{a}	127.3^{a}	117.6^{b}	0.0412	3.66	0.8039
Triglyceride (mg dl^{-1})	71.2^{b}	100.9^{a}	0.0001	14.58	90.5^{a}	87.7^{a}	79.9^{b}	0.0164	3.19	0.2181
N	*12*	*12*			*8*	*8*	*8*			

ab – means on the same row having different superscripts are significantly different (P <0.05); *z* – added dietary copper to basal copper concentration; SEM – pooled standard of mean; *N* – number of observation per mean

weeks in lay significantly influenced the total weight gain. The birds fed diets containing 100 mg kg^{-1} Cu gained the highest (P <0.05) weight compared to those fed 50 mg kg^{-1} and 150 mg kg^{-1} Cu. Cu source × Cu concentration did not influence (P >0.05) the growth and production performance throughout the laying period.

Table 3 shows the main effects of Cu sources and Cu concentration on the Cu in yolk, and cholesterol in yolk and blood and yolk triglyceride of laying hens during 0–42 weeks in lay. During the early and mid periods in lay, birds fed Cu-P had higher (P <0.05) accumulation of Cu in the yolk compared to those fed $CuSO_4$. The yolk and blood cholesterol and triglycerides measured were significantly higher (P <0.05) in birds fed $CuSO_4$. However, the effect of Cu sources on blood cholesterol was not noticed during late lay. The Cu content in yolk increased with increased Cu concentrations in the birds fed 100 and 150 mg kg^{-1} Cu having significantly higher (P <0.05) Cu in the yolk compared to those fed 50 mg kg^{-1} Cu. The values of cholesterol in yolk and blood decreased as the levels of Cu supplementation increased. The triglycerides were not significantly (P >0.05) affected by Cu concentration during early lay, but were significantly reduced (P <0.05) as Cu concentration increased during mid and late lay. The interaction of Cu source and Cu concentration significantly influenced plasma triglycerides. Birds fed diets containing 50 mg kg^{-1} $CuSO_4$ had the highest (P <0.05) triglyceride value, while those fed 150 mg kg^{-1} Cu-P had the least (P <0.05) value. Other parameters measured were not significantly influenced (P >0.05) by the interaction "Cu source × Cu concentration" throughout the laying period.

The performance of the laying birds during 0–42 weeks in lay show that the sources of Cu in the diet resulted in a significant variation in feed conversion ratio and hen´s day egg production. This trend was similar throughout the three phases (0–14, 15–28 and 29–40 weeks of lay) representing early, mid and late laying phases of the laying period, respectively. Less feed was consumed by birds fed Cu-P to lay a kilogram and a dozen egg compared to those fed $CuSO_4$. This suggests a better utilization of the diet containing the former and its effectiveness in enhancing better egg production. Idowu *et al.* (2006) reported a better feed conversion ratio (feed consumed per dozen eggs) when layers were fed diets supplemented with organic Cu. The hen´s day egg production was higher in birds fed Cu-P supplemented diets. The better feed consumption per kg egg and per dozen eggs converted to a better hen day egg production. Sheidder and Ceyland (1999) and Tucker *et al.* (2003) noticed significant improvement in egg production when laying hens diets were supplemented with organic minerals. Tanika (2004) reported that the addition of organic Cu increased egg production. Lim and Paik (2006) reported a variable effect of organic Cu, Zn and Mn on the egg production and egg quality. The body weight, weight gain and feed intake of laying birds were not influenced by Cu sources and Cu concentration throughout the laying period.

Increase in Cu concentration over 50 mg kg^{-1} during early lay and 100 mg kg^{-1} during mid lay resulted in a decline in the egg production. Hen´s day egg production decreased with increased levels of Cu. It is evident from this study, that high concentration of Cu inhibited egg production. The decrease in the egg production observed after feeding high Cu concentration for 28 weeks was unexpected though consistent with the results of Pearce *et al.* (1983) and Stevenson *et al.* (1983) who reported a decreased egg production following Cu supplementation in the diets of laying hens.

Moderately high levels of dietary copper seem to reduce cholesterol levels in eggs and meat products, although these effects are sometimes associated with loss of performance (Leeson, 2009). Ankari *et al.* (1998) reported that the reduced egg cholesterol was at the expense of 10 % reduction in the egg production when feeding high levels of Cu. The results of Jackson (1977), Thomas and Goatcher (1976) and Idowu *et al.* (2006) are contrary to the result of this study. The beneficial effect of additional Cu supplementation on egg production was not evident after the mid laying period.

The higher concentration of Cu in yolk of birds fed Cu-P suggests an increased bioavailability of Cu in Cu-P compared to $CuSO_4$. Organic Cu is reported to be more bioavailable in the tissues and organs of broiler chickens (Jegede *et al.*, 2011) The yolk cholesterol, blood cholesterol and triglyceride were significantly lower in laying birds fed Cu-P diets compared to those fed $CuSO_4$ diets throughout the laying period. Lien *et al.* (2004) reported a significant reduction in egg yolk and serum cholesterol of laying birds fed supplemental Cu. The significant reduction in yolk and blood cholesterol by feeding Cu-P shows that proteinate form of Cu was more effective in reducing cholesterol level than sulphate, when fed to laying birds. This observation agreed with the report of Idowu *et al.* (2006). Similar observation was reported by Chromwell *et al.* (1989), that sulphate form of Cu resulted in higher yolk and serum cholesterol level. Egg yolk cholesterol has been reported to be synthesized in the liver of laying hens and transported to the developing follicles via plasma very low density lipoprotein (VLDL) where it is deposited by receptor mediated endocytosis (Nimpt and Shneider, 1991). Cu in yolk was increased as the level of Cu supplementation increased. Similar trend was observed throughout the laying phases. Yolk and blood cholesterol decreased as Cu levels increased from 50–150 mg kg^{-1} in each of the laying phases. This indicated that dietary Cu intake

reduced cholesterol concentration. It is possible that high Cu concentration reduced hepatic glutathione through the stimulation of the enzyme 3-hydroxyl-3-methylglutaryl coenzyme reductase (Kim *et al.*, 1992). The activity of the enzyme is the rate-limiting step of mevalonate and ultimately cholesterol biosynthesis (Valsala and Kurup, 1987). Eggs are rich source of dietary cholesterol and consumption of high level of dietary cholesterol increases the risk of coronary heart disease (CHD; Kritchevsky, 2004). Producing eggs low in cholesterol will be of great interest to egg consumers and this can be better achieved by supplementing the diets of laying birds with Cu-P.

CONCLUSION

More Cu was accumulated in the yolk of laying hens fed Cu-P. The cholesterol content in the yolk and blood of laying hens was reduced in birds fed Cu-P. The overwhelming evidence in this study was that Cu-P® is more bioavailable than $CuSO_4$ and it is more effective in reducing blood and egg yolk cholesterol.

REFERENCES

ALLAIN, C.C – POON, L.S – CHAN, C.S – RICHMOND, W. – FU, P.C. 1974. Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, vol. 20, 1974, p. 470–475.

AOAC.1995. Association of Official Analytical Chemists, 15th ed. Official Method of Analyses, Washington DC.

ANKARI, A. – NAJIB, H. – AL HOZAB, A. 1998. Yolk and serum cholesterol and Production traits, as affected by incorporating a supra-optimal amount of copper in the diet of the Leghorn hen. *British Poultry Science*, vol. 39, 1998, p. 393–397.

BAKALLI, R.I – PESTI, G.M – RAGLAND, W.L. – KONJUFCA, V.H. 1995. Dietary copper in excess of nutritional requirement reduces plasma and breast muscle cholesterol of chicken. *Poultry Science*, vol. 74, 1995, p. 360–365.

CHIOU, P.S – CHEN, K.L. – YU, B. 1998. Effect of dietary organic arsenicals and Cu-Pric sulphate on copper toxicity, liver accumulation and residue in eggs and excreta of laying hens. *Animal Feed Science and Techology*, vol. 73. 1998. p 161–171.

CHOWDHURY, S.D. – PAIK, I.K. – NAMKUNG, H. – LIM, H.S. 2004. Response of broiler chickens to organic copper fed in the form of copper-methionine chelate. *Animal Feed Science and Technology*, vol. 115, 2004, p. 281–283.

CLOSE, W.H. 1998. The role of trace mineral proteinates in pig nutrition. In: Biotechnology In the Feed Industry, *Proceedings of the 14th Annual Symposium* (T.P. Lyons and K.A. Jacques, eds.) Nottingham University Press. Nottingham, U.K. 1998, p. 469–474.

CROMWELL, G.L. – STHLY, T.S. – MONGUE, H.J. 1989. Effect of source and levels of copper on performance and liver copper store in weanling pig. *Journal of Animal Science*, vol. 67, 1989, p. 296–302.

DUNCAN, D. B. 1955. Multiple range and multiple F test. *Biometrics*, vol. 1, 1955, p. 11–42.

FOLCH, G. – LEES, M. – STANLEY, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, vol. 226, 1957, p. 497–509.

IDOWU, O.M.O. – LANIYAN, T.F. – KUYE, O.A. – OLADELE-OJO, V.O. – ERUVBETINE, D. 2006. Effect of copper salts on performance, cholesterol, residues in liver, eggs and excreta of laying hens. *Separata de Archivos de Zootecnia*, vol. 55 (212), 2006.p. 327–338.

JACKSON, N. 1977. The effect of dietary copper sulphate on laying performance, nutrient intake and tissue copper and iron levels of the mature, laying, domestic fowl. *British Journal of Nutrition*, vol. 38, 1977, p. 93–100.

JAMES, C.S. 1996. Analytical Chemistry of Foods. Blackie Academic and professionals, Glascow. 1996.

JEGEDE, A.V. – ODUGUWA, O.O. – BAMGBOSE, A.M. – FANIMO, A.O. – NOLLET, L. 2011. Growth response, blood characteristics and Cu accumulation in organs of broilers fed on diets supplemented with organic and inorganic dietary Cu sources. *British Poultry Science*, vol. 52, 2011, p. 133–139.

JEGEDE, A.V. – ODUGUWA, O.O. – OSO, AO. – FAFIOLU, A.O. – IDOWU, O.M.O. – NOLLET, L. 2012. Growth performance, blood characteristics and plasma lipids of growing pullet fed dietary concentrations of organic and inorganic copper sources. *Livestock Science*, vol. 145, 2012, p. 298–302

KLASING, K.C. 1998. Nutritional modulation of resistance to infectious diseases. *Poultry Science*, (77), pp. 1119–1125.

KIM, J.W. – CHAO, P.Y. – ALLEN, A. 1992. Inhibitionof elevated hepatic glutathione abolishes copper deficiency cholesterolemia. *FASEB journal*, vol. 6, 1992, p. 2467–2471.

KRITCHEVSKY, S.B. 2004. A review of scientific research and recommendations regarding eggs. *Journal of American College of Nutrition*, vol. 23 (6), 2004, p. 596S–600S

LEESON, S. 2009. Copper metabolism and dietary needs. *World's Poultry Science Journal*, vol. 65, 2009, p. 353–366. 2009.

LIEN, T.F. – CHEN, K.L. – WU, C.P. – LU, J.J. 2004. Effect of supplemental copper and chromium on the serum and egg traits of laying hens. *British Poultry Science*, vol. 45 (4), 2004, p. 535–539.

LIM. H.S. – PAIK, I.K. 2006. Effect of dietary supplementation of copper chelates in the form of methionine, chitosan and yeast in laying hens. *Asian-Australian Journal of Animal Science*, vol.19, 2006, p. 1174–1178. 2006.

NATIONAL RESEARCH COUNCIL. 1994. Nutrient Requirements of Poultry, 9th Revised edition. National Academy Press, Washington, D.C. 1994.

NIMPT, J. – SCHNEIDER, W.J. 1991. Receptor – mediated lipoprotein transport in laying hens. *Journal of Nutrition*, vol. 121, 1991, p. 1471–1474.

OLOMU, J. M.1995. Monogastric Nutrition: Principle and practice. 1st edition, Benin, Nigeria. 1995, p. 75–82.

PAUZENGA, U. 1985. Feeding parent stock. *Zootechnica International*, pp. 22–24.

PEARCE, J. – JACKSON, N. – STEVENSON, M.H. 1983. The effects of dietary intake and of dietaryconcentration of copper sulphate on the laying domestic fowl: Effects of some aspects of lipid, carbohydrate and amino acid metabolism. *British Poultry Science*, vol. 24, 1983, p. 337–348.

PESTI, G.M. – BAKALLI, R.I. 1996. Studies on the feeding of Cu-Pric sulfate pentahydrate and Cu-Pric citrate to broiler chickens. *Poultry Science*, vol. 75, 1996, p. 1086–1091.

PESTI, G.M. – BAKALLI, R.I. 1998.Studies on the effect of feeding Cu-Pric sulfate pentahydrate to laying hens on egg cholesterol content. *Poultry Science*, vol.77, 1998, p. 1540–1545.

SHEIDDER, S.E. – CEYLAND, N. 1999. Effects on Mn and Zn Proteinates (Eggshell 49) on egg shell quality of laying hens. *Proceedings of the VIII Euopean Symposium* on the quality of eggs and egg products, Bologna, Italy. vol. 2, 1999, p.187–192.

STEVENSON, M.H. – PEARCE, J. – JACKSON, N. 1983.The effects of dietary intake and of dietary concentration of copper sulphate on the laying domestic fowl: Effects on laying performance and tissue mineral contents. *British Poultry Science*, vol. 24, 1983, p. 327–335.

TANIKA, O.D. 2004. Mineral utilization in poultry as affected by virginiamycin or mineral source. An MSc thesis submitted to graduate faculty of the Louisiana State University. 2004.

THOMAS, M.C – GOATCHER, W.D. 1976. High levels of copper in layers diets. *Poultry Science*, vol. 55, 1976, p. 1608. (Abstract).

TURKER, L.A. – KENYON, S. – WILSON, S. 2003. Benefits of organic minerals in Commercial laying hen performance and egg quality. *Proceedings of the 16th European Symposium* on the quality of eggs and egg products. Sept. 23–26 Ploutragan France, 2003. p. 198–202.

VALSALA, P. – KURUP, P.A. 1987. Investigation on the mechanism of hypercholesterolemia observed in Cu deficiency in rats. *J. Bioscience* (Bangalore), vol. 12, 1987, p. 137–142.

WEDEKIND, K.J. – LEWIS, A.J. – GIESMANN, M.A. – MILLAR, P.S. 1994. Bioavailability of zinc from inorganic and organic sources for pigs fed corn-soybean meal diets. *Journal of Animal Science*, vol. 72, 1994. p. 2681–2689.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 48, 2015 (1): 37–42

ISSN 1337-9984

THE INFLUENCE OF THE OOPLASM ON DNMT1 AND DNMT3A GENE EXPRESSION

M. MOROVIČ[1]*, F. STREJČEK[1], M. PERNIŠ[1], O. ØSTRUP[2, 3], A. LUCAS-HAHN[1], B. PETERSEN[4], H. NIEMANN[4], P. HYTTEL[2], J. LAURINČÍK[1]

[1]Constantine the Philosopher University, Nitra, Slovakia
[2]University of Copenhagen, Faculty of Life Sciences, Department of Basic Animal and Veterinary Sciences, Denmark
[3]Norwegian Center for Stem Cell Research, Stem Cell Epigenetics Laboratory, IBM, Faculty of Medicine, University of Oslo, Oslo, Norway
[4]Institute of Farm Animal Genetics (FLI), Mariensee, Neustadt, Germany

ABSTRACT

Developmental incompetence of embryos constructed by somatic cell nuclear transfer is caused by insufficient reprogramming of the transferred nucleus to a state equivalent to that of an early embryonic nucleus. Preceding studies have shown that the transcription of DNA methyltransferase (DNMT) genes in mammalian oocytes and preimplantation embryos is a species-dependent process and the incomplete DNA methylation correlates with the nuclear transfer failure rate in mammals. In the present study the transcription of DNMT1 and DNMT3a genes in early embryonic stages of intergeneric nuclear transfer (iSCNT) embryos (bovine, porcine) was detected by RT-PCR. Based on the diverse timing of major genome activation during embryonic development in bovine and porcine embryos, the strong influence of the ooplasm on transferred somatic cell nucleus was expected. Despite the presence of DNMT1 and DNMT3a mRNA of maternal origin, expression of somatic DNMT1 and DNMT3a genes was not detected and the development of intergeneric embryos stopped at 4-cell stage. These results indicate that the species-specific epigenetic reprogramming during early embryogenesis is strongly influenced by ooplasm environment.

Keywords: bovine embryos; porcine embryos; intergeneric nuclear transfer; DNMT1 and DMT3a gene expression

INTRODUCTION

During embryonic development different cells and tissues gain different programs of gene expression. It is thought that this is mainly regulated by epigenetic modifications such as DNA methylation, histone tail modifications and non-histone proteins that bind to the chromatin (Bird, 2002; Li, 2002). For most cell types in the body, these epigenetic marks become fixed when the cells differentiate or exit the cell cycle. In normal developmental situations, some cells may undergo major epigenetic reprogramming, involving the removal of epigenetic marks in the nucleus, followed by establishment of a different set of marks (Rideaut *et al.*, 2001).

Epigenetic modifications occur during the life cycle in two phases: during gametogenesis and preimplantation development. Primordial germ cells (PGCs) originate from somatic tissue and develop into mature gametes over an extended period of time (Morgan *et al.*, 2005). Their genome undergoes DNA demethylation in the embryo between E11.5 and E12.5, including imprinted genes. Following demethylation, the genomes of the gametes are *de novo* methylated and acquire imprints; this process continues up to E18.5 in males and in maturing oocytes before ovulation in

***Correspondence:** e-mail: mmorovic@ukf.sk
Martin Morovič, Constantine the Philosopher University, Nitra, Slovakia,
Tr. A. Hlinku 1, 949 74, Nitra, Slovakia,
phone.: +421 37 640 87 12

Received: October 22, 2014
Accepted: March 30, 2015

females (Saitou *et al.*, 2012). After the fertilization the next round of reprogramming begins and lasts during the early embryonic development. The paternal genome actively decreases its methylation level and its histones absent some modifications of polypeptide tails in comparison to histones in the maternal pronucleus. The embryo genome is passively demethylated during early cell cycles before blastulation. Despite this methylation loss, imprinted genes maintain their methylation through this preimplantation reprogramming. *De novo* methylation roughly coincides with the differentiation of the first two lineages of the blastocyst stage, and the inner cell mass (ICM) is hypermethylated in comparison to the trophectoderm (TE). These early lineages set up the DNA methylation status of their somatic and placental derivatives. Histone modifications may also reflect this DNA methylation asymmetry. Particular classes of sequences may not conform to the general genomic pattern of reprogramming shown (Mann *et al.*, 2003).

DNA methylation critically depends on the activity of specific enzymes, the DNA methyltransferases (DNMTs). Five mammalian cytosine DNA methyltransferases have been identified to date (Bestor, 2000). DNA-methyltransferase 1 (DNMT1) is a maintenance enzyme that is responsible for restoring methylation of hemi-methylated CpG dinucleotides after DNA replication (Bestor, 1992). An oocyte-specific form, DNMT1o, is present at high concentrations in mature oocytes and lasts during early zygotic stages. Gene targeting experiments suggest that DNMT1o has a role in maintaining methylation marks at maternally imprinted genes in mice (Howell *et al.*, 2001).

Additional types of the vertebrate cytosine methyltransferase are DNMT3a and DNMT3b. These enzymes catalyze *de novo* methylation and are thus essential for establishing DNA methylation during development (Okano *et al.*, 1998).

The aim of this study was to detect the expression of DNMT1 and DNTM3a genes at different stages of embryonic development of bovine vs. porcine intergeneric nuclear transfer (iSCNT) embryos and compare these results with expression in parthenogenetically activated porcine and bovine oocytes. The influence of different ooplasm environment on DNMT1 and DNMT3a genes expression was expected.

MATERIAL AND METHODS

Oocyte recovery and *in vitro* maturation

Bovine cumulus-oocyte complexes (COCs) were isolated by ovarian slicing from slaughtered cattle of different origin. Selected COCs were matured *in vitro* in tissue culture medium 199 (TCM 199; Sigma-Aldrich, Germany) containing L-glutamine and 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Sigma, St. Louis, MO, USA) supplemented with 22 mg mL^{-1} pyruvate, 2.2 mg mL^{-1} $NaHCO_3$, 50 mg mL^{-1} gentamicin, 10 IU mL^{-1} eCG, and 5 IU mL^{-1} of hCG (Suigonan, Intervet, Tönisvorst, Germany). Oocytes were *in vitro* matured in 100 ml droplets of maturation medium under silicone oil for 19 h at 39 °C and 5 % CO_2 in humidified atmosphere. After maturation, cumulus cells were completely removed by vortexing with 0.1 % hyaluronidase in phosphate-buffered saline (PBS). MII oocytes were chosen for further experiments.

Ovaries from pre-pubertal gilts were collected from local abattoirs. Oocytes with at least three complete layers of cumulus cells were collected and matured *in vitro* for 40 h (Holker *et al.*, 2005). Then, matured oocytes were transferred to TL-HEPES supplemented with 0.1 % hyaluronidase and incubated for 5 min. Cumulus cells were removed by repeated pipetting. Denuded oocytes were transferred to Tyrode's lactate HEPES (TL HEPES) (Sigma, St. Louis, MO, USA) covered with mineral oil (Silicone fluid DC200, Serva Biochemica, Heidelberg).

iSCNT and parthenogenetic activation

For production of nuclear transfer embryos the MII oocytes were placed into TCM 199 medium enriched with gentamicin, Na-pyruvat, $NaHCO_3$ and BSA (TCM-air) containing 5 mg mL^{-1} Hoechst 33342 and 7.5 mg mL^{-1} cytochalasin B for 8 min.

The oocytes were enucleated by aspirating the first polar body and the MII plate. A single fibroblast was transferred into the perivitelline space of the recipient enucleated oocyte. Oocyte-fibroblast cell couplets were electrically fused in a 0.285 mM mannitol-based medium containing 0.1 mM $MgSO_4$ and 0.05 bovine serum albumin (BSA) with Multipolator® machine (Eppendorf AG, Germany). Fused cell hybrids (iSCNT) and intact oocytes (parthenogenetic activation) were chemically activated by 5 µM ionomycin. After activation, the embryos were washed and cultured in 30 ml droplets of synthetic oviduct fluid medium supplemented with amino acids and BSA (SOFaa; Sigma-Aldrich, Germany) supplemented with 0.4 % BSA at 39 ºC in 5 % O_2, 5 % CO_2 and 90 % N_2 in modular incubation chambers. The embryos were collected at 2-, 4-, 8- and 16-cell stages for further processing.

RNA extraction and RT-PCR

Total RNA was extracted from pools (triplicates) of 10 embryos each at 2-cell, 4-cell, 8-cell and 16-cell stages from iSCNT/parthenogenetic embryos using Dynabeads® mRNA DIRECT™ kit (Life Technologies, USA). Subsequently, RT was carried out in total volume of 20 µl using 2.5 µM random hexamers. The species-specific primers for DNMT1 and DNMT3a genes were designed for determination of *de novo* synthesis

Table 1: Species specific primer sequences designed for detection of DNMT1 and DNMT3a genes expression in bovine and porcine embryos

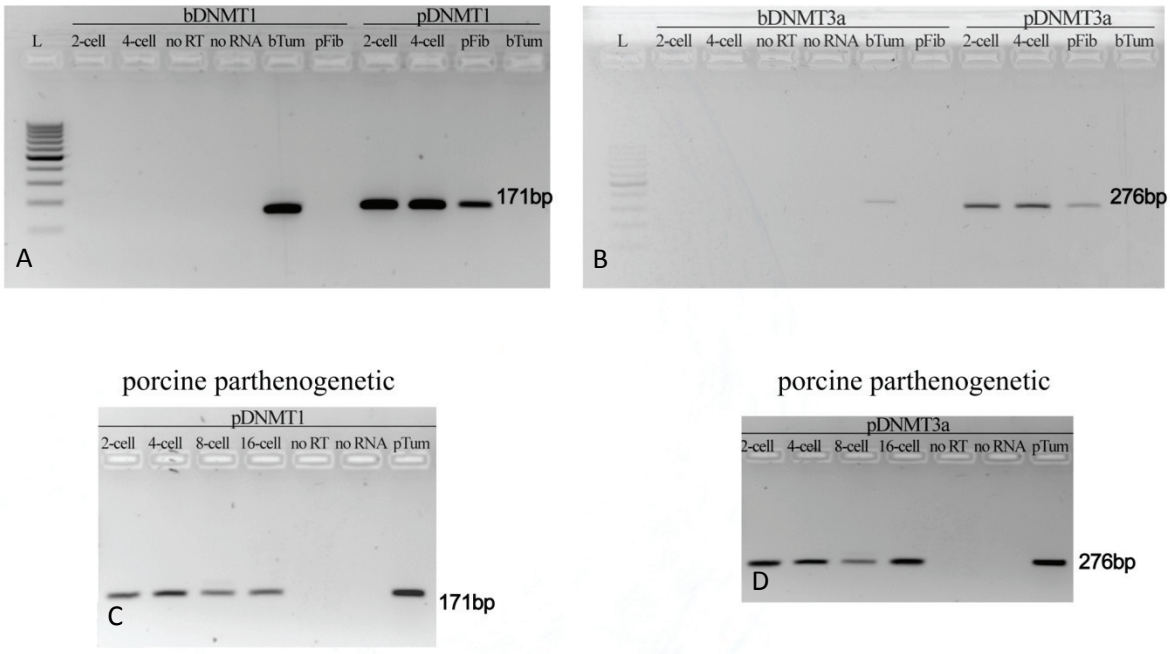
Gene	Sequence (5´-3´)	Length
bDNMT1	**F**-ACCGAGTGCTTGCAGTACCT **R**-GCTGAGGCAAATCCTCGTAA	154 bp
bDNMT3a	**F**-CAAAGCAGCTGACGATGAAC **R**-GCAGGACCTCGTAGATAGCC	296 bp
pDNMT1	**F**-AGTGCGTTCAGTGTGGACAG **R**-CGGTCAGTTTGTGTTGGAGA	171 bp
pDNMT3a	**F**-CAGTACGACGATGACGGCTA **R**-GTCAAATTCCTGGTCGTGGT	276 bp

b – bovine specific, p – porcine specific

of epigenetic enzymes (Tab. 1). As positive controls, porcine and bovine parthenogenetic embryos were used.

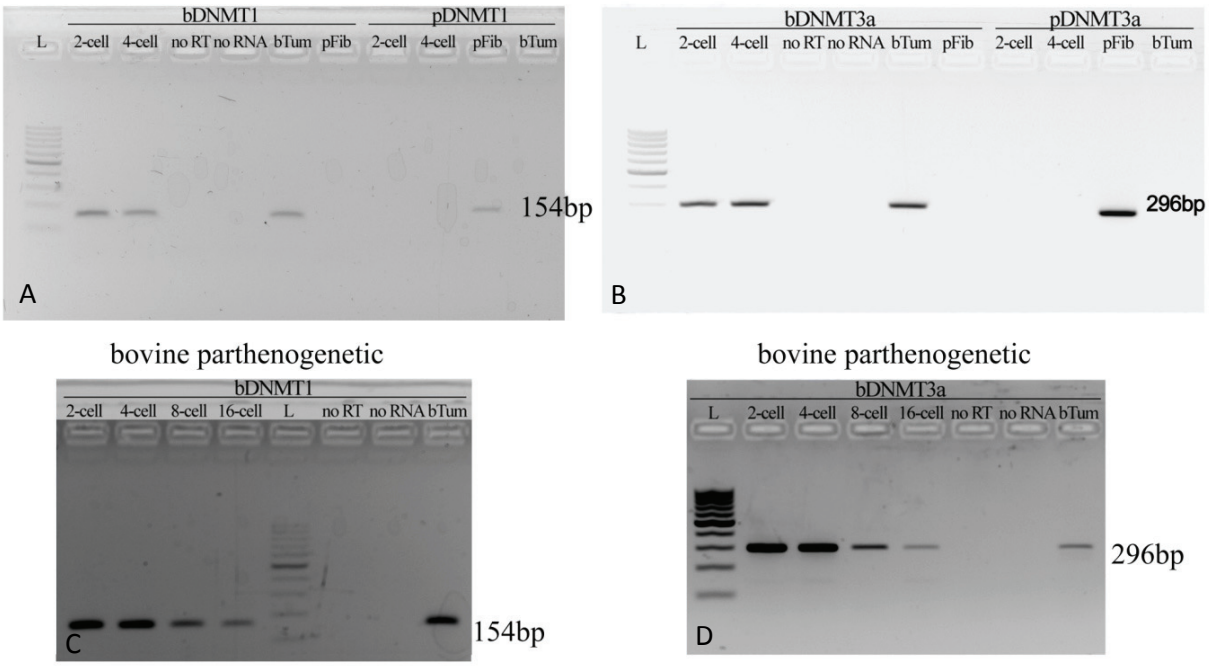
RESULTS AND DISCUSSION

All the pools were done in triplicate and contained iSCNT embryos (n = 10). The species-specific primers for DNMT1 and DNMT3a genes were designed for determination of *de novo* synthesis of epigenetic enzymes. Gene transcription for bovine DNMT1 (bDNMT1) and DNMT3a (bDNMT3a) was not observed in 2- and 4-cell stage embryos generated by bovine fibroblast transfer into the porcine ooplasm, however, positive results were obtained by using primers for pig DNMT1 (pDNMT1) and DNMT3a (pDNMT3a) (Fig. 1A, B). As positive



bTum – bovine tumour cells (control of primer specificity), pFib – porcine fibroblasts (control of primer specificity), pTum – porcine tumour cells (control of primer specificity), noRT – no reverse transcriptase in mastermix (negative PCR control), no RNA – no RNA in mastermix (negative PCR control), L – 100 bp DNA ladder (Abnova, Germany)

Fig. 1: (A-B) bovine DNMT1 (bDNMT1) (A) and bovine DNMT3a (bDNMT3a) (B) gene expression in comparison with porcine DNMT1 (pDNMT1) (A) and porcine DNMT3a (pDNMT3a) (B) in iSCNT embryos constructed by injecting the bovine fibroblast into the porcine oocyte indicates the lack of *de novo* transcription of bovine-specific epigenetic enzymes. (C-D) porcine DNMT1 (pDNMT1) (C) and porcine DNMT3a (pDNMT3a) (D) gene expression in porcine embryos after parthenogenetic activation indicates the presence of porcine-specific transcripts of epigenetic enzymes from 2-cell to 16-cell stage



bTum – bovine tumour cells (control of primer specificity), pFib – porcine fibroblasts (control of primer specificity), noRT – no reverse transcriptase in mastermix (negative PCR control), no RNA – no RNA in mastermix (negative PCR control), L – 100 bp DNA ladder (Abnova, Germany)

Fig. 2: (A-B) bovine DNMT1 (bDNMT1) (A) and bovine DNMT3a (bDNMT3a) (B) gene expression in comparison with pig DNMT1 (pDNMT1) (A) and pig DNMT3a (pDNMT3a) (B) in iSCNT embryos constructed by injecting the porcine fibroblast into the bovine oocyte indicates the lack of *de novo* transcription of porcine-specific epigenetic enzymes. (C-D) bovine DNMT1 (pDNMT1) (C) and bovine DNMT3a (pDNMT3a) (D) gene expression in bovine embryos after parthenogenetic activation indicates the presence of bovine-specific transcripts of epigenetic enzymes from 2-cell to 16-cell stage

controls, porcine parthenogenetic embryos were used (Fig. 1C, D).

In the 2- and 4-cell stage embryos constructed using porcine fibroblast and bovine ooplasm only the bovine specific primers (bDNMT1, bDNMT3a) showed positive signals (Fig. 2A, B). Based on the different timing of major genome activation during the embryonic development in bovine and porcine embryos, the strong influence of ooplasm on introduced fibroblast was expected. Despite the mRNA presence of DNMT1 and DNMT3a enzyme of the oocyte origin, *de novo* transcription of somatic DNMT1 and DNMT3a genes was not detected and iSCNT embryos did not develop beyond the 4-cell stage. In parthenogenetic embryos, the continuous supplementation of epigenetic enzyme transcripts is maintained by the major genome activation including the expression of DNMT1 and DNMT3a genes (Fig. 2C, Fig. 2D).

DNA methyltransferase 1 (DNMT1) is very intensively studied DNA methyltransferase and is thought to be responsible for maintaining the methylation patterns following the DNA replication (Dean *et al.*, 1998). DNMT3a and DNMT3b are responsible for *de novo* DNA methylation (Okano *et al.*, 1999). Previous studies have shown species-dependent expression patterns of DNMT genes in mammalian oocytes and preimplantation embryos (Vassena *et al.*, 2005) and also a correlation between incomplete DNA methylation and the lack of NT success in mammals (Dean *et al.*, 2001; Bortvin *et al.*, 2003). DNMT1 and DNMT3a mRNA in cattle were detected continuously from the 2-cell stage to the blastocyst stage produced *in vitro* (Golding and Westhusin, 2003), but the relative abundance of the Dnmt1 transcript significantly varied between *in vivo* and *in vitro* produced bovine embryos, with *in vivo* produced embryos expressing significantly less DNMT1 (Wrenzycki *et al.*, 2001). Similarly, a significant increase in DNMT1 expression at the 8-cell stage was reported in porcine fibrillar sphere NT embryos compared with either *in vivo* produced or fetal porcine skin originated sphere

stem cell NT embryos (Zhu *et al.*, 2004). Comparing IVF and *in vivo* derived embryos, the relative abundance of DNMT1 transcript was significantly increased in NT embryos and only a low level of DNMT1 transcription was found for *in vivo* derived embryos (Kumar *et al.*, 2007). DNMT3a mRNA was expressed at all stages analyzed with a significant increase in abundance between the 8-cell and morula to blastocyst stages which coincides with initiation of cell differentiation processes.

Intergeneric SCNT has been considered as a very effective technique for studying the influence of the ooplasm on epigenetic reprogramming of introduced genome which subsequently leads to different aberrations during the embryogenesis. Therefore, the production of live offspring from intergeneric embryos has not been reported, and embryonic development beyond the stage of major embryonic genome activation is significantly hindered (Østrup *et al.*, 2011). Remodeling and reprogramming of the transferred genome is essential for successful embryonic development following SCNT.

The presented study is focused on the influence of different ooplasms (porcine and bovine) on the activation of *de novo* synthesis of DNMT1 and DNMT3a mRNA. It is already known that the matured mammalian oocytes are equipped with efficient amount of epigenetic enzymes and their transcripts required for initial epigenetic processes during early embryogenesis (Vassena *et al.*, 2005). However, these enzymatic supplies are not sufficient for complete reprogramming of transferred nuclei during SCNT, what results in embryonic development breakdown. The low efficiency of NT seems to be related to the inability of a somatic nucleus to undergo the normal changes in methylation as indicated by increased levels of DNMT1 or to the lack of *de novo* methylation triggered by low DNMT3a expression (Kumar *et al.*, 2007). To distinguish the source of epigenetic enzymes (ooplasmic and *de novo* synthesized) intergeneric nuclear transfer (pig vs. bovine and *vice versa*) was applied in combination with species-specific RT-PCR primers detecting DNMT1 and DNMT3a transcription. Our results significantly show the incompetence of introduced somatic nucleus to initiate and establish the continual expression of observed genes in the environment of ooplasm of different origin. This malfunction has led to embryonic development breakdown at the 4-cell stage.

CONCLUSION

Our results strongly indicate species-dependent and maternally controlled regulation of epigenetic reprogramming during early embryogenesis and importance of epigenetic enzymes in proper embryonic development.

ACKNOWLEDGMENTS

This work was co-funded by the European Community under project no 26220220180: Building Research Centre "AgroBioTech".

REFERENCES

BESTOR, T. H. 1992. Activation of mammalian DNA methyltrasferase by cleavage of Zn binding regulatory domain. *EMBO J*. 11, 1992, p. 2611–2617.

BESTOR, T. H. 2000. The DNA methyltransferases of mammals. *Hum Mol Genet*, vol. 9, 2000, p. 2395–2402.

BIRD, A. 2002. DNA methylation patterns and epigenetic memory. *Genes Dev.*, 16, 2002, p. 6–21.

BORTVIN, A. – EGGAN, K. – SKALETSKY, H. – AKUTSU, H. – BERRY, D. L. – YANAGIMACHI, R. – PAGE, D. C. – JAENISCH, R. 2003. Incomplete reactivation of Oct4 related genes in mouse embryos cloned from somatic nuclei. *Development*, 130, 2003, p. 1673–1680.

DEAN, W. – BOWDEN, L. – AITCHISON, A. – KLOSE, J. – MOORE, T. – MENESES, J. J. – REIK, W. - FEIL, R. 1998. Altered imprinted gene methylation and expression in completely ES cell-driven mouse fetuses: association with aberrant phenotypes. *Development*, 125, 1998, p. 2273–2282.

DEAN, W. – SANTOS, F. – STOJKOVIC, M. – ZAKHARTCHENKO, V. – WALTER, J. – WOLF, E. – REIK, W. 2001. Conservation of methylatio reprogramming in mammalian development: aberant reprogramming in cloned embryos. *Proc Natl Acad Sci USA*, 98, 2001, p. 13734–13738.

GOLDING, M. C. – WESTHUSIN, M. E. 2003. Analysis of DNA (cytosine 5) methyltransferase mRNA sequence and expression in bovine preimplantation embryos, fetal and adult tissues. *Gene Expr Patterns,* 3, 2003, p. 551–558.

HOLKER, M. – PETERSEN, B. – HASSEL, P. 2005. Duration of *in vitro* maturation of recipient oocytes affects blastocyst development of cloned porcine embryos. *Cloning Stem Cells,* 7, 2005, p. 35–44.

HOWELL, C. Y. – BESTOR, T. H. – DING, F. 2001. Genomic imprinting disrupted by a maternal effect mutation in the Dnmt1 gene. *Cell*, 104, 2001, p. 829–838.

KUMAR, B. M. – JIN, H. F. – KIM, J. G. – OCK, S. A. – HONG, Y. – BALASUBRAMANIAN, S. – CHOE, S. Y. – RHO, G. J. 2007. Differential gene expression patterns in porcine nuclear transfer embryos reconstructed with fetal fibroblasts and mesenchymal stem cells. *Developmental Dynamics*, vol. 236, issue 2, 2007, p. 435–446.

LI, E. 2002. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat. Rev. Genet.*, 3, 2002, p. 662–673.

MANN, M. R. W. – CHUNG, Y. G. – NOLEN, L. D. – VERONA, R. I. – LATHAM, K. E. – BARTOLOMEI, M. S. 2003. Disruption of Imprinted Gene Methylation and Expression in Cloned Preimplantation Stage Mouse Embryos. *Biology of Reproduction*, vol. 69, no. 2, 2003, p. 902–914.

MORGAN, H. D. – SANTOS, F. – GREEN, K. – DEAN, W. – REIK, W. 2005. Epigenetic reprogramming in mammals. *Hum. Mol. Genet*. vol. 14 (suppl. 1), 2005, p. 47–58.

OKANO, M. – XIE, S. – LI, E. 1998. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat. Genet*., 19, 1998, p. 219–220.

OKANO, M. – BELL, D. – HABER, D. – LI, E. 1999. DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell*, 99, 1999, p. 247–257.

ØSTRUP, O. – STREJCEK, F. – PETROVICOVA, I. – HAHN, A. L. – MOROVIC, M. – LEMME, E. – PETERSEN, B. – LAURINCIKOVA, N. – NIEMANN, H. – LAURINCIK, J. – HYTTEL, P. 2011. Role of ooplasm in nuclear and nucleolar remodeling of intergeneric somatic cell nuclear transfer embryos during the first cell cycle. *Cellular Reprogramming*, vol. 13, no. 2, 2011, p. 145–155.

RIDEOUT, W. M. – EGGAN, K. – JAENISCH, R. 2001. Nuclear cloning and epigenetic reprogramming of the genome. *Science*, 293, 2001, p. 1093–1098.

SAITOU, M. – KAGIWADA, S. – KURIMOTO, K. 2012. Epigenetic reprogramming in mouse pre-implantation development and primordial germ cells. *Development,* vol. 139, 2012, p. 15–31.

VASSENA, R. – DEE SCHRAMM, R. – LATHAM, K. E. 2005. Species-dependent expression patterns of DNA methyltransferase genes in mammalian oocytes and preomplantation embryos. *Mol. Reprod. Dev.*, 72, 2005, p. 430–436.

WRENZYCKI, C. – HERRMANN, D. – KESKINTEPE, L. – MARTINS, A. Jr. – SIRISATHIEN, S. – BRACKETT, B. – NIEMANN, H. 2001. Effects of culture system and protein supplementation on mRNA expression in preimplantation bovine embryos. *Hum. Reprod.*, 16, 2001, p. 893–901.

ZHU, H. – CRAIG, J. A. – DYCE, P. W. – SUNNEN, N. – LI, J. 2004. Embryos derived from porcine skin-derived stem cells exhibit enhanced preimplantation development. *Biol. Reprod.*, 71, 2004, p. 1890–1897.

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 48, 2015 (1): 43–48

ISSN 1337-9984

Short communication

THE EFFECT OF *CURCUMA LONGA* DRIED POWDER IN THE DIET ON WEIGHT GAIN OF RABBIT DOES

M. FÖLDEŠIOVÁ[1]*, A. BALÁŽI[2], Ľ. CHRASTINOVÁ[2], P. CHRENEK[1, 2]

[1]Slovak University of Agriculture, Faculty of Biotechnology and Food Science, Nitra, Slovak Republic
[2]National Agricultural and Food Centre, Research Institute for Animal Production Nitra, Lužianky, Slovak Republic

ABSTRACT

The aim of the present study was to evaluate the effect of different concentrations of *Curcuma longa* plant additive to the diet on the weight gain of rabbit does. Rabbit females (n = 45) of New Zealand White breed of the same age (2 months) were used in the experiment. Rabbit does in the control group (C; n = 15) were fed a commercially available feed. In the experimental groups 5 g (E1; n = 15) and 20 g (E2; n = 15) of *Curcuma longa* dried powder was added to 100 kg feed mixture. The highest average weight gain per week was observed in the first experimental group (E1; 235.7 ± 22.35 g) when compared to the control (C; 216.2 ± 25.59 g) and the second experimental (E2; 220.5 ± 31.94 g) groups. The highest total average weight gain of rabbit does was observed in the E1 (2103.3 ± 63.22 g) compared to the second (E2, 2045 ± 84.36 g) and control (C, 1950 ± 126.88 g) groups. In conclusion, supplementation of *Curcuma longa* plant powder to the commercially diet for rabbit positively affected weight gains in rabbit does. Therefore, to improve growth performance, further studies are required to define an optimal supplementation of *Curcuma longa* to the rabbit diet.

Keywords: rabbit does; *Curcuma longa*; weight gain

INTRODUCTION

In the last years, rabbit production on the commercial level acquired increasing interest due to their prolificacy, rapid growth rate and meat yields (Savietto *et al.*, 2015; Mínguez, 2014; Ricke *et al.*, 2012; Gondret *et al.*, 2005).

More suitable composition of feed mixture or administration of natural additives at a suitable concentration might be beneficial in livestock farming without negative effect on the environment and the animal as an individual (Wareth *et al.*, 2014; Githiori *et al.*, 2003; Amber *et al.*, 2004; Namkung *et al.*, 2004).

Positive effect of biologically active substances and extracts from *Acacia saligna* (Tamir and Asefa, 2009), *Morus australis* (Wu *et al.*, 2013), *Yucca schidigera* (Földešiová *et al.*, 2013), *Agave tequilana* (Sáyago-Ayerdi *et al.*, 2014), *Saposhnikovia divaricata*, *Lonicera japonica*, *Chelidonium majus* (Park *et al.*, 2014) a. o. on weight gain were observed in lambs, mice, rabbits, rats and broilers.

Curcuma longa Linn, a member of *Zingiberaceae* family, commonly known as turmeric, originate in tropical and subtropical regions of India and China. Medicinal properties of *Curcuma longa* have been attributed primarily to curcuminoids, which are located

***Correspondence:** e-mail: mt.foldesiova@gmail.com
Martina Földešiová, Institute of Farm Animal Genetics and Reproduction, Research Institute for Animal Production in Nitra, National Agricultural and Food Centre, Lužianky, Slovak Republic, phone: +421 37 6546285, Fax: +421 37 654 61 89

Received: February 3, 2015
Accepted: March 3, 2015

in the plant rhizome. Curcumin (diferuloylmethane) is the most important fraction of *Curcuma longa* (Araújo and Leon, 2001). Polyphenol curcumin, extracted from dried rhizomes of the plant, acts through inhibition of mitogen-activated protein kinases (Jeon *et al.*, 2013). Although turmeric is consumed every day in Asian countries, no toxic effect on the health of population was found (Ammon and Wahl, 1991).

Beneficial effects of curcuma on the animal liver protection (Deshpande *et al.*, 1998), treatment of the human diabetes (Eshrat and Hussain, 2002), steroidogenesis, proliferation and apoptosis in porcine ovaries (Kádasi *et al.*, 2012) were found.

In particular, yellow rhizome, containing curcumin, is used to treat digestive, neuropsychiatric diseases (Mei *et al.*, 2011) and osteoarthritis in combination with a ginger (Low Dog, 2006).

Furthermore, the plant has also been shown to possess high antioxidant (Maheshwari *et al.*, 2006; Wojdyło *et al.*, 2007) anticarcinogenic (Hatcher *et al.*, 2008), antibacterial (De *et al.*, 2009) and anti-inflammatory (Jurenka, 2009) effect.

The objective of this study was to evaluate the effect of different concentrations of *Curcuma longa* dried powder as an additive to the diet on the average weight gain (g) per week and the total average weight gain of the rabbit does.

MATERIAL AND METHODS

Animals

Two months old clinically healthy rabbit does of the New Zealand White line (NAFC Nitra, SR) were used in this experiment. The animals were housed in individual cages, under a constant photoperiod of 14 hours of light day, average relative humidity 60 ± 5 % and temperature 17 ± 3 ºC. The rabbits were fed *ad libitum* and water was provided *ad libitum* with nipple drinkers.

Rabbit does (n = 45) were divided into three groups: control (C; n = 15) and two experimental groups (E1; n = 15 and E2; n = 15). The does in the control group were fed a commercially available complete feed mixture. In experimental groups the complete feed mixture was enriched with *Curcuma longa* dried powder at the concentrations of 5 g (E1) and 20 g (E2) per 100 kg. The animals were fed for 63 days (9 weeks) and weighted weekly.

The treatment of the animals was approved by the Ministry of Agriculture and Rural Development of the Slovak Republic, no. SK P 28004 and Ro 1488/06-221/3a.

Statistical analysis

The data were analysed by the t-test using Sigma Plot statistical package (Systat Software Inc., Germany).

RESULTS AND DISCUSSION

In our study we tested effect of the addition of *Curcuma longa* dried powder to the complete feed mixture on the average weight gain (g) per week and the total average weight gain (g) of rabbit does.

The highest average weight gain of rabbit does per week (g) was found in the first experimental group (E1; 235.7 ± 22.35) when compared to the control (C; 216.2 ± 25.59) and the second experimental group (E2; 220.5 ± 31.94) (Table 1).

Total average weight gain (g) was higher in the first experimental group (E1; 2103.3 ± 63.22) compared to the second (E2; 2045 ± 84.36) and control (C; 1950 ± 126.88) groups (Table 2, Figure 1).

In accordance to Holder *et al.* (1978) we suggest that slightly lower weight gain in the second experimental group compared to the first experimental group might be due to the higher concentration of *Curcuma longa* in feed mixture, which can cause poor absorption from the intestine.

In our study, we found that the addition of both concentrations (5 g and 20 g kg^{-1} diet) of *Curcuma longa* dried powder into rabbit complete feed mixture had a positive effect on average weight gain per week and total average weight gain of analysed rabbit does.

Positive effect of *Curcuma longa* powder to the diet was also found in broiler chickens. Higher weight gain was observed in the birds fed the diet containing *Curcuma longa* at level of 0.5 %, compared to the birds receiving 0.25 %, 1 % and control birds (Al-Sultan, 2003). Osava *et al.*, (1995) and Al-Sultan (2003) attributed the increase in the body weight gain to the antioxidant activity of *Curcuma longa*.

Moreover, Durrani *et al.* (2006) reported significantly positive effect of curcuma at the level of 0.5 % on weight gain of birds. It was also shown, that curcumin added to the diet of kids during the hot summer months significantly improved the final live body weight and average daily body gain of kids compared to the control (Habeeband and Tarabany, 2012). On the other hand, similarly to our results no significant effect of the supplementation of curcuma powder in the broiler rabbit (Basavaraj *et al.*, 2010) and in the broiler chicks feed a mixture (Mehala and Moorthy, 2008) were reported.

Table 1: Weight gain (g) per week of analysed rabbit does fed with *Curcuma longa* enriched feed

Groups	Weight gain per week (g)									Average weight gain per week (g) (Mean ± S.E.M.)
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	
C (*n* = 15)	286.20 ± 80.77	280.77 ± 27,86	258.46 ± 23.26	245.38 ± 28.43	223.08 ± 27.29	185.38 ± 31.88	227.69 ± 47.73	183.85 ± 19.29	59.23 ± 29.41	216.20 ± 25.59
E1 (*n* = 15)	258.00 ± 29.13	291.30 ± 17.75	310.70 ± 19.28	283.30 ± 21.86	278.00 ± 29.18	217.30 ± 28.07	208.00 ± 22.28	170.70 ± 20.31	86.00 ± 17.91	235.70 ± 22.35
E2 (*n* = 15)	222.86 ± 27.08	263.57 ± 30.69	327.10 ± 40.35	299.30 ± 16.82	254.30 ± 18.53	232.90 ± 14.76	211.40 ± 22.84	165.00 ± 32.39	69.30 ± 27.60	220.50 ± 31.94

C – control group, commercially available diet (normal diet) ; E1 – 5 g of *Curcuma longa* dried powder added to 100 kg of normal diet; E2 – 20 g of *Curcuma longa* dried powder added to 100 kg of normal diet

Table 2: Average weight per week (g) and total average weight gain of analysed rabbit does fed with *Curcuma longa* enriched feed

Groups	Average weight of rabbit does per week (g)										Total average weight gain (g) (Mean ± S.E.M.)
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	
C (*n* = 15)	1713.08 ± 83.30	1999.23 ± 103.41	2280.00 ± 116.59	2538.46 ± 128.04	2783.85 ± 148.68	3006.92 ± 150.51	3192.31 ± 156.99	3420.00 ± 183.50	3603.85 ± 187.85	3663.08 ± 171.73	1950.00 ± 126.88
E1 (*n* = 15)	1842.67 ± 44.37	2100.67 ± 53.24	2392.00 ± 58.98	2702.67 ± 65.03	2986.00 ± 64.66	3264.00 ± 72.93	3481.33 ± 73.03	3689.33 ± 86.68	3860.00 ± 78.22	3946.00 ± 77.07	2103.30 ± 63.22
E2 (*n* = 15)	1818.57 ± 47.31	2041.43 ± 57.87	2305.00 ± 63.48	2632.14 ± 65.84	2931.43 ± 77.90	3185.71 ± 77.78	3418.57 ± 86.23	3630.00 ± 102.61	3795.00 ± 118.74	3864.29 ± 104.18	2045.00±84.36

C – control group, commercially available diet (normal diet) ; E1 – 5 g of *Curcuma longa* dried powder added to 100 kg of normal diet; E2 – 20 g of *Curcuma longa* dried powder added to 100 kg of normal diet

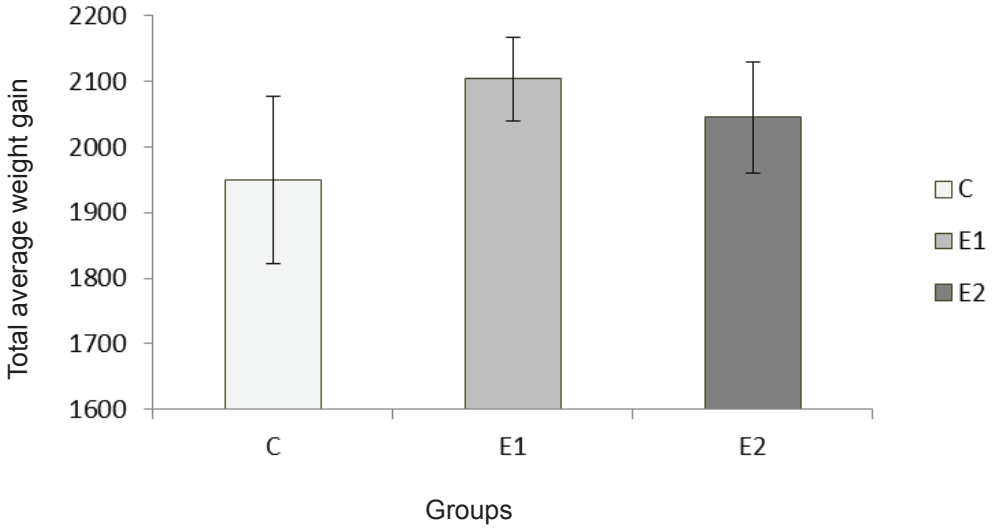


Fig. 1: Total average weight gain (g) of analysed rabbit does fed with *Curcuma longa* dried plant added into complete feed mixture

CONCLUSION

The supplementation of *Curcuma longa* plant powder to the commercially available diet for rabbits positively affects weight gains in rabbit does. Therefore, for improving growth performance, further studies are required to define an optimal supplementation of *Curcuma longa* to the rabbit diet.

ACKNOWLEDGMENT

We thank Mr. Milan Dobiaš for technical support. The research leading to these results has received funding from the European Community under project no 26220220180: Building Research Centre „AgroBioTech“.

REFERENCES

AL-SULTAN, S. I. 2003. The Effect of *Curcuma longa* (Tumeric) on Overall Performance of Broiler Chickens. *International Journal of Poulry Science*, vol. 2 (5), 2003, p. 351–353.

AMBER, K. H. – YAKOUT, H. M. – HAMED RAWYA, S. 2004. Effect of feeding diets containing yucca extract or probiotic on growth, digestibility, nitrogen balance and caecal microbial activity of growing New Zealand White rabbits. In: *Proceedings – 8th World Rabbit Congress*. Puebla, Mexico, p. 737–745.

AMMON, H. P. – WAHL, M. A. 1991. Pharmacology of *Curcuma longa* L. *Planta Medica*, vol. 57, 1991, p. 1–7.

ARAÚJO, C. A. C. – LEON, L. L. 2001. Biological activities of *Curcuma Longa* L. *Memorias Do Instituto Oswaldo Cruz*, vol. 96 (5), 2001, p. 723–728.

BASAVARAJ, M. – NAGABHUSHANA, V. – PRAKASH, N. – MALLIKARJUNAPPA, S. – APPANNAVAR, M. M. – WAGMARE, P. 2010. Effect of dietary supplementation of Pulvis *Curcuma Longa* on the voluntary feed intake, nutrient digestibility and growth performance of Broiler rabbits under summer stress. *Veterinary World*, vol. 3 (8), 2010, p. 369–372.

DE, R. – KUNDU, P. – SWARNAKAR, S. – RAMAMURTHY, T. – CHOWDHURY, A. – NAIR, G.B. – MUKHOPADHYAY, A. K. 2009. Antimicrobial Activity of Curcumin against *Helicobacter pylori* Isolates from India and during Infections in Mice. *Antimicrobial Agents and Chemotherapy*, vol. 53 (4), 2009, p. 1592–1597.

DESHPANDE, U.R. – GADRE, S. G. – RASTE, A. S. – PILLAI, D. – BHIDE, S. V. – SAMUEL, A. M. 1998. Protective effect of turmeric (*Curcuma longa* L.) extract on carbon tetrachloride-induced liver damage in rats. *Indian Journal of Experimental Biology*, vol. 36 (6), 1998, p. 573–577.

DURRANI, F.R. – ISMIAL, M. – SULTAN, A. – SHHAIL, S. M. – CHAND, N. – DURRANI, Z. 2006. Effect of different levels of feed added tumeric

(*Curcuma longa*) on the performance of broiler chicks. *Journal of Agricultural and Biological Science*, vol. 1 (2), 2006, p. 9–11.

ESHRAT, H. – HUSSAIN, A. 2002. Hypoglycemic, hypolipidemic and antioxidant properties of combination of Curcumin from *Curcuma longa*, Linn, and partially purified product from *Abroma augusta*, Linn. In streptozotocin induced diabetes. *Indian Journal of Clinical Biochemistry*, vol. 17 (2), 2002, p. 34–43.

FÖLDEŠIOVÁ, M. – BALÁŽI, A. – CHRASTINOVÁ, Ľ. – CHRENEK, P. 2013. Effect of *Yucca schidigera* herbal extract in diet on rabbit does weight gains. *Slovak Journal of Animal Science*, vol. 46 (2), 2013, p. 81–86.

GITHIORI, J. B. – HÖGLUND, J. – WALLER, P. J. – LEYDEN BAKER, R. 2003. Evaluation of antihelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmers in Kenya against *Heligmosomoides polygyrus* infections in mice. *Veterinary Parasitology*, vol. 118 (3–4), p. 215–226.

GONDRET, F. – LARZUL, C. – COMBES, S. – DE ROCHAMBEAU, H. 2005. Carcass composition, bone mechanical properties, and meat quality traits in relation to growth rate in rabbits. *Journal of Animal Science*, vol. 83 (7), p. 1526–1535.

HABEEBAND, A. A. M. – EL-TARABANY, A. A. 2012. Effect of *Nigella sativa* or Curcumin on daily body weight gain, feed intake and some physiological functions in growing Zaraibi Goats during hot summer season. *Arab Journal of Nuclear Science and Applications*, vol. 45 (2), 2012, p. 238–249.

HATCHER, H. – PLANALP, R. – CHO, J. – TORTI, F. M. – TORTI, S. V. 2008. Curcumin: From ancient medicine to current clinical trials. *Cellular and Molecular Life Sciences*, vol. 65 (11), 2008, p. 1631–1652.

HOLDER, G.M. – PLUMMER, J.L. – RYAN, A. J. 1978. The metabolism and excretion of curcumin (1,7-bis-(4-hydroxy-3methoxyphenyl)-1,6-hepadiene-3,5-dione) in the rat. *Xenobiotica*, vol. 8 (12), 1978, p. 761–768.

JEON, Y. – KIM, CH. E. – JUNG, D. – KWAK, K. – PARK, S. – LIM, D. – KIM, S. – BAEK, W. 2013. Curcumin Could Prevent the Development of Chronic Neuropathic Pain in Rats with Peripheral Nerve Injury. *Current Therapeutic Research*, vol.74, 2013, p. 1–4.

JURENKA, J.S. 2009. Anti-inflammatory Properties of Curcumin, a Major Constituent of *Curcuma longa*: A Review of Preclinical and Clinical Research. *Alternative Medicine Review*, vol. 14 (2), 2009, p. 141–153.

KÁDASI, A. – SIROTKIN, A. V. – MARUNIAKOVÁ, N. – KOLESÁROVÁ, A. – BULLA, J. – GROSSMAN, R. 2012. The effect of curcumin on secretory activity, proliferation and apoptosis of the porcine ovarian granulosa cells. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2 (1), 2012, p. 349–357.

LOW DOG, T. 2006. A Reason to Season: The Therapeutic Benefits of Spices and Culinary Herbs. *The Journal of Science and Healing*, vol. 2 (5), 2006, p. 446–449.

MAHESHWARI, R. K. – SINGH, A. K. – GADDIPATI, J. – SRIMAL, R. C. 2006. Multiple biological activities of curcumin: A short review. *Life Sciences*, vol. 78 (18), 2006, p. 2081–2087.

MEI, X. – XU, D. – XU, S. – ZHENG, Y. – XU, S. 2011. Gastroprotective and antidepressant effects of new zinc (II)-curcumin complex in rodent models of gastric ulcer and depression induced by stresses. *Pharmacology, Biochemistry and Behavior*, vol. 99 (1), 2011, p. 66–74.

MEHALA, C. – MOORTHY, M. 2008. Production performance of broilers fed with Aloe vera and *Curcuma longa* (Tumeric). *International Journal of Poultry Science*, vol. 7 (9), 2008, p. 852–856.

MÍNGUEZ, B. C. 2014. Genetic analyses of growth, carcass and meat quality traits in maternal lines of rabbits and their diallel cross: tesis doctoral. Valencia : Universitat Politecnica de Valencia. 261s.

NAMKUNG, H. – LI, M. – GONG, J. – YU, H. – COTTRILL, M. – DE LANGE, C. F. M. 2004. Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. *Canadian Journal of Animal Science*, vol. 84 (4), p. 697–704.

OSAWA, T. – SUGIYAMA, Y. – INAYOSHI, M. – KAWAKISHI, S. 1995. Antioxidative activity of tetrahydrocurcuminoids. *Bioscience, Biotechnology and Biochemistry*, vol. 59 (9), p. 1609–1612.

PARK, J. H. – KANG, S. N. – CHU, G. M. – JIN. S. K. 2014. Growth performance, blood cell profiles, and meat quality properties of broilers fed with *Saposhnikovia divaricata*, *Lonicera japonica* and *Chelidonium majus* extracts. *Livestock Science*, vol. 165, 2014, p. 87–94.

RICKE, S. C. – VAN LOO, E. J. – JOHNSON, M. G. – O´BRYAN, C. A. 2012. *Organic Meat Production and Processing*. The first edition. USA : Wiley-Blackwell. 464 p. ISBN 978-0-8138-2126-9.

SAVIETTO, D. – FRIGGENS, N. C. – PASCUAL, J. J. 2015. Reproductive robustness differs between generalist and specialist maternal rabbit lines: the role of acquisition and allocation of resources. *Genetics, Selection, Evolution*, vol. 47, 2015, p. 1–12.

SÁYAGO–AYERDI, S. – MATEOS, R. – ORTIZ-BASURTO, R. I. – LARGO, C. – SERRANO, J. – BELÉN GRANADO-SERRANO, A. – SARRIÁ, B. – BRAVO, L. – TABERNERO, M. 2014. Effects of consuming diets containing *Agave tequilana* dietary fibre and jamaica calyces on body weight gain and redox status in hypercholesterolemic rats. *Food Chemistry*, vol. 148 (1), 2014, p. 54–59.

TAMIR, B. – ASEFA, G. 2009. Effects of different forms of *Acacia saligna* leaves inclusion on feed intake, digestibility and body weight gain in lambs fed grass hay basal diet. *Animal Feed Science and Technology,* vol. 153 (1–2), 2009, p. 39–47.

ABDEL-WARETH, A. A. A. – HAMMAD, S. – AHMED, H. 2014. Effects of Khaya *Senegalensis* levaes on performance, carcass traits, haematological and biochemical parameters in rabbits. *EXCLI Journal*, vol. 13, 2014, p. 502–512.

WOJDYŁO, A. – OSZMIANSKI, J. – CZEMERYS, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, vol. 105 (3), 2007, p. 940–949.

WU, T. – QI, X. – LIU, Y. – GUO, J. – ZHU, R. – CHEN, W. – ZHENG, X. – YU, T. 2013. Dietary supplementation with purified mulberry (*Morus australis* Poir) anthocyanins suppresses body weight gain in high-fat diet fed C57BL/6 mice. *Food Chemistry*, vol. 141 (1), 2013, p. 482–487.

Slovak Journal of Animal Science

http://www.vuzv.sk/index.php/slovak-journal-of-animal-science

INSTRUCTIONS FOR AUTHORS

The scientific journal Slovak Journal of Animal Science publishes original papers, review articles, short communications, reviews of important works, chronicles, and reports on participation in important international conferences. Topics of the journal include problems in biology of animals, animal production and veterinary medicine.

The author is responsible for originality of the paper, subject-matter and formal attributes of the paper. Papers are published in English language. The author is responsible for translation into English. In case of linguistic shortcomings the paper will be sent back to the author.

Copyright. The journal is protected by copyright held by the publisher after the manuscript has been accepted for publication. As regards the transfer of rights, the corresponding author assumes responsibility for all the authors.

MANUSCRIPT SUBMISSION

- **Manuscripts** should be sent by e-mail as attachments. Alternatively, they can be submitted on floppy disk, USB key, in hard copy and a properly labeled Compact Disk (CD) with identical contents, including figures. Unique MS Word file must be saved under the first author's surname only. They are to be sent to the following address:

  Slovak Journal of Animal Science - Editorial Office
  National Agricultural and Food Centre - Research Institute
  for Animal Production Nitra
  Hlohovecká 2, 951 41 Lužianky
  Slovak Republic
  Phone: +421 37 6546 249 Fax: +421 37 6546 311 e-mail: editor@vuzv.sk
- **The author's declaration** (find at www.cvzv.sk/index.php/slovak-journal-of-animal-science) that the paper has not been published anywhere else should be enclosed. The Declaration must be carefully completed and signed by the first author.
- **The Corresponding Author** should include his or her full name including all academic, scientific and pedagogic titles and the detailed address of the institution with postal code, telephone and fax numbers, and e-mail address.

REVIEWERS' EVALUATION

All scientific contributions will be peer-reviewed on the criteria of originality and quality. Two reviewers, who have appropriate knowledge of the subject, are appointed for each paper. If need be third refree will be asked. The reviewers are asked to return papers within 3 weeks. Editorial board of the journal decides on contributing publications, taking into consideration the standpoint of reviewers, scientific significance and quality of manuscript. The manuscript can be rejected if it does not comply with the subject of the journal. The editor makes every effort to explain to authors the reasons of rejection.

AFTER REVIEWERS' EVALUATION

After the author receives the reviewers' opinion he owes to return the corrected manuscript to the editorial office within three weeks. The authors may, however, request an extension of the re-submission deadline if necessary. After incorporation of revisions it is necessary to send the new complete manuscript with tables and figures, also in case of minor revisions in the original manuscript. In an enclosure the author has to answer all the important comments of the reviewers clearly and in detail. The author is not obliged to respect the comments of reviewers but he has to explain in the enclosure why he does not accept the reviewers' comments. If the author does not keep the term of sending back the revised manuscript, his paper is liable to be excluded from further processing.

SENDING THE CORRECTED PROOF

The author has to return the made-up manuscript for proof-reading. He must send it back within two days. Within this stage of manuscript preparation for printing, corrections are done only for such errors that arise during the work in editorial office.

TECHNICAL LAYOUT OF MANUSCRIPT

TYPE OF CONTRIBUTIONS

Following categories of scientific contributions are accepted for publication:

A. **Original articles** – original full-length papers which have not been published yet, should not exceed 8 000 words (including tables and illustrations).
B. **Review articles** – focusing on special topics of interest and summarising latest scientific information, up to 10 000 words (including tables and illustrations). Contributions of authors with experimental experience and publications on the given topic are highly preferred.
C. **Short communications** – these may be of a preliminary nature but sufficient for immediate publication, up to 2 500 words (including tables and illustrations)
D. **Chronicles, and reports** on participation in important international conferences - should not exceed 1 500 words.

TEXT

- The paper should be delivered in text editor Microsoft Office (Word, Excel) for Windows. The text should be in non-formatted style (unjustified).
- It is necessary to number all the lines in the text and pages.
- If any abbreviations are used in the paper, they must be appropriately explained when they are used in the text for the first time. It is not advisable to use any abbreviation in the title of the paper or in the abstract;
- Latin words are to be written *in italics*;
- SI units must be used;
- Tables, photographs, graphs and figures should not comprise a part of the text – approximate place is marked within the text where they should be placed as follows: one empty line, then the table No. should be written, followed by an empty line and then the text. They are to be submitted on separate pages appended to the article.
- The words should not be divided; between words just one stroke should be left; no style, macro should be adjusted; should be written only as basic text, no reduction and increase in font size is admissible, indices should be written with upper index and lower index; only ‘enter’ should be used to end the paragraph.

TABLES

Font type : Times New Roman, font size: 9; simple spacing; well-arranged and as much simple as possible, typed in format – *Table : Insert* (each entry has to be done in separate cells); width 16.4 cm x height 23 cm = one page per table (maximum size inclusive of title and explanatory notes below the table); Arabic numerals to be used for marking the tables (e.g. Table 1: …); no vertical lines should be used; significance of differences is marked in the same line as numbers, not as an index (e.g. 0.55++ or 0.55ab); abbreviations in description of tables should not be used; SI units have to be used.

GRAPHS

In MS Excel editor or as a figure; simple and well-arranged, without shadowy outline and background, and without grid, of width 16.4 × height 23 cm = one page per graph (maximum size of graph together with all explanatory notes below the graph); charts in MS Excel format (xls) will be accepted when submitted with their underlying table data.

PHOTOGRAPHS AND FIGURES

Format tiff, gif, jpg, psd, eps – in CMYK; resolution min. 300 dpi; they are published black and white for the present, the author must expect the coloured photograph to be published in degrees of grey colour (on www.cvzv.sk are photographs in colour); width 16.4 × height 23 cm = one page per figure (maximum size inclusive of title and explanatory notes); photographs and figures including written text has to be in group format; photographs, graphs and drawings to be indicated as figures (Fig. 1: … etc).

Slovak Journal of Animal Science
http://www.vuzv.sk/index.php/slovak-journal-of-animal-science

Subscription and distribution

All orders should be sent direct to:
Slovak Journal of Animal Science
Editorial Office
NAFC - Research Institute for Animal Production Nitra
Hlohovecká 2
951 41 Lužianky, Slovak Republic
Phone: +421 37 6546 249
Fax: +421 37 6546 311
E-mail: editor@vuzv.sk

Subscription rates 2015 (journal + postage)

Print:
65,- € collecting in editorial office directly
75,- € Slovak Republic
85,- € Czech Republic
95,- € Europe
105,- € ROW

Offprints:
Editorial office will send on request the offprint in Portable Document Format (PDF) via e-mail.

Slovak J. Anim. Sci., 48, 2015 (1)

Contents

ISSN 1337-9984 (Print)
ISSN 1338-0095 (Online)