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IČO: 42337402, Phone: +421 376 546 249, e-mail: sjas@nppc.sk
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SEMEN CHARACTERISTICS, GONADAL AND EXTRAGONADAL SPERM RESERVES IN COCKS FED DIETS CONTAINING DIFFERENT INCLUSION LEVELS OF MONOSODIUM GLUTAMATE

Olumuyiwa Joseph OLAROTIMI[1,2]*, Olufemi Adesanya ADU[1]

[1]Department of Animal Production and Health, School of Agriculture and Agricultural Technology, The Federal University of Technology Akure, P.M.B. 704, Akure, Ondo State, Nigeria
[2]Department of Animal Science, Adekunle Ajasin University, Akungba-Akoko, P.M.B. 001, Akungba-Akoko, Nigeria

ABSTRACT

The investigation was carried out to assess the semen characteristics, gonadal and extragonadal sperm reserves of cocks fed dietary monosodium glutamate (MSG) at varied inclusion levels (0.00, 0.25, 0.50, 0.75, 1.00 and 1.25 g.kg^{-1} diet). A total of 240 sexually matured barred Plymouth Rock cocks of twenty 24 weeks of age were used in a 16-week feeding trial. The cocks were weighed and allotted to the 6 treatment diets. Each treatment was replicated 5 times with 8 cocks per replicate in a completely randomized design (CRD). At the end of the feeding trial, 4 cocks per replicate were humanely sacrificed and their reproductive tracts were dissected. The testes and epididymides were carefully sampled, weighed and processed. The data collected were subjected to analysis of variance followed by Tukey's Honestly Significant Difference ($\alpha_{0.05}$) where significant differences occurred. The results showed that all the semen characteristics were significantly and negatively affected by inclusion of MSG above 0.50 g.kg^{-1} diet. The paired epididymides and *vas deferens* weights were significantly ($P<0.05$) reduced by the inclusion levels of MSG above 0.75 g.kg^{-1} diet, while their lengths were significantly ($P<0.05$) reduced above 0.50 g MSG.kg^{-1} diet. Nevertheless, the testicular parameters were not significantly ($P>0.05$) affected by the varying inclusion levels of MSG when compared with the control. The paired testicular sperm reserves (TSR/testis and TSR/g testis) were not significantly ($P>0.05$) influenced by the MSG inclusion when compared with the control diet. However, the paired epididymides sperm reserves were significantly lowered at the inclusion levels of 1.00 and 1.25 g MSG.kg-^{1} diet. This study suggests that dietary MSG has the potential to significantly affect the sperm characteristics and sperm reserves of cocks when administered above 0.50 g.kg^{-1} diet.

Key words: cocks; sperm reserves; characteristics; epididymis; testis; monosodium glutamate

INTRODUCTION

An important feed factor to give a deserved attention is feed palatability and acceptability. Non-palatability nature of feeds could prevent birds from consuming adequate quantity required for optimum performance. The purpose of investing so much on the feed will be totally defeated if the feeds are not acceptable, non-palatable and inadequately utilized by the animals. This may predispose the animal to feed refusal and/or feed wastage and this will confer a serious economic risk on the part of the farmers. Feed additives may be included into diets for the purpose of improving performance such as weight gain, increased laying performance, enhancing the sperm production capacity for breeding purposes, improving hatchability, preventing diseases, enhancing palatability and

***Correspondence:** E-mail: olarotimioj@futa.edu.ng
Olumuyiwa Joseph Olarotimi, Department of Animal Science, Adekunle Ajasin University, P.M.B. 001, Akungba-Akoko, Nigeria

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providing enhanced digestibility of the feed materials (Windisch, 2008). The additive of interest in this study to enhance feed palatability is monosodium glutamate (MSG).

Therefore, fortifying poultry feeds with taste enhancers, such as MSG, in order to increase the palatability of such feeds for better acceptability and subsequent improved productivity, such as increased body weight, improved hen day production, enhanced reproductive potentials, among others, will be highly welcomed (Olarotimi *et al*., 2019). MSG has also been reported to have a toxic effect on the testis by causing a significant oligozoospermia and increase in abnormal sperm morphology in a dose-dependent fashion in male Wistar rats (Onakewhor *et al.*, 1998). It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology (Oforofuo *et al.*, 1997). Another study also reported that there were no gross morphological changes on the physical examination of testes of Wistar rats administered 250 mg – 2 g.ml^{-1} MSG solution with only slight variations observed in the sperm concentration of all the treatment groups (Kianifard, 2016). Progressive motility of sperm reduced equally with all the treatment groups having fair forward directional movement, compared to the control, which had an excellent forward directional movement (Kianifard, 2016). Since the potential of MSG as useful and cheaper taste enhancer both for animal and human feeding has been previously emphasized, this study, therefore, aims to evaluate the possible effects it may confer on cocks' sperm characteristics, gonadal and extragonadal sperm reserves, which are traits used in assessing good quality sperm for breeding purposes.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Poultry Unit, Teaching and Research Farm, the Federal University of Technology Akure, Nigeria. The geographical coordinates of the location are between 7° 17' North and 5° 9' East (Mapzoom, 2015). The climatic condition of Akure follows the pattern of southwest Nigeria where the climate is influenced mainly by the rain-bearing southwest monsoon winds from the ocean and the dry northwest winds from the Sahara desert. The rainy season lasts for about seven months (April to October). The rainfall is about 1524 mm per year. The atmospheric temperature ranges between 28 °C and 31 °C and mean annual relative humidity of about 80 % (Ajibefun, 2011). It was conducted in accordance with the research ethics and guidelines of the Animal Production and Health Department of the institution (FUTA/APH/15/4750).

Experimental design and diet

Six (6) experimental diets (Table 1) were formulated with varying inclusion levels (0.00, 0.25, 0.5, 0.75, 1.00 and 1.25 g.kg^{-1}) of MSG (Ajinomoto brand) sourced from the open market. The diets met the nutrient requirements of adult cocks according to NRC (1994) recommendations. The proximate analyses of the diet samples were carried out with the use of a Near-Infra Red Spectrophotometer. The metabolizable energy (ME) of the feed samples was calculated using the prediction equation by Pauzenga (1985) as follows:

ME = (37 x CP + 81.8 x EE + 35.5 x NFE).

Where:

CP = crude protein, EE = ether extract, NFE = nitrogen free extract

Experimental cocks and management

A total of 240 sexually matured barred Plymouth Rock cocks of twenty-four (24) weeks of age were used for the study. They were caged for two weeks before the onset of the experiment for stabilization. They were fed commercial grower ration throughout the stabilization period with fresh and cool water given *ad libitum*. At the end of the stabilization period, the cocks were weighed and randomly allotted to the experimental treatments. Each treatment was replicated 5 times with 8 cocks per replicate in a completely randomized design. The experimental diets were given according to the body weight twice daily and drinking water was also provided *ad libitum* throughout the sixteen weeks period of the experiment. All required managerial practices, such as strict bio-security measures, were ensured as at and when due, appropriate vaccines, and prophylactic treatments were administered. The birds were housed in an open-sided building in a thoroughly cleaned, washed and disinfected three tier cage system of 32 x 38 x 42 cm dimension. Two (2) birds were conveniently housed

Table 1. Composition of the cocks' experimental diets

	Diets with MSG inclusion (g.kg^{-1})					
Ingredients (kg)	T1	T2	T3	T4	T5	T6
Maize	330	330	330	330	330	330
Soya Bean Meal	28	28	28	28	28	28
Ground Nut Cake	35	35	35	35	35	35
Corn Bran	110	110	110	110	110	110
Palm Kernel Meal	160	160	160	160	160	160
Wheat Offal	300	300	300	300	300	300
Limestone	15	14.75	14.5	14.25	14	13.75
Bone Meal	14	14	14	14	14	14
Lysine	1	1	1	1	1	1
Methionine	1	1	1	1	1	1
Salt	3.5	3.5	3.5	3.5	3.5	3.5
Layer Premix	2.5	2.5	2.5	2.5	2.5	2.5
MSG	0	0.25	0.5	0.75	1	1.25
Total	1000	1000	1000	1000	1000	1000
ANALYSED VALUES						
Crude protein (%)	15.15	15.2	15.19	15.23	15.21	15.11
ME (kcal.kg^{-1})	2520.73	2516.96	2520.37	2516.17	2513.69	2519.8
Ca (%)	1.3	1.26	1.18	1.23	1.31	1.19
Crude fibre (%)	9.45	10.19	10.08	10.22	9.98	10.01
Moistiure (%)	11.5	10.88	11.01	10.89	10.99	11.47
Crude fat (%)	2.19	2.13	2.25	2.02	2.22	2.34
NFE (%)	50.17	50.15	49.98	50.35	49.84	49.84
Crude ash (%)	11.54	11.45	11.49	11.29	11.76	11.23

NFE = Nitrogen Free Extract; ME = Metabolizable Energy * Composition of premix (Nutrivitas®): 2.5 kg of premix contains: Vit. A (10,000,000 iu), Vit. D3 (2,500,000 iu), Vit. E (12,000 iu), Vit. B1 (2000 mg), Niacin (25000 mg), Vit. B6 (1500 mg), Vit. B12 (10 mg), Vit. K3 (2500 mg), Biotin (75 mg), Folic Acid (2000 mg), Panthothenic Acid (7000 mg), Chlorine Chloride (50 %) (200000 mg), Manganese (80000 mg), Iron (40000 mg), Copper (10,000 mg), Zinc (60000mg), Selenium (200 mg), Iodine (1500 mg), Magnesium (100 mg), Ethoxyquine (500 g), BHT (700 g), Cobalt (250 mg)

in a unit. At the end of the feeding trial, four (4) cocks per replicate were selected and fasted overnight for semen evaluation.

Semen Collection

Semen was harvested from the already trained cocks selected before sacrificing for evaluation. The collection of semen was done between 6 to 8 am. The cocks were handled with care to avoid fright and undue physical stress. Semen was collected by the manual massage technique, as described by Udeh *et al.* (2011). Labeled plain sample tubes were used for the collection of the semen.

Semen Evaluation

Each semen sample was evaluated for volume, gross motility, live-dead count and concentration within 6 min after collection as described by Ewuola and Akinyemi (2017). Semen volume was determined by drawing the semen with a graduated tuberculin syringe of 1.0 ml capacity and reading directly to the nearest 0.01 ml. Progressive motility was evaluated by diluting a drop of semen with two drops of normal saline on a clean pre-warmed (37 °C) glass slide and covered with a clean coverslip. The observation was done under a microscope at x 40 magnification and scored 0 – 100 % with 0 representing no progressive motility.

A dilution of 0.1 ml of the ejaculate with 5 ml normal saline was prepared in a clean test tube for the evaluation of the sperm concentration. Sperm concentration was evaluated using a Neubauer Haemocytometer and a binocular microscope (Olympus CH-2 CHS Binocular Microscope, Olympus

Corporation, Japan) at 100 x magnification, as described by Ewuola and Egbunike (2010). The mass activity was estimated as described by Ogunlade (2015). A drop of raw undiluted semen was examined on pre-warmed slides under a microscope at 10 x magnification. The mass activity was scored subjectively according to the intensity of the wave motion, from the absence of wave motion (0) to slow motion (+), rapid motion (+ +) or turbulent motion (+ + +) characterized by the appearance of dark prominent wave in a rapid motion. The ratio of live sperm cells to dead sperm cells was evaluated by eosin-nigrosin vital staining technique. A drop of the ejaculate was placed onto a clean pre-warmed glass slide and two drops of eosin-nigrosin stain were added. This was mixed and a smear was prepared from the mixture and then viewed under a microscope at × 40 magnification. Dead sperm cells absorbed the stain while live sperm cells repelled it. The dead sperm percentage was obtained by dividing the number of dead cells in a field by the total number of sperm cells counted in the same field multiplied by 100. Percentages of live sperm cells (Liveability/Viability) were obtained by subtracting the value of the dead cell percentage from 100. From the values obtained above, the following characteristics were determined:

Sperm Concentration (Sperm Cells.ml^{-1}) = N x C x D (Maina *et al.*, 2006).

Where:
N = number of sperm cells counted; C = constant = 52000 (Ewuola *et al.*, 2014); D = dilution factor = vol. normal saline/volume of semen

$$\text{Liveability} = \frac{\text{Total number of counted cells} - \text{Dead cells} \times 100}{\text{Total number of counted cells}}$$

$$\text{Total sperm cells/Ejaculate} = \text{Sperm concentration/ml} \times \text{Volume of ejaculate}$$

$$\text{Total live sperm/ml} = \frac{\text{Liveability} \times \text{Sperm concentration/ml}}{100}$$

$$\text{Total motile sperm cells/ml} = \frac{\text{Motility} \times \text{Sperm concentration/ml}}{100}$$

Assessment of Gonadal and Extragonadal Morphometric Indices

After semen collection, the cocks were humanely sacrificed through cervical dislocation and eviscerated for a gross examination of organs *in situ*. The reproductive tracts of the slaughtered cocks were carefully harvested. Testicular weights were recorded using a highly sensitive weighing balance in the laboratory. The testes and epididymides were separated free of adhering connective tissues and fats. The left and right testes and epididymides were measured separately and their weights recorded. The volumes of the testes were measured volumetrically using Archimede's principle of water displacement in a measuring cylinder, as described by Olarotimi *et al.* (2015) and the result were recorded. The testes densities were calculated from the testicular weights and volumes and expressed as g.ml^{-1} (Olarotimi *et al.*, 2015).

$$\text{Testis density} = \frac{\text{Testis weight (g)}}{\text{Testis volume (ml)}}$$

Estimation of gonadal sperm reserves

The reproductive organs were carefully dissected; the testes and epididymides were carefully collected, trimmed off adhering tissues and weighed using a sensitive electronic balance. Testicular and epididymal morphometric characteristics were determined. Gonadal/testicular spermatozoa reserves (GSR/TSR) were determined haemocytometrically as described by Orlu and Egbunike (2009). A sample of each testis was sectioned and weighed. The samples were homogenized separately with a pair of sharp scissors in 0.9 % NaCl (normal/physiological saline) at the rate of 5 ml.g^{-1} testis. The testicular homogenate sample was stored overnight at 4 °C to allow the spermatozoa ooze out of the organ. The suspensions were mixed and filtered through a double layer of sterile gauze into clean glass test tubes and the filtrate diluted with distilled water to the ratio 1:10 (Ewuola, 2013; Amao and Akanbi, 2017). Some drops of the homogenate were placed into an improved Neubauer haemocytometer counting chamber. All the elongated spermatids and mature sperm cells in the four diagonal and the centre squares of the haemocytometer were counted in each diluted homogenate. The concentration of the sperm cells

per gram of testis parenchyma was calculated as follows:

RTSR/LTSR = N x C x D

Where:

N = number of sperm cells count

D = dilution factor = $d_1 \times d_2$

$$d_1 = \frac{\text{Volume of normal saline for homogenization}}{\text{Weight of sample homogenized}}$$

$$d_2 = \frac{\text{Volume of normal saline for filtrate dilution}}{\text{The volume of filtrate diluted}}$$

C = constant = 52,000

RTSR = right testicular sperm reserve; LTSR = left testicular sperm reserve

Right/Left Testis Sperm Reserve (RTSR/LTSR)/testis =

$$= \frac{\text{RTSR/LTSR x Total weight of right/left testis}}{\text{Weight of homogenated right/left testis}}$$

Therefore, Paired TSR (PTSR)/testis = RTSR/testis + LTSR/testis

$$\text{RTSR/LTSR per gram testis} = \frac{\text{RTSR/testis}}{\text{Total weight of right testis}} \text{ OR } \frac{\text{LTSR/testis}}{\text{Total weight of left testis}}$$

Therefore, Gonadal Sperm Reserve (GSR) or paired TSR (TSR)/gram testis = RTSR/gram testis + LTSR/gram testis

Estimation of extragonadal (epididymal) sperm reserves (ESR)

The left and right epididymides were removed from the testes and separated into the caput, corpus and cauda epididymides. They were separately homogenized in normal saline at 5ml.g^{-1}. The homogenate was kept refrigerated overnight. It was then filtered through double layers of gauze into graduated test tubes. Extragonadal sperm reserves were determined by direct counting of sperm cells in a haemocytometer after 1:1(v/v) dilutions as described by Ewuola *et al.* (2014). The extragonadal sperm reserve was the total number of spermatozoa in all the sections of epididymal tissues expressed in millions.

ESR = ESR (caudal) + ESR (corpus) + ESR (caput)

ESR (caudal/corpus/caput) = N x C x D

Statistical analysis

All data obtained were subjected to a One-Way Analysis of Variance (ANOVA) of the GraphPad Prism, software version 6.01 (2012). Significant differences between the treatment means were revealed using the Tukey's Honestly Significant Difference (α 0.05) option of the same software.

RESULTS

The semen characteristics of the cocks fed diets supplemented with varying inclusion levels of MSG are shown in Table 2. It was observed that cocks on the diet containing 1.25 g MSG.kg^{-1} did not produce any ejaculate at all; therefore, no semen characteristics could be studied on the cocks placed on this diet. The cocks on the diets containing 0.25 and 0.50 g MSG.kg^{-1} did not show a significant (P > 0.05) effects of MSG inclusions when compared with one another and

Table 2. Semen characteristics of cocks fed diets with different levels of MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
Ejaculate Volume (ml)	0.60 ± 0.01^{a}	0.48 ± 0.18^{ab}	0.43 ± 0.12^{ab}	0.32 ± 0.09^{b}	0.22 ± 0.06^{bc}	0.00 ± 0.00^{c}	0.0003*
Sperm Motility (%)	94.30 ± 1.17^{a}	81.00 ± 4.09^{ab}	89.30 ± 1.17^{a}	67.70 ± 4.80^{b}	23.30 ± 4.41^{c}	0.00 ± 0.00^{d}	< 0.0001*
Sperm Viability (%)	97.44 ± 0.71^{a}	96.94 ± 0.86^{a}	91.95 ± 1.62^{a}	85.78 ± 2.11^{ab}	72.57 ± 7.15^{b}	0.00 ± 0.00^{c}	< 0.0001*
Sperm Conc. (x 10^8 ml^{-1})	7.32 ± 0.72^{a}	5.53 ± 0.29^{ab}	5.47 ± 0.27^{ab}	5.56 ± 1.53^{ab}	4.53 ± 0.56^{b}	0.00 ± 0.00^{c}	0.0122*
TSC/ejacualte (x 10^8 ml^{-1})	4.39 ± 0.56^{a}	2.65 ± 0.39^{ab}	2.35 ± 0.41^{ab}	1.78 ± 0.15^{b}	1.21 ± 0.22^{c}	0.00 ± 0.00^{d}	< 0.0001*
Total Live Cells.ml^{-1} (x 10^8)	7.13 ± 1.67^{a}	5.34 ± 1.02^{ab}	5.00 ± 1.80^{ab}	4.73 ± 1.84^{ab}	3.28 ± 0.09^{b}	0.00 ± 0.00^{c}	0.0325*
Total Motile Cells.ml^{-1} (x 10^8)	6.90 ± 1.54^{a}	4.48 ± 1.02^{ab}	4.88 ± 1.92^{ab}	3.76 ± 1.15^{b}	1.29 ± 0.42^{c}	0.00 ± 0.00^{d}	0.0269*
Mass Activity Grade	+++	+++	+++	++	+	0.00	

Values are means ± SEM; Means in a row without common superscripts are significantly (P < 0.05) different. Level of significance = ns (non significant) = P > 0.05; * = P < 0.05, Conc. = Concentration; TSC = Total Sperm Cells; MSG levels in g.kg^{-1} diet. + + +: Very turbulent motion; + +: Rapid wave motion; +: Slow wave motion.

those on the control diet for semen characteristics such as ejaculate volume, sperm motility, testicular sperm cells/ejaculate and total motile cells.ml^{-1} but recorded a significantly (P < 0.05) higher means than those on the diets containing above 0.50 g MSG.kg^{-1} diet for the same parameters. However, the sperm viability, concentration and total live cells.ml^{-1} among the cocks fed diets containing up to 0.75 g MSG.kg^{-1} did not show any significant (P > 0.05) difference when compared with one another and those on the control diet but significant reductions in these parameters were observed among the cocks fed diet containing 1.00 g MSG.kg^{-1}. The mass activity ranged from very turbulent motion for the cocks on the control diet to slow motion for those on the diet containing 1.00 g MSG.kg^{-1}.

The results of the extragonadal lengths and weights for left and right epididymides, as well as *vas deferens* of the cocks, fed different levels of MSG are shown in Table 3. It was observed that all of the epididymal and *vas deferens* lengths of the cocks across all the dietary treatments were progressively reduced with increasing inclusion levels of MSG. The cocks on the diets containing up to 0.50 g MSG.kg^{-1} diet showed statistical (P > 0.05) similarities among themselves and those on the control diet which were significantly (P < 0.05) higher than those on the diets containing 0.75 to 1.25 g MSG.kg^{-1} for left, right and paired epididymides and vas deference lengths. Similarly, the cocks on the control diets recorded the highest significant (P < 0.05) left, right and paired epididymal and *vas deferens* weights when compared with cocks on other diets. The cocks on the diets containing 0.25 to 0.75 g MSG.kg^{-1} did not differ significantly (P > 0.05) when the weights recorded by them for right, left and paired epididymides as well as right, left and paired *vas deferens* were compared with those on the control diet. However, increasing levels of MSG above 0.75 g.kg^{-1} diet significantly (P < 0.05) reduced the weights of the right, left and paired epididymides and *vas deferens* of the cocks. For gonadal characteristics (Table 4), it also was observed that the cocks on the diet containing 0.50 g MSG.kg^{-1} had the higher non-significant (P > 0.05) values for the right testicular volume; left testicular

Table 3. Extra gonadal lengths and weights of cocks fed diets MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
Extra Gonadal Length (cm)							
Epididymis							
Left	2.83 ± 0.12^{a}	2.80 ± 0.14^{a}	2.70 ± 0.14^{a}	2.07 ± 0.06^{b}	2.07 ± 0.02^{b}	2.17 ± 0.02^{b}	< 0.0001*
Right	3.47 ± 0.12^{a}	3.00 ± 0.18^{ab}	2.87 ± 0.07^{abc}	2.63 ± 0.27^{bc}	2.53 ± 0.17^{bc}	2.27 ± 0.06^{c}	< 0.0001*
Paired	6.30 ± 0.21^{a}	5.80 ± 0.29^{ab}	5.57 ± 0.11^{abc}	4.70 ± 0.08^{bc}	4.60 ± 0.18^{c}	4.44 ± 0.27^{c}	< 0.0001*
Vas deferens							
Left	12.53 ± 0.19^{a}	8.37 ± 0.19^{ab}	8.37 ± 0.35^{ab}	8.77 ± 0.71^{b}	7.63 ± 0.19^{bc}	5.67 ± 0.03^{c}	< 0.0001*
Right	10.93 ± 1.26^{a}	10.40 ± 0.46^{ab}	8.33 ± 0.18^{abc}	7.74 ± 0.36^{bc}	7.46 ± 0.82^{c}	7.97 ± 0.21^{bc}	< 0.0001*
Paired	23.46 ± 0.48^{a}	18.77 ± 1.15^{ab}	16.67 ± 0.57^{ab}	16.51 ± 0.49^{b}	15.09 ± 0.79^{bc}	13.64 ± 0.21^{c}	< 0.0001*
Extra Gonadal Weight (g)							
Epididymis							
Left	0.63 ± 0.10^{a}	0.60 ± 0.06^{ab}	0.53 ± 0.06^{ab}	0.50 ± 0.02^{ab}	0.40 ± 0.03^{bc}	0.37 ± 0.02^{c}	0.0063*
Right	0.73 ± 0.11^{a}	0.53 ± 0.12^{ab}	0.60 ± 0.06^{ab}	0.63 ± 0.09^{ab}	0.43 ± 0.06^{b}	0.30 ± 0.00^{b}	0.0094*
Paired	1.37 ± 0.20^{a}	1.13 ± 0.07^{ab}	1.13 ± 0.05^{ab}	1.13 ± 0.08^{ab}	0.83 ± 0.03^{bc}	0.67 ± 0.02^{c}	0.0002*
Vas deferens							
Left	0.70 ± 0.02^{a}	0.57 ± 0.07^{ab}	0.56 ± 0.08^{ab}	0.53 ± 0.15^{ab}	0.47 ± 0.07^{b}	0.30 ± 0.02^{c}	< 0.0001*
Right	0.66 ± 0.14^{a}	0.53 ± 0.03^{a}	0.56 ± 0.09^{a}	0.52 ± 0.07^{ab}	0.46 ± 0.15^{b}	0.30 ± 0.08^{b}	0.0006*
Paired	1.36 ± 0.24^{a}	1.10 ± 0.08^{ab}	1.13 ± 0.14^{ab}	1.05 ± 0.09^{ab}	0.93 ± 0.10^{bc}	0.61 ± 0.03^{c}	< 0.0003*

Values are means ± SEM; Means in a row without common superscripts are significantly (P < 0.05) different. Level of significance = ns (non significant) = P > 0.05; * = P < 0.05, MSG levels in g.kg^{-1} diet.

Table 4. Gonadal weights, volume and density of cocks fed different levels of MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
Parameters Right Testicle (g)							
Whole Weight	15.16 ± 2.40	18.28 ± 1.742	17.96 ± 0.58	15.59 ± 0.86	16.02 ± 1.60	13.12 ± 0.39	0.1404^{ns}
Parenchymal Weight	14.10 ± 2.23	17.00 ± 1.62	16.70 ± 0.54	14.50 ± 0.78	14.90 ± 1.49	12.20 ± 0.37	0.1404^{ns}
Albuginea Weight	1.06 ± 0.18	1.28 ± 0.12	1.26 ± 0.04	1.09 ± 0.06	1.12 ± 0.11	0.92 ± 0.03	0.1404^{ns}
Volume (ml)	16.00 ± 2.57	18.00 ± 1.89	18.70 ± 0.17	16.70 ± 1.20	13.30 ± 1.67	16.70 ± 0.44	0.2350^{ns}
Density (g.ml^{-1})	0.97 ± 0.03^{b}	1.03 ± 0.04^{b}	0.96 ± 0.03^{b}	0.96 ± 0.06^{b}	1.23 ± 0.04^{a}	0.79 ± 0.01^{c}	< 0.0001*
Left Testicle (g)							
Whole Weight	14.89 ± 2.03	13.40 ± 0.29	15.64 ± 0.63	15.42 ± 0.69	14.15 ± 1.36	14.89 ± 2.17	0.8806^{ns}
Parenchymal Weight	14.00 ± 1.91	12.60 ± 0.27	14.70 ± 0.59	14.50 ± 0.65	13.30 ± 1.28	14.00 ± 2.04	0.8806^{ns}
Albuginea Weight	0.89 ± 0.12	0.80 ± 0.02	0.94 ± 0.04	0.92 ± 0.04	0.85 ± 0.08	0.89 ± 0.13	0.8806^{ns}
Volume (ml)	15.30 ± 2.20	17.30 ± 0.17	17.70 ± 0.17	16.30 ± 0.44	12.00 ± 1.15	15.00 ± 2.31	0.0742^{ns}
Density (g.ml^{-1})	0.99 ± 0.03^{ab}	0.77 ± 0.01^{b}	0.88 ± 0.03^{ab}	0.96 ± 0.07^{ab}	1.19 ± 0.01^{a}	1.16 ± 0.21^{a}	0.0156*
Paired Testicles (g)							
Whole Weight	30.06 ± 4.42	26.52 ± 0.48	33.12 ± 1.06	31.01 ± 1.09	30.28 ± 2.84	33.18 ± 3.794	0.4977^{ns}
Parenchymal Weight	28.10 ± 4.13	24.80 ± 0.44	31.40 ± 1.03	29.00 ± 1.02	28.30 ± 2.65	31.00 ± 3.55	0.5003^{ns}
Albuginea Weight	1.96 ± 0.29	1.72 ± 0.03	1.72 ± 0.03	2.01 ± 0.07	1.98 ± 0.19	2.18 ± 0.24	0.4599^{ns}
Volume (ml)	31.30 ± 4.76	34.00 ± 0.50	36.30 ± 0.33	33.00 ± 1.50	25.30 ± 2.73	33.00 ± 4.19	0.1632^{ns}
Density (g.ml^{-1})	1.96 ± 0.06^{ac}	1.56 ± 0.01^{c}	1.85 ± 0.05^{bc}	1.91 ± 0.12^{bc}	2.42 ± 0.04^{a}	2.19 ± 0.24^{ab}	< 0.0001*

Values are means ± SEM; Means in a row without common superscripts are significantly (P < 0.05) different. Level of significance = ns (non significant) = P > 0.05; * = P < 0.05, MSG levels in g.kg^{-1} diet.

parenchymal weight, albuginea weight, volume, the whole left testicular weight and paired testicular volume. The right testicular parenchymal weight, albuginea weight and whole right testicular weight as well as the paired testicular parenchymal weight, albuginea weight and whole testicular weight on the diet containing 1.25 g MSG.kg^{-1} were higher but statistically similar (P > 0.05) when compared to those recorded on other diets. However, cocks on the diet containing 1.00 g MSG.kg^{-1} showed the highest significant (P < 0.05) values for the right testicular, left testicular and paired testicular densities.

The epididymal and testicular sperm reserves of cocks on each of the experimental diets are shown in Table 5. A common trend was noticed for each of the studied parameters as there were elevations in epididymal sperm reserves as the inclusion rates of MSG in the diets were increased from 0.00 to 0.50 g MSG.kg^{-1} diet with the cocks on 0.50 g MSG.kg^{-1} diet recording the highest significant (P < 0.05) means for all the parameters. Also, progressive decreases were observed in the epididymal sperm reserves of the cocks fed 0.75 to 1.25 g MSG.kg^{-1} diet with the cocks on 1.25 g MSG.kg^{-1} diet recording the least significant (P < 0.05) values for the studied parameters. The highest significant (P < 0.05) value for the testicular sperm reserve per testis (TSR/testis) for the right testis was recorded for birds on the control diet, while those of the left and paired testes were found to be significantly (P < 0.05) highest among the cocks on the diet containing 0.50 g MSG.kg^{-1}. TSR.g^{-1} testis (Testicular Sperm Reserve per gram testis) for the right (P < 0.05) and paired testes (P > 0.05) were also found to be highest on the control diet, while cocks on the diets containing 0.50 g MSG.kg^{-1} had the highest significant (P < 0.05) value for that of the left testis.

DISCUSSION

A high dose of monosodium glutamate has been implicated to be responsible for various abnormalities in the micro-architecture of the testis as well as semen

Table 5. Gonadal and extra-gonadal sperm reserves of cocks fed MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
TSR/g testis (x 10^8)							
Left	0.65 ± 0.08^{ab}	0.81 ± 0.07^{ab}	1.02 ± 0.08^{a}	0.91 ± 0.10^{ab}	0.59 ± 0.02^{b}	0.71 ± 0.14^{ab}	0.0122*
Right	1.07 ± 0.10^{a}	0.83 ± 0.07^{ac}	0.62 ± 0.07^{bc}	0.56 ± 0.06^{c}	0.91 ± 0.10^{ab}	0.57 ± 0.08^{c}	< 0.0001*
Paired	1.72 ± 0.18	1.64 ± 0.11	1.64 ± 0.05	1.47 ± 0.14	1.50 ± 0.11	1.28 ± 0.15	0.2079^{ns}
TSR/testis (x 10^9)							
Left	0.78 ± 0.03^{c}	1.01 ± 0.08^{bc}	1.45 ± 0.10^{a}	1.32 ± 0.15^{ab}	0.78 ± 0.06^{c}	0.82 ± 0.15^{c}	< 0.0001*
Right	1.32 ± 0.12^{a}	1.00 ± 0.07^{ab}	0.85 ± 0.07^{b}	0.92 ± 0.09^{ab}	1.25 ± 0.05^{ab}	0.98 ± 0.14^{ab}	0.0053*
Paired	2.11 ± 0.15^{ab}	2.01 ± 0.12^{ab}	2.30 ± 0.03^{a}	2.25 ± 0.16^{a}	2.03 ± 0.02^{ab}	1.79 ± 0.03^{b}	0.0149*
ESR (x 10^7)							
Left	1.72 ± 0.09	1.73 ± 0.26	1.85 ± 0.43	1.76 ± 0.19	1.65 ± 0.23	1.49 ± 0.23	0.4850^{ns}
Right	3.05 ± 0.10^{ab}	3.06 ± 0.12^{ab}	3.32 ± 0.16^{a}	2.46 ± 0.27^{bc}	2.14 ± 0.25^{c}	1.64 ± 0.25^{c}	< 0.0001*
Paired	4.77 ± 0.42^{a}	4.79 ± 0.46^{a}	5.17 ± 0.17^{a}	4.22 ± 0.17^{ab}	3.79 ± 0.30^{b}	3.13 ± 0.34^{b}	0.0033*

Values are means ± SEM; Means in a row without common superscripts are significantly ($P < 0.05$) different. Level of significance = ns (not significant) = $P > 0.05$; * = $P < 0.05$; TSR/testis (Testicular Sperm Reserve per testis); TSR/g testis (Testicular Sperm Reserve per gram testis), ESR (Epididymal/Extra-gonadal Sperm Reserve) MSG levels in g.kg^{-1} diet.

characteristics (Eweka and Om'Iniabohs, 2007). In the present study, the inclusion of MSG in the cocks' diets at 0.25 to 0.50 g.kg^{-1} did not adversely affect the ejaculate volume significantly. While cocks on 0.75 and 1.00 g MSG.kg^{-1} diet were hypospermic, 1.25 g MSG.kg^{-1} diet inclusion level was observed to have caused aspermia among the cocks on this diet. This has supported the reports of earlier findings that high level of MSG consumption causes oligozoospermia, increased abnormal sperm morphology and various degenerative changes, and also, causing deleterious effects on the Sertoli cells and Leydig cells of the testis, thereby, adversely impacting on spermatogenesis, spermiogenesis and testosterone production in adult Wistar rat males (Oforofuo *et al.*, 1997; Onakewhor *et al.*, 1998).

Furthermore, while MSG inclusion levels of 0.25 and 0.50 g.kg^{-1} did not significantly affect semen characteristics such as sperm motility, total live cells, sperm concentration and viability, no adverse effects were documented for total live cells.ml^{-1} and total motile cells.ml^{-1} in the present study, though a slight decrease was observed in certain parameters as MSG inclusion increases. However, a significant effect occurred at an inclusion rate above 0.50 g MSG.kg^{-1} diet. This agreed with the report of Kianifard (2016) that high administration of MSG (60 mg.kg^{-1} body weight) altered sperm parameters in preadolescent rats, thereby, leading to a significant reduction in the sperm count, sperm viability and sperm motility. Dong and Robbins (2015) also supported this finding by reporting that high dose intake of MSG negatively impacted sperm count and concentrations in adult rats fed 4 g.kg^{-1} MSG. The significant decrease observed for all the sperm characteristics in the group fed 1.25 g MSG.kg^{-1} diet could be attributed, probably, to the anti-fertility effect of MSG applied at higher doses.

The quality and quantity of testicular sperm production, as well as storage capacity, played a key role in the selection for breeding purposes (Ewuola and Akinyemi, 2017). Testicular and epididymal parameters, such as weight and length, are usually used in assessing their normality, thus, improving the detection of any deviation from normal that might result during the experimental process (Franca and Russel, 1998). These parameters are usually positively correlated with the spermatogenic activity of the testis (Nosseir *et al.*, 2012). The significant gradual decrease observed in the paired epididymal and *vas deferens* lengths and weights in the present study suggests that MSG might have a structural toxic effect on the epididymides and *vas deferens* at an inclusion level above 0.75 g MSG.kg^{-1} diet. This

agreed with the finding of Fernandes *et al.* (2012), who reported significant reductions in the absolute and relative weight of epididymis and testis in MSG-treated Wistar rats at the rate of 4 mg.kg^{-1} bodyweight, but disagreed in the case of testis weight, as no significant difference was observed in this experiment. They assumed that the reduction in the epididymal weight and length resulted in reduced sperm count in the epididymis and it may be responsible for the acceleration of the sperm transit time through the epididymis. Acceleration in sperm transit time has been reported to have an impairment tendency on sperm maturation and cause a reduction in the number of sperm cells available for ejaculation and fertility (Kempinas and Klinefelter, 2010).

Comparison of the gross testicular weights, volume, density, parenchymal and albuginea weights of the left, right and paired testes revealed that MSG inclusion up to 0.75 g.kg^{-1} diet did not have significant adverse effects on the studied parameters. The result of this finding upheld the report of Franca et al (2006), who found very similar results for most of the testis parameters evaluated in MSG-treated and control rats. Extragonadal sperm reserves (ESR), which are reflected in the amount of sperm storage in the epididymis, has been known to be correlated to sperm production by the testes (Azubuike *et al.*, 2016). In this study, the paired total epididymal sperm reserves of the cocks fed varying levels of MSG significantly reduced with increasing inclusion levels in the diets. This trend is suggestive of the dietary influence of MSG, since all the cocks were fed isocaloric and isonitrogenous diets with only the inclusion levels of MSG being the varying factor. The result of the study corroborates with the findings of Igwebuike *et al.* (2011) who reported a significant decrease in the mean caudal epididymal sperm reserves of the rats that were given medium- to-high doses of monosodium glutamate relative to the control group and the low dose group. The dose-related significant reduction in the ESR of the cocks on diets 0.75, 1.00 and 1.25 g MSG.kg^{-1} could be that the normal process of spermatogenesis was adversely affected in the cocks that consumed the diets containing MSG above 0.50 g.kg^{-1} diet. Since testosterone plays a major role in the spermatogenesis (Oforofuo *et al.*, 1997; Nayataraet *et al.*, 2008) the dose-related reduction in the ESR might be an indication that there could have been a considerable decline in the influence of testosterone on spermatogenesis in the significantly affected cocks (Dong and Robbins, 2015). The paired testicular sperm reserves (TSR) of the cocks decreased, though not significantly, with an inclusion level of 0.50 g MSG.kg^{-1} diet and above despite higher testes weight. The decline may possibly be the resultant effects of MSG on the degeneration of Sertoli cells that provide nourishment for the growth and survival of sperm cells within the seminiferous tubules. This corroborates with the opinion of Igwebuike *et al.* (2011) that monosodium glutamate may have a negative impact on spermatogenesis through its disruption of the hypothalamic-pituitary-testis regulatory (HPG) axis and not through any direct toxic effect on the testis. The results of our findings in the present study may be limited due to the length of exposure of the cocks to the diets treated with MSG, since adult birds were used and the exposure time to MSG was sixteen weeks. However, further research is required to assess the impact of dietary MSG on cocks fed treated diets from day old to the maturity stage.

CONCLUSION

Supplementation of cocks' diets with MSG up to 0.5 g.kg^{-1} did not compromise the semen characteristics, gonadal and extragonadal sperm reserves in the treated birds. Enhancing feed palatability for optimum feed utilization could be achieved with MSG in cocks' diet if the tolerable limit is not exceeded. This study reveals the possible taste-enhancing effect of monosodium glutamate in cocks' diet as a feed flavour additive. This study will help the farmers and feed millers to enhance the palatability of poultry feeds for optimum feed utilization. This will also add value to the use of phytogenic feed additives and non-conventional feedstuffs, which are reported to possess great potentials in poultry production but poor palatability might be a limiting factor. Thus, a new theory on the combined use of monosodium glutamate and non-conventional feedstuffs may be developed.

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USE OF ENTEROCIN M SUBSTANCE APPLIED IN DRINKING WATER AND NATURAL ZEOLITE AS DIETARY SUPPLEMENTS FOR GROWING RABBITS

Ľubica CHRASTINOVÁ[1]*, Mária CHRENKOVÁ[1], Zuzana FORMELOVÁ[1], Andrea LAUKOVÁ[2], Monika POGÁNY SIMONOVÁ[2], Matúš RAJSKÝ[1], Mária POLÁČIKOVÁ[1], Iveta PLACHÁ[2], Kristína BAČOVÁ[2], Ondřej BUČKO[3], Gabriela ŠTRKOLCOVÁ[4], Zuzana MLYNEKOVÁ[1], Tomáš RAŠI[5], Ľubomír ONDRUŠKA[1], Rastislav JURČÍK[1]

[1]NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[2]Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Košice, Slovak Republic
[3]Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Nitra, Slovak Republic
[4]University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic
[5]Zeocem Company, Bystré, Slovak Republic

ABSTRACT

A total number of 72 post-weaned rabbits (aged 35 days, meat line M91 a P91 hybrid rabbits), reared in an intensive breeding in the Slovak Republic, were used in the experiment. The rabbits were randomly divided into 3 similar groups, 24 animals in each group (control -EG1, experimental groups – EG2 and EG3) and kept in standard metal cages, two animals per cage. This study investigated the effects of different feeding on growth performance, chemical composition, fatty acid profile and content of essential amino acids of the *Musculus longissimus thoracis* and *lumborum* of growing rabbits. This *in vivo* study was designed to reveal whether the antimicrobial effect of Enterocin M (produced by non-rabbit origin strain *Enteroccocus faecium* AL41-CCM8558), is able to influence the meat quality of rabbits. Every day at the same time in the morning the rabbits in EG 2 received 50 µl dose of Ent M per animal per day, administered into their drinking water. The rabbits of experimental group (EG3) had commercial diet enriched with the supplement of 1 % natural zeolite (a product of ZEOCEM Company, Bystré, Slovakia) and the drinking water did not contain any coccidiostatic drugs during the experiment. The experiment was lasted for 42 days.
The fat of rabbits in the 2EG had a higher ($P < 0.05$) concentration of n-3 and n-6, MUFA, while the ratio of SFA in the control was lower (32.92 %) than in 2EG and 3EG reared rabbits (34.61 % vs. 34.71 %). Feeding of natural substances to rabbits did not influence biochemical and zootechnical parameters and it had no negative effect on growth performance in rabbits. It had positive effect on health status and it reduced the number of *Eimeria* spp. Oocysts were not detected in rabbits' intestinal tract.

Key words: rabbit meat; enterocin; zeolite

INTRODUCTION

In recent years, naturally occurring antimicrobial and antioxidant compounds have been preferably employed in meats because of their potential health benefits and safety compared to synthetic preservatives (Lauková *et al.*, 2015, 2016; Strompfová *et al.*, 2017). Among those compounds/substances enterocins have proteinaceous character and produced mostly by the representatives of the genus *Enterococcus*. Enterocins can inhibit the growth of both Gram-positive and Gram-negative bacteria (Franz *et al.*, 2007) and were used with beneficial influence on rabbit husbandry (Lauková *et al.*, 2012).

Zeolite is a substance of crystal structure, containing cations of alkaline-earth metals. Zeolite

***Correspondence:** E-mail: lubica.chrastinova@nppc.sk
Ľubica Chrastinová, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic

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is used to modify fermentation processes including buffers, which regulate gut pH and favour the activity of cellulolytic bacteria, compounds to suppress methane production and bloat-preventing compounds, which prevent the build-up of gas trapped in foam (CO_2, NH_3, H_2S, CH_4 and some nitrogen combinations) in the caecum fluid (Shibaev and Butko, 1986; Shadrin, 1988). Because breeders have been looking for ways to maintain or improve animal health status in association with meat production, they are more open to use natural substances.

The aim of this *in vivo* study was to determinate the effect of Enterocin (Ent) M, applied to drinking water and zeolite, as dietary supplements for growing rabbits, on growth performance, selected physico-chemical parameters and nutritional quality of rabbits meat.

MATERIALS AND METHODS

A total number of 72 post weaned rabbits (aged 35 days, meat line M91 a P91 hybrid rabbits), reared in an intensive breeding in the Slovak Republic, were used in the experiment. The rabbits were randomly divided into 3 similar groups (control-EG1, experimental group EG2, experimental group-EG3), 24 animals in each group, and kept in standard metal cages, two animals per cage.

A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Temperature and humidity in the building were recorded continuously by a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed the building air temperature to be maintained within 21 ± 4 ˚C throughout the experiment. Relative humidity was about 70 ± 5 %. The rabbits were fed a commercial diet (KV; TEKRO Nitra, Ltd. Slovak Republic) pellets of 3.5 mm in diameter *ad libitum* and water was provided *ad libitum* using nipple drinkers. The experiment was performed in co-operation with the Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences in Košice. All care and experimental procedures were approved by the Slovak Veterinary and Food Administration and by the Ethic Commission of both institutes. Every day at the same time in the morning the rabbits in EG2 received 50 µl dose of Ent M per animal per day, administered into their drinking water. The rabbits of the experimental group (EG3) were fed a commercial diet enriched with supplement of 1 % natural zeolite, and the drinking water did not contain any coccidiostatic drugs during the experiment. The zeolite (ZeoFeed) was supplied by Zeocem Company (Zeocem a.s., Bystré, Slovakia) from the quarry of Nižný Hrabovec, Slovakia. Zeolite used in the experiment had a particle size of 0.2 – 0.5 mm and contained more than 80 % of clinoptilolite, as determined by X-ray powder diffraction. Its chemical composition was as follows: SiO_2 – 70.98 %, Al_2O_3 – 11.72 %, Fe_2O_3 – 1.26 %, CaO – 2.89 %, MgO – 0.53 %, K_2O – 3.25 %, Na_2O – 0.56 % and loss on ignition – 7.17 %.

To detect *Eimeria* sp. oocysts, the quantitative McMaster method (1986) was used; the oocyst counts were expressed in OPG.g^{-1} (detected oocysts per gram of faecal sample).

The ingredients and chemical composition of this diet is presented in Table 1, according to procedures of the AOAC (2005) and Van Soest *et al.* (1991).

The body weight of each experimental animal was recorded weekly during the whole study. Weight feed mixture was checked daily and average daily weight gain and feed conversion were calculated mathematically, as well as mortality, at the end of the experiment. Five animals (at the age of 70 days) from each group were slaughtered and samples were taken. After electro-stunning (90 V for 5 s), rabbits were slaughtered in an experimental slaughterhouse by cutting the carotid and jugular veins and bleeding out. *Musculus longissimus thoracis* and *lumborum* (*MLTL*) was separated by removing the skin and connective tissue chilled and stored at 4 °C for 24 h until physico-chemical analysis started. The pH value was determined after 24 h (*post-mortem*) using a Radelkis OP-109 measure device (Jenway, England) with a combined electrode penetrating 3 mm into samples. The electrical conductivity (µS.cm^{-1}) defined as locations of muscles were evaluated using PMV 51 (Tecpro GmbH, Germany), colour characteristic were expressed by CIE L*a*b system (lightness-L*, 0: black and 100: white), (redness and greenness-a*; yellowness and blueness-b*) using a Lab. Miniscan, Lightness measurements at room temperature were also performed. Physico-chemical characteristics and

Table 1. Composition and nutrient content of granulated diet for growing rabbits (g.kg^{-1} in original matter)

Ingredients	%	Chemical analysis	Control diet	Control diet + 1 % Zeolite
Lucerne meal	36.0	Crude protein (N*6,25)	187.74	197.96
Extracted sunflower meal	5.5	Crude fibre	170.15	180.43
Extracted rapeseed meal	5.5	Fat	34.24	36.05
Wheat bran	9.0	Ash	67.09	81.06
Oats	13.0	Starch	127.76	95.19
Malt sprouts	15.0	Organic matter	826.65	803.65
DDGS	5.0	ADF	203.17	210.79
Sodium chloride	0.3	ADF	349.46	321.06
Mineral and vitamin mixture*	1.7	Calcium	9.09	8.60
Barley grains	8.0	Phosphorus	5.74	6.50
Limestone	1.0	ME (MJ.kg^{-1})	10.95	10.82

*Premix contains per kg: calcium, 6.73 g; phosphorous, 4.13 g; magnesium, 1.90 g; sodium, 1.36 g; potassium, 11.21 g; iron, 0.36 g; zinc, 0.13 g; copper, 0.03 g; selenium, 0.2 mg; Vitamin mixture provided per kg of diet: Vitamin A 1 500 000 IU; Vitamin D3 125 000 IU; Vitamin E, 5 000 mg; Vitamin B1, 100 mg; Vitamin B2, 500 mg; Vitamin B6, 200 mg; Vitamin B12, 0.01 mg; Vitamin K3, 0.5 mg; biotin,10 mg; folic acid, 25 mg; nicotinic acid, 4 000 mg, choline chloride, 100 000 mg. DDGS: dried distillers grains with solubles; ADF: Acidodetergent fibre; NDF: Neutraldetergent fibre; ME: metabolisable energy

chemical composition were determined by standard methods (STN 570185). The content of water, protein and fat were estimated using a FoodScaneTM – Meat Analyser (FOSS, Denmark) by a FT IR method (Fourier Transform infrared Spectroscopy); expressed in g.100 g^{-1}. From these values, the energy value was calculated according to the equation of Strmiska *et al.* (1988):

Energy value (kJ.100 g^{-1}) = 16.75 x protein content + 37.65 x fat content.

The water holding capacity was determined by the compress method at constant pressure (Hašek and Palanská, 1976; Rafay *et al.*, 2008). The analysed samples (0.3 g in weight) were placed on filter papers (Schleicher and Shuell No. 2040B, Dassel, Germany) with tweezers previously weighed. Together with the papers samples were sandwiched between Plexiglas plates and then subjected to a pressure of 5 kg for 5 min. The results were calculated

Table 2. Effect of treatment on performance of rabbits (mean ± SD)

Parameter (n = 24)	EG1 control	EG2 control + Ent M	EG3 control + 1 % Zeolite
Number of animals in groups	24	24	24
Initial live weight (35 d), g	1059 ± 99	1000 ± 98	1153 ± 136
Intermediate live weight (56 d), g	1981 ± 207	1953 ± 164	2077 ± 237
Final weight (77 d), g	2779 ± 300	2635 ± 334	2832 ± 264
Feed conversion ratio between 35th and 56th day (g.g^{-1})	3.466 ± 0.354	3.434 ± 0.347	3.537 ± 0.287
Feed conversion ratio per kg gain	3.882 ± 0.320	3.769 ± 0.461	3.849 ± 0.258
Mortality (n)	4	4	4
Daily weight gain, (g.d^{-1})	40.95	38.93	40.38
Carcass value (%)	55.67 ± 1.41	55.36 ± 1.05	55.42 ± 1.15

P > 0.05; not significant differences from control; EG-experimental group

from the difference in weight between the slips with aspirating spot and the pure filter paper. The ash content was determined by mineralization of the samples at 550 °C according to STN 570185. The fatty acid (FA) composition of MLD samples was determined by the method of Ouhayoun (1992) and Bannon *et al.* (1982) by gas chromatography of fatty acid methyl ester (FAME) on GC 6890N (Agilent Technologies, Switzerland). The results were expressed as percentages of total fatty acids. Fatty acid composition varies a lot and it is expressed as share of SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acids), P/S and n6/n3 index. The amino acid composition of the diet was analysed by an ion-exchange chromatography on AAA (Ingos Prague, Czech Republic) after acid hydrolysis with 6M HCl and methionine and cystine after oxidation hydrolysis.

The results were expressed as the mean ± SD. Mean values within the same row having different superscripts indicate significant difference using Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Among the experimental groups no significant differences were found in feed intake, feed conversion ratio and carcass value in the fattening experiment. Furthermore, rabbits reared in the EG3 group weighed more but the difference was not significant. The average daily weight gain was lower in the both experimental groups comparing to the control group: EG1 – 40.95 g, EG2 (*Ent* M) – 38.93 g, EG3 (1 % zeolite) – 40.38 g.

Table 3. Physio-chemical characteristics of rabbit meat (*MLTL*) 24 h *post-mortem* (n = 5)

Characteristic Age at slaughter (70d)		EG1 control		EG2 control + Ent M		EG3 control + 1 % Zeolite	
		mean	SD	mean	SD	mean	SD
Water	g.100g^{-1}	74.41	0.43	74.18	0.62	74.72	0.26
Protein	g.100g^{-1}	23.19	0.19	22.98^{a}	0.14	23.11	0.19
Fat	g.100g^{-1}	1.16	0.26	1.30^{a}	0.26	1.16	0.16
Collagen fibre	g.100g^{-1}	0.89	0.18	0.77	0.16	0.81	0.05
Energy value	kJ.100g^{-1}	432.07	12.48	433.83	8.04	430.58	5.75
Water holding capacity	g.100g^{-1}	23.02	4.81	24.19	3.77	29.09	6.16
Ash	g.100g^{-1}	0.62	0.15	0.57	0.14	0.53	0.10
Cholesterol	g.100g^{-1}	0.38	0.03	0.36^{a}	0.036	0.39	0.02
pH 24		6.05	0.05	5.99	0.06	5.96	0.05
* Colour L	L	50.66	1.25	48.24	2.57	50.01	3.76
Electric conductivity	µS.cm^{-1}	1.08	0.26	1.51^{a}	0.27	1.01	0.59
	Fatty acid composition in intramuscular fat (% of total fatty acids)						
SFA	%	32.924	1.135	34.606	1.760	34.710	1.710
MUFA	%	47.862	1.152	48.176^{a}	0.351	47.180	0.681
PUFA	%	13.224	1.195	12.280	1.210	12.228	0.769
Essential FA	%	9.236	0.091	8.178^{a}	0.863	8.913	0.520
ω 6	%	8.676	0.521	9.012^{a}	0.847	8.670	0.656
ω 3	%	0.442	0.026	0.452	0.030	0.440	0.037

[a] $P < 0.05$ significant differences from control; EG-experimental group

SFA: saturated fatty acids, include C8:0, C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0.

MUFA: monounsaturated fatty acids, include C16:1 n-7, C18:1 n-9c, C18:1 n-9t, C20:1, C22:1.

PUFA: polyunsaturated fatty acids, include C18:2 n-6, C18:3 n-3, C20:4 n-6, C20:5n-3, C22:5 n-6, C22:6n-3.

The feed conversion ratio also decreased in the groups administering enterocins: in the EG2 group (*Ent* M) – 3.434; in the EG3 (1 % zeolite) – 3.537. These findings are in agreement with our previous results (Pogány Simonová *et al.*, 2015; Szabóová *et al.*, 2011; Lauková *et al.*, 2016). Results regarding the growth parameters are shown in Table 2. After Ent M applying into the water (50 µl per animal per day for 6 weeks), growth performance of rabbits and physio-chemical parameters of meat quality and nutritional value were influenced. Moreover, also non-autochthonous strain can have protective and beneficial effect on broiler rabbits (Lauková *et al.*, 2012; 2016; Strompfová *et al.*, 2017; Chrastinová *et al.*, 2018). No significant differences were observed in the mean carcass yield ratio, the weights of liver, heart and kidney in the tested variants. *Eimeria* sp. oocysts were not detected in the faecal samples of rabbits.

The selected meat quality parameters (content of water, content of proteins, fat, content of amino acids and fatty acids profile, the electric conductivity, ultimate pH 24 (*p.m.*), colour meat characteristic) are presented in Table 3. The oral administration of Ent M lead to a significant increase in the electrical conductivity, compared to control, and to decrease in cholesterol contents ($P \leq 0.05$) of the MLTL. The fat of rabbits in the 2EG had a higher concentration of n-3 and n-6 and MUFA ($P < 0.05$), while the ratio of SFA in control was lower (32.924 %) than in 2EG and 3EG groups of reared rabbits (34.61 % vs. 34.71 %).

Meat is a major source of proteins, essential amino acids, minerals and fatty acids. Protein and lipid contents of meat are closely related to the energy value (Dalle Zotte, 2002; Dalle Zotte and Szendrö, 2011; Pogány Simonová *et al.*, 2015). Chemical composition of meat is closely related to the age. This results are in agreement with the studies investigating effects of age and genotype on muscle composition (Gondret *et al.*, 1998; Chrastinová *et al.*, 2010; Combes, 2004; Bianch *et al.*, 2006; Kalafová, *et al.*, 2014; 2018). The amino acid composition in *MLTL* muscle is shown in Table 4. The essential amino acid composition is one of the most important nutritional qualities of protein.

Only slight differences were found between the individual components, which corresponds with the results of the other authors. Dietary supplementation with 1 % natural zeolite reduced feed conversion ratio, but differences were not significant ($p > 0.05$).

CONCLUSION

Feeding natural substances to rabbits did not negatively influence biochemical and zootechnical parameters and it also had no negative effects on growth performance in rabbits. It had a beneficial effect on health status and reduced the number of *Eimeria* spp. Oocysts were not revealed in the rabbit intestinal ecosystem. In this way, rabbit meat could also be considered to be a functional food.

Table 4. The content of essential amino acids in *MLTL* muscles 24 h *post-mortem* of rabbits (g.100g^{-1}) tissue

Characteristic (n = 5)	EG1 control	EG2 control + Ent M	EG3 control + 1 % Zeolite
Threonine	0.783 ± 0.068	0.709 ± 0.148[a]	0.742 ± 0.057
Valine	0.792 ± 0.053	0.758 ± 0.118	0.767 ± 0.051
Methionine	0.549 ± 0.038	0.514 ± 0.099	0.520 ± 0.032
Cystine	0.231 ± 0.012	0.214 ± 0.034[a]	0.222 ± 0.003
Isoleucine	0.703 ± 0.080	0.635 ± 0.163	0.654 ± 0.075
Leucine	1.405 ± 0.133	1.291 ± 0.293[a]	1.321 ± 0.120
Phenylalanine	0.728 ± 0.065	0.676 ± 0.147[a]	0.687 ± 0.057
Histidine	0.702 ± 0.053	0.665 ± 0.147[a]	0.680 ± 0.054
Lysine	1.529 ± 0.160	1.397 ± 0.342[a]	1.428 ± 0.142
Arginine	1.151 ± 0.122	1.049 ± 0.257[a]	1.073 ± 0.109

[a] $P < 0.05$ significant differences from control

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ASSESSMENT OF SPLINE FUNCTIONS AND NON-LINEAR MODELS FOR ESTIMATING GROWTH CURVE PARAMETERS OF FUNAAB-ALPHA CHICKENS

Hameed Akande BASHIRU*, Saidu Oyarekhua OSENI, Louis Adebayo OMADIME

Department of Animal Sciences, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria

ABSTRACT

The study assessed the growth of FUNAAB-Alpha chickens (FAC) using spline and non-linear functions in order to establish the most appropriate growth function(s) for FAC. Three hundred (300) day-old chickens of FAC were used for the study. They were raised intensively under a deep litter system for 20 weeks and body weight records were taken weekly with the aid of a digital scale. Spline models of different numbers of, and locations of, knots were fitted using the REG procedure of SAS® while four non-linear models (Gompertz, Logistic, Bertalanffy and Richards') were fitted using the NLIN procedure of SAS®. The estimated hatch weight (β_0) for the male and female chickens ranged from 30.77 g to 74.71 g and from 15.56 g to 38.19 g, respectively. The regression coefficients ranged from -38.47 to 47.46 and -39.40 to 40.47 for the male and female, respectively. The highest magnitudes of these coefficients were estimated at early ages (3 to 10 weeks), implying that growth rate at early stage of life might be a key response to selection for later growth performance. For non-linear models, parameter A (or asymptotic weight) for all the models ranged from 3716 g to 2050 g and 1591 g to 3330 g for male and female, respectively. The parameter (B), the scaling parameter (constant of integration), ranged from 0.7541 to 15.441. Likewise, parameter K, which is the maturity index, ranged from 0.0463 to 0.2002. The age at inflection point for FUNAAB-Alpha chickens ranged between 13.30 and 17.63 weeks for male chickens and between 14.23 and 19.94 weeks for female chickens while the corresponding body weight at inflection point ranged between 754 and 1528 g and 586 and 1261 g for male and female chickens, respectively. Based on Akaike Information Criterion and Bayesian Information Criterion as best fit model selection criteria, it was concluded that the spline models of 3 and 4 knots were the best fit linear spline models while Bertalanffy and Gompertz models were selected as the best fit non-linear models.

Key words: spline model; non-linear models; knots; growth parameters; regression coefficients

INTRODUCTION

Nigerian indigenous chickens have been characterized as hardy, good scavengers and highly adapted to the harsh, hot and humid tropical environment (Peters *et al*., 2007). These chickens are said to be flighty, good mothers and resistant to many diseases and they play an integral role in rural economy (Sonaiya and Swan, 2004). However, Nwosu and Asuquo (1985) described them as small bodied, slow growing, poor feed converters, poor layers and poor meat birds. This is as a result of long-term natural selection for fitness in the harsh tropical and disease-prevalent environment (Adebambo *et al*., 2010). These shortcomings led to the intensification of efforts towards the development of indigenous chicken breeds with improved meat and egg production through the exchange of germ plasm with established exotic breeds. For instance, the ShikaBrown breed was developed by the National Animal Production

***Correspondence:** E-mail: hameedbashiru@gmail.com
Hameed Akande Bashiru, Department of Animal Sciences,
Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria

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Research Institute (NAPRI), Ahmadu Bello University, Zaria, Nigeria (Ikanni and Annatte, 2000). Similarly, the Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB) has developed the FUNAAB-Alpha breed, described as an improved, indigenous, tropically adapted and dual-purpose breed. FUNAAB-Alpha has attributes and potentials for improved meat and egg production while maintaining adaptation to the tropical environment characterized by heat stress and infectious diseases (Adebambo, 2015). Males of FUNAAB-Alpha chickens have been reported to weigh about 1.5 – 2.0 kg at 20 weeks of age while their females usually weigh between 1.2 to 1.8 kg at 18 – 21 weeks of age when they lay their first egg (Adebambo *et al*., 2018).

The characterization of poultry breeds is a key to understanding their distinctiveness, growth, production potential, management requirements and their ability to thrive under various climatic environments. The Global Plan of Action (GPA) for Animal Genetic Resources (FAO, 2007) recognizes that a better understanding of the characteristics of livestock breeds is necessary for guiding decision making in the development of breeding strategies to enhance sustainable use of animal genetic resources. Specifically, Strategic Priority Area 6 of the GPA specifically entails "Support indigenous and local production systems and associated knowledge systems of importance to the maintenance and sustainable use of animal genetic resource". Hence, there is the need for a detailed performance characterization and evaluation study on FUNAAB-Alpha chickens. One major and important trait for such evaluation is growth.

Growth can be defined as body weight gain or weight gain of body parts with age. The process of growth has often been summarized using mathematical equations fitted to growth curves and the objective of this curve fitting is to describe the course of body weight increase over time or age with mathematical parameters that are biologically interpretable (Aggrey, 2002). These parameters have a biological interpretation in terms of growth process and their values, as well as their relationships with other parameters, they provide a genetic basis for understanding growth process and for development of breeding strategies to alter or modify the trajectory of growth.

Many mathematical models have been applied to the study of growth performance in poultry research (Laird *et al*., 1965; Grossman *et al*., 1985; Aggrey, 2002). Most of these models are non-linear and fitted curves that relate the age of the bird with its weight, characterize the different phases of growth of the bird, allow the estimation of the animal's growth rate, the age at which the animal stops growing, and when it reaches sexual maturity (Galeano-Vasco *et al*., 2014).

The spline model has been suggested for the study of sigmoidal growth (Aggrey, 2002), and as an alternative to non-linear growth models. The spline linear model is a compound function consisting of a series of linear equations which meet at certain points known as knots. The spline linear regression model can be used as an alternative to high order polynomials and complicated non-linear models (Aggrey, 2002; Meyer, 2005) and can also serve as an alternative means to model complex growth processes, since it can easily be modified to accommodate more knots. Harrell (2004) suggested that linear splines could be modifiable by varying the number and position of the knots to obtain the best fit model to the dataset. Extensive information on the growth curve parameters of FAC with non-linear and spline functions will enhance making effective management and production decisions that are integral to the sustainable use of FAC as a genetic resource for income generation and poverty alleviation.

Most of the growth models available for poultry have been fitted using non-linear models. Consequently, there is a need to fit alternative growth models using spline functions so as to compare and establish model superiority for describing the growth curve of FAC. The objective of the study, therefore, was to assess the growth of FUNAAB-Alpha chickens using spline and non-linear functions in order to establish the most appropriate growth function(s) for FAC.

MATERIAL AND METHODS

Experimental location

This experiment was conducted at the Poultry Unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The farm

is located at Longitude 04° 33' E and Latitude 07° 28' N at an altitude of 224 m above sea level.

Experimental birds

Three hundred (300) day-old chickens of the FUNAAB-Alpha chickens (FAC) were obtained from the Hatchery Unit of the Federal University of Agriculture, Abeokuta (FUNAAB). They were brooded for two weeks. Adequate temperature of 40 °C – 45 °C was provided during brooding using electric bulbs and gas burner as the source of heat. They were thereafter transferred to a deep litter pen at the end of the fourth week.

Management practices

The deep litter pen, containing thirty cells (each 1.5 m × 1.5 m), was made of wood and wire netting while the floor was made of concrete. The bushes around the building were cleared, the pen was properly fumigated and wood shavings were thoroughly spread on the concrete floor before the birds were transferred. Feeders and drinkers were provided for each cell in the deep litter pen. The chickens were fed starter ration containing 20 % crude protein (CP) and 2800 kcal.kg^{-1} of metabolizable energy (ME) from day old till the fifth week, after which they were fed with grower ration containing 18 % CP and 2900 kcal.kg^{-1} till the twentieth week when the experiment was terminated. Clean water was provided *ad libitum*. The feed was placed in standard and specialized feeding tray made of red colour to attract the chickens to the feed while water was provided in a standard and specialized 3.0 litre plastic drinker, placed upside down for proper water dispensation and to avoid water spillage.

Health management

Proper hygiene was ensured all the time. Biosecurity was guaranteed by barring visitors and strangers from entering the pen while a foot dip was provided at the entrance and replaced daily. Drinkers and feeders were thoroughly washed and cleaned daily while left-over feeds and water were removed in order to prevent build-up of parasites and pathogens. The litter was kept dry at all times. The chickens were vaccinated against Newcastle disease on the 10th day and other medications were administered when due, following the standard practice in poultry management.

Data collection

Each bird was wing tagged for identification and weighed weekly using a sensitive digital weighing scale (Model SF-400) with a maximum capacity of 10 kg and a sensitivity of 1 g throughout the conduct of this experiment. The bodyweight records were taken early in the morning before feeding following FAO (2012). The feather morphology (frizzle feathered or normal feathered) and feather distribution pattern (naked neck or normal neck) were also observed and recorded. The cross-tabulation of FUNAAB-Alpha chickens across sexes, feather distribution and morphology subgroups used in this study is presented in Table 1. These chickens are as shown in Plate 1 below.

Data Analysis

The raw data was first plotted to determine the appropriate locations of the knots following Aggrey (2002). Based on this preliminary step, splines of 3, 4, 5 and 6 knots corresponding to varied age ranges (in weeks) on the growth trajectory were fitted. The equations, location of the knots (age in weeks

Table 1. Distribution of FUNAAB-Alpha chickens across sexes, feather distribution and morphology sub-groups

Feather distribution/Morphology	Male	Female	Total
Normal	106	138	244
Naked neck	22	34	56
Total	128	172	300
Normal	96	127	223
Frizzled	32	45	77
Total	128	172	300

Plate 1. Naked neck, frizzled-feathered and normal feathered FUNAAB–Alpha chickens

along the trajectory) of these models are presented in Table 2. The spline functions were fitted to the body weight records using the REG procedure of SAS®.

Four classical non-linear growth models including von Bertalanffy, Gompertz, Logistic and Richards' models were also fitted to the body weight records using PROC NLIN of SAS®. Parameter estimates of these non-linear models were thereafter compared with the fitted spline functions. These non-linear models were fitted using the NLIN procedure of SAS® using Marquardt iterative option (Marquardt, 1963)

Table 2. Equations for the spline functions

Spline model	Number of knots	Location of knots (age in weeks)	Equation of the model
SP3	3	4, 10, 16	$W_t = W_0 + b_1t + b_2$ (t -4) + b_3 (t -10) + b_4 (t -16) + e
SP4	4	4, 8, 12, 16	$W_t = W_0 + b_1t + b_2$ (t -4) + b_3 (t -8) + b_4 (t -12) + b_5 (t -16) + e
SP5	5	4, 7, 10, 14, 18	$W_t = W_0 + b_1t + b_2$ (t -4) + b_3 (t -7) + b_4 (t -10) + b_5 (t -14) + b_6 (t -18) + e
SP6	6	3, 6, 9, 12, 15, 18	$W_t = W_0 + b_1t + b_2$ (t -3) + b_3 (t -6) + b_4 (t -9) + b_5 (t -12) + b_6 (t -15) + b_7 (t -18) + e

Where W_t = body weight at time t; W_0 is the intercept of the model (body weight at hatch); $b_1 ... b_7$ are the regression coefficients (growth rates of the specified periods that constitute the entire spline); and e is the residual error.

Table 3. Non-linear growth model equations

Model	Equation	Inflection time	Inflection point	Relative growth rate
Gompertz	$W_t = A*exp(-B*exp(-k*t))$	A/e	$\ln(B)/k$	$k\left(\frac{A-W_{(t)}}{A}\right)$
Logistic	$W_t = \frac{A}{1+B*exp(-k*t)}$	$A/2$	$\ln(B)/k$	$k*\log\left(\frac{A}{W_{(t)}}\right)$
Bertalanffy	$W_t = A(1-B*e^{-k*t})^3$	$8/27\,(A)$	$\frac{1}{k}\,ln.3\,(B)$	$3k\left[\left(\frac{A}{W_{(t)}}\right)^{1/3}-1\right]$
Richards'	$1+B*exp(-k*t)^{1/d}$	$A/(d+1)^{1/d}$	$\frac{1}{k}*ln\lvert d/B\rvert$	$dk\left[\left(\frac{A}{W_{(t)}}\right)^{1/d}-1\right]$

Where W_t = body weight at t weeks of age; t = bird's age in weeks; A = asymptotic weight or mature weight; B = scaling parameter (constant of integration); k = maturity index; d = shape parameter for Richards' model which allows a variable point of inflection.

according to the equations presented in Table 3. The most appropriate model(s) was/were selected using Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) following Kaps and Lamberson (2004). According to these authors, a model with the lowest AIC and BIC values represent the best fit model.

RESULTS

The least squares means and standard error of body weight of FUNAAB-Alpha chickens raised under a deep litter system from hatch till the birds were 20 weeks old are shown in Table 4. The body weight of the male and female chickens were similar at hatch ($P > 0.05$). However, from hatch, the males were heavier than the female chickens across different ages ($P < 0.05$). The difference in their body weight increased linearly from hatch till the 20th week of age and reaching two peaks at 16th and 18th week of age.

The estimated hatch weights and regression coefficients for FUNAAB-Alpha chickens using spline functions of 3 (SP3), 4 (SP4), 5 (SP5) and 6 (SP6) knots are presented in Table 5. For both sexes, SP3 estimated the highest hatch weight while SP6 estimated the least values. The regression coefficients ranged from -38.47 to 47.46 for male chickens, while it ranged from -39.40 to 40.47 for female chickens. Highest magnitudes of these coefficients were estimated at early ages.

Table 4. Least squares means for body weight of FUNAAB-Alpha Chickens raised under a deep litter system from day old to 20 weeks of age

Age (weeks)	LSM (g) ± SE (male)	LSM (g) ± SE (female)
Day old	33.40 ± 3.45	31.25 ± 1.28
2	126.80 ± 4.06^a	118.07 ± 3.95^b
4	296.65 ± 10.81^a	254.27 ± 10.28^b
6	408.05 ± 14.12^a	321.76 ± 14.23^b
8	606.10 ± 18.25^a	418.70 ± 21.35^b
10	805.29 ± 21.60^a	568.23 ± 26.30^b
12	901.65 ± 23.66^a	665.19 ± 30.28^b
14	1305.41 ± 33.62^a	853.65 ± 36.44^b
16	1509.78 ± 39.89^a	953.86 ± 43.05^b
18	1669.22 ± 42.03^a	1129.33 ± 48.78^b
20	1894.80 ± 45.95^a	1321.71 ± 52.43^b

LSM = least squares means, SE = standard error of the means.
^{ab}Means within the same column having different superscript are significantly different ($P < 0.05$).

Table 5. Estimated coefficients for spline regression model parameters of FUNAAB-Alpha chickens raised under a deep litter system

	Male				Female			
Parameters	SP3	SP4	SP5	SP6	SP3	SP4	SP5	SP6
Hatchweight (β_0)	74.71	52.32	68.45	30.77	34.40	29.57	38.19	15.56
β_1	47.46	38.01	33.39	39.50	36.68	35.34	40.22	31.90
β_2	-38.39	35.07	35.52	33.52	-29.05	31.85	28.47	37.84
β_3	-21.81	-30.49	-38.47	-29.13	-24.35	-23.84	-27.88	-18.83
β_4	23.91	-28.60	-15.80	-23.00	19.33	-21.56	-29.14	-19.40
β_5		25.89	-29.44	-21.38		28.72	-24.35	-19.89
β_6			29.43	22.78			18.77	24.28
β_7				19.51				15.23

Table 6 showed the estimated growth model parameters for male and female FUNAAB-Alpha chickens using Gompertz, Logistic, Bertalanffy and Richards' growth functions. For all the models, parameter (A), which is the asymptotic weight (or maximum stationary weight), ranged from 2050.8 g to 3716.6 g for the male and from 1591.7 g to 3330 g for the female chicken respectively. Parameter (B), the scaling parameter (constant of integration) ranged from 0.7541 g to 15.441 g. Likewise, parameter K, which is the maturity index ranged from 0.0463 g to 0.2002 g. The Bertalanffy model estimated the highest asymptotic weight while the Logistic model estimated the least.

Table 7 showed the body weight and age at inflection point for FUNAAB-Alpha chickens as estimated by Gompertz, Logistic, Bertalanffy and Richards' models. For all the models fitted, age at inflection point for FUNAAB-Alpha chickens ranged between 13.30 and 17.63 weeks for male chickens and 14.23 to 19.94 weeks for female chickens. The corresponding body weight at inflection point ranged between 754 and 1528 g and 586 and 1261 g for male and female chickens respectively. For both

Table 6. Estimates of growth model parameters for FUNAAB-Alpha chickens

	Male			
Model	A	B	K	D
Gompertz	3056.3 ± 462.7	3.5503 ± 0.1046	0.0860 ± 0.011	-
Logistic	2050.8 ± 178.5	15.441 ± 1.4930	0.2002 ± 0.0178	-
Bertalanffy	3716.6 ± 951.3	0.7541 ± 0.0116	0.0463 ± 0.00923	-
Richards'	3056.2 ± 462.5	2.521 ± 0.153	0.150 ± 0.0111	0.343 ± 0.0367
	Female			
Gompertz	2521.0 ± 362.0	3.5813 ± 0.0859	0.080 ± 0.0091	-
Logistic	1591.7 ± 118.4	15.7189 ± 1.1851	0.1964 ± 0.0140	-
Bertalanffy	3330.6 ± 1262.7	0.7672 ± 0.0124	0.0417 ± 0.00889	-
Richards'	2520.9 ± 361.9	2.852 ± 0.964	0.147 ± 0.0190	0.352 ± 0.0224

Where A, B, K and D represent the asymptotic weight, the scaling parameter, maturity index and the shape parameter (for Richards' model) respectively.

Table 7. Body weight (g) and age (weeks) at inflection point

	Male		Female	
Model	T (weeks)	W (g)	T (weeks)	W (g)
Gompertz	14.73	1528	15.95	1261
Logistic	13.67	754	14.03	586
Bertalanffy	17.63	1101	19.94	987
Richards'	13.30	1294	14.23	1070

Where T is the age (weeks) and W is the body weight (g) at inflection point.

Table 8. Best fit model selection criteria using Goodness-of-Fit tests

	Male		Female	
Model	AIC	BIC	AIC	BIC
SP3	46.735	55.221	44.138	54.343
SP4	50.167	61.256	43.867	54.201
SP5	49.204	59.544	45.867	56.425
SP6	54.660	65.298	46.623	57.188
Gompertz	50.42	61.528	44.460	55.102
Logistic	53.23	64.488	47.10	58.342
Bertalanffy	49.42	60.122	44.21	54.154
Richards'	50.42	61.778	46.76	57.813

Where AIC and BIC are Akaike Information Criterion and Bayesian Information Criterion respectively.

sexes, the Gompertz model estimated the highest body weight at inflection while the Logistic model estimated the least. Similarly, the Richards' model predicted the earliest age at inflection point while the Bertalanffy model estimated the latest age at inflection. For all the models, males had higher body weight at inflection than females. However, the females had higher ages at inflection point than the corresponding males for all the models.

Goodness-of-fit tests for spline models as well as for the non-linear models (Gompertz, Logistic, Bertalanffy and Richards') are presented in Table 8. These included the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). The lower the values of AIC and BIC, the better fit is the data (Kaps and Lamberson, 2004). For the male, SP3 had the lowest AIC and BIC and was adjudged the best fit model followed by SP5, SP4 and SP6 in that order. For the female, SP3 and SP4 had the lowest AIC and BIC values and were selected as the best fit model followed by SP6 and SP5 in that order.

Table 9 showed the correlation coefficients among model parameters. High and negative correlation coefficients ($r < -0.90$) were observed between parameters A (asymptotic weight) and K (maturity index), both male and female, for all the models. Between parameters B and K, there was high positive correlation for the Logistic and Richards' models, for both male and female. For the Gompertz model, negative correlation was observed for the male while positive correlation was observed for the female. The correlation coefficients between parameter A (asymptotic weight) and B (constant of integration), ranged from -0.933 to 0.735 for all models. For the Richards' model, both male and female, there was a highly negative correlation ($r < -0.90$) between these parameters,

Table 9. Correlation coefficients among model parameters for nonlinear models

Male				Female			
Gompertz	Logistic	Bertalanffy	Richards'	Gompertz	Logistic	Bertalanffy	Richards'
Parameter A and B							
0.00266	-0.176	0.406	-0.918	0.263	-0.106	0.735	-0.933
Parameter A and K							
-0.981	-0.915	-0.993	-0.981	0.982	-0.918	-0.995	-0.983
Parameter B and K							
-0.181	0.533	0.309	0.962	0.0931	0.467	-0.669	0.986

A = asymptotic weight or mature weight; B = scaling parameter (constant of integration); and k = maturity index

which indicated that chickens with higher constant of integration had lower asymptotic weight and vice-versa. Positive correlation was observed between these parameters based on Bertalanffy model which implied that high asymptotic weight is associated with higher values of the constant of integration.

Figures 1 and 2 show graphical representations of growth rate of male and female FUNAAB-Alpha chickens, respectively, as predicted by spline functions of 6 (SP6), 5 (SP5), 4 (SP4) and 3 (SP3) knots. The growth curves of the male and female chickens by the non-linear models are depicted in Figures 3 and 4. Generally, body weight increased with age but at different rates as predicted by different spline functions. There are overlaps in the growth rates predicted by these functions from hatch till about 4th to 6th week for most cases. For the male FUNAAB-Alpha chickens, such overlaps were obvious between SP4, SP5 and SP6 from hatch till the 14th week before the growth rate of the SP5 became higher than the rest. The growth rate predicted by the 3-knot function (SP3) was found to be lowest. For the female FUNAAB-Alpha chickens, the growth rate predicted by the SP5

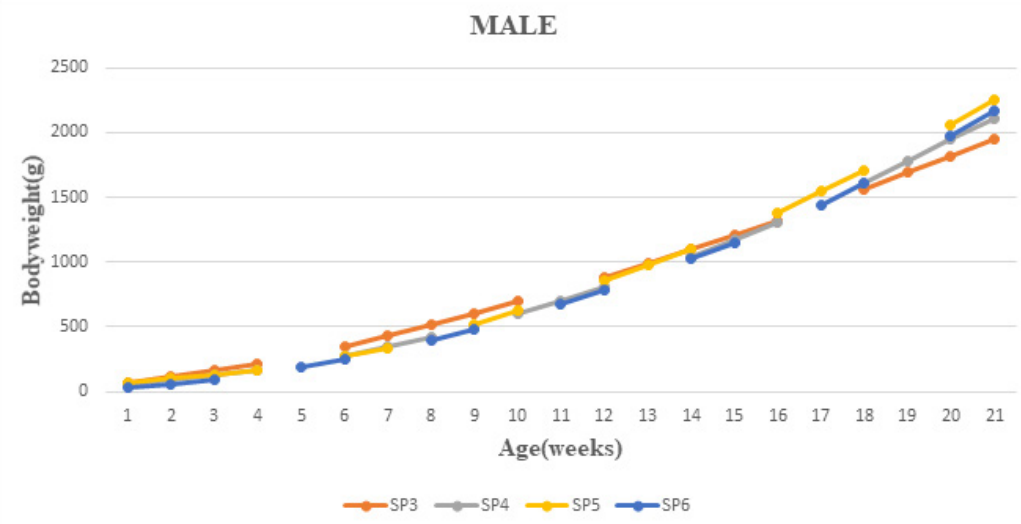

Figure 1. Growth curve of FAC as predicted by spline models (male chickens)

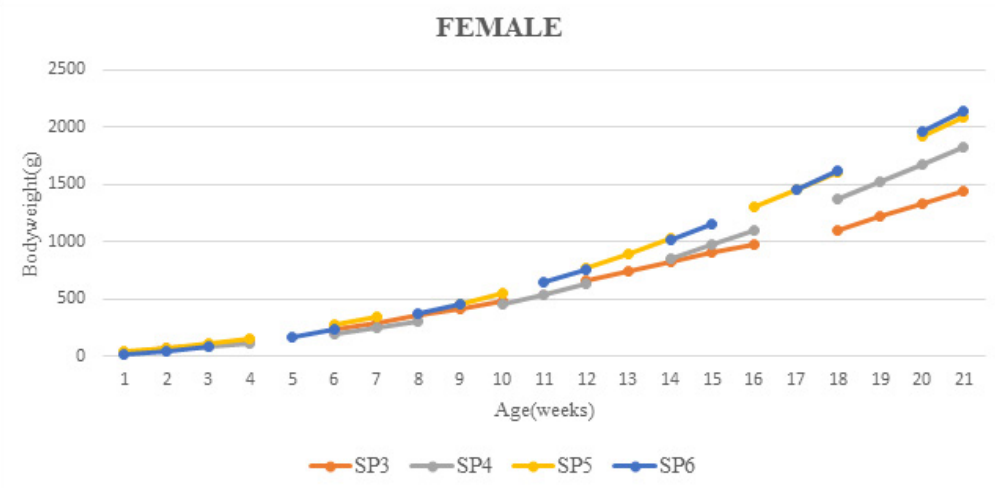

Figure 2. Growth curves of FAC as predicted by spline models (female chickens)

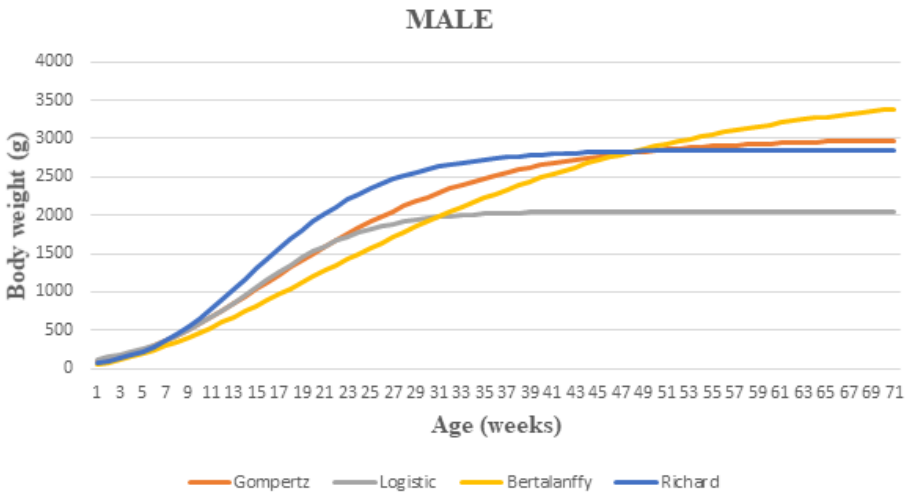

Figure 3. Growth curves for FAC (male chickens) predicted by Richards', Gompertz, Logistic and Bertalanffy models

and SP6 functions were similar and higher and higher than the growth rate predicted by SP3 which was observed to be the lowest.

Relative growth rate (RGR) of FAC across sex, as estimated by Gompertz, Logistic, Bertalanffy and Richards' models are presented in Figures 5 (a-d).The RGR represent chickens' growth rate relative to body size at various ages. Based on all non-linear models fitted, the initial relative growth rate was observed to be maximum at the first week, and decreased steadily till the curve flattens out, indicating that RGR was almost zero after the point of inflection had been reached. Further, the RGR decreased at a lower rate from 0 to 8 weeks of age, while the rate of decrease was rapid after the inflection point was reached.

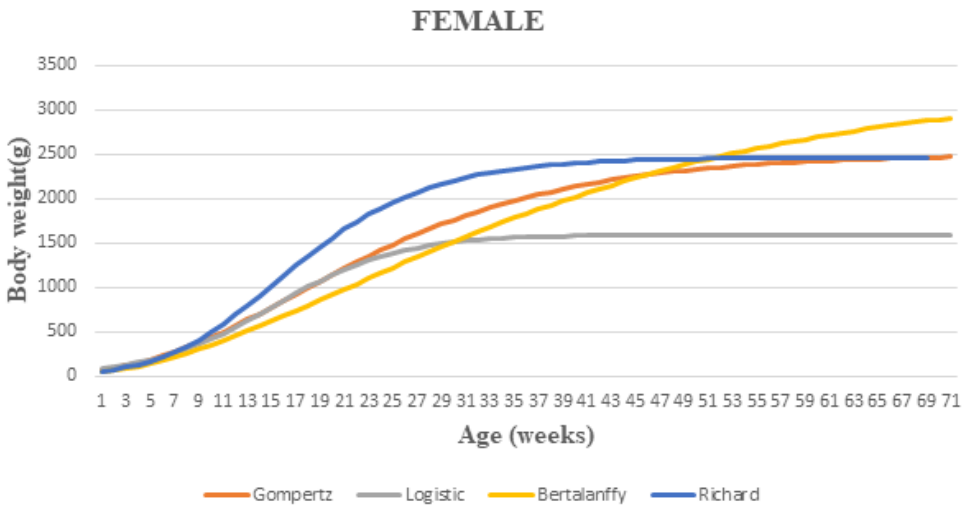

Figure 4. Growth curves for FAC (female chickens) predicted by Richards', Gompertz, Logistic and Bertalanffy growth models

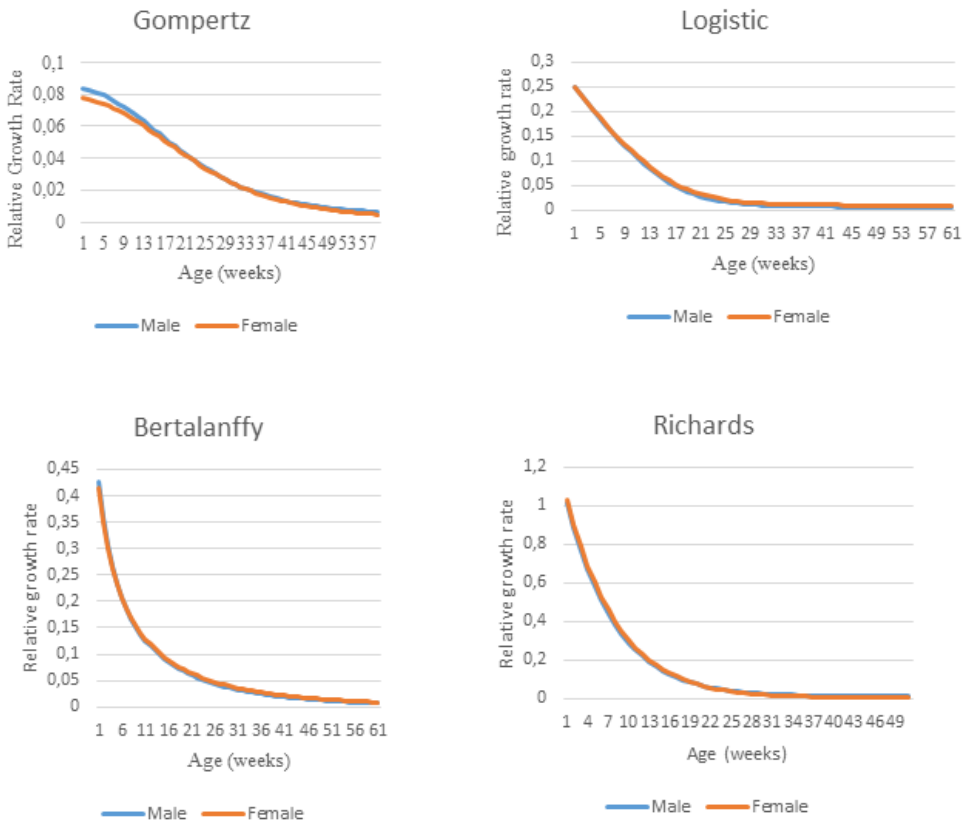

Figure 5 (a-d). Relative growth rate for FUNAAB-Alpha chickens raised under a deep litter system based on Gompertz, Logistic, Bertalanffy and Richards' model

DISCUSSION

Weight at hatch obtained in this study are higher than 29.00 ± 1.0 g and 23 ± 1.6 g and 24 ± 0.8 g and 25.6 ± 0.7 g for male and female, respectively, as reported by Adedokun and Sonaiya (2001) for some indigenous chickens of Nigeria in derived savanna and rainforest agro-ecological zone of Nigeria, respectively. The higher hatch weight obtained in this study could be attributed to the fact that FUNAAB-Alpha chickens have been improved genetically as they have undergone selection over many generations for improved growth performance (Adebambo *et al*., 2018). The body weight at maturity (20th week) obtained in this study for the male was lower than an average of 2.10 kg reported by Adebambo *et al*. (2018) for improved FUNAAB-Alpha breeds that were reared across 5 agro-ecological zones of Nigeria under the African Chicken Genetic Gain Programme (www.africacgg.net). However, the body weight obtained in this study at 4th, 8th, 12th and 16th week of age were similar to 287.13 ± 6.17 g, 844.30 ± 21.84 g, 1158.15 ± 25.71 g, and 1587.93 ± 40.00 g, respectively, reported by Oleforuh-Okoleh *et al*., (2017) for FUNAAB-Alpha.

The hatch weight predicted by SP3 for the male (74.71 g) was higher than 32.80 g reported by Aggrey (2002) who fitted linear splines of 3 knots at 6, 18 and 113 days of age to describe the growth patterns of Athens-Canadian chickens. This could possibly be due to the differences in the location of the knots utilized. The value of 33.60 g obtained by Aggrey (2002) for female chickens was however, similar to 34.40 g obtained in this study. The range of linear regression coefficients obtained were also much higher than the range of 5.70 to 17.90 reported by Aggrey (2002). An important factor that may hinder direct comparison of the values of regression coefficients is the fact that the locations of the knots utilized in these studies are different, and may be data-specific. The knots were placed at specific locations based on observed growth patterns obtained from preliminary analysis of the data. Highest growth rates for all the spline models were predicted for the first 3 to 10 weeks of growth. This is in agreement with the report of Aggrey (2002) that the highest growth rate was attained between days 18 and 113 for the female chickens, while for the male chickens it was from hatch to day 6. Therefore, growth rate at early stage of life may be an indicator trait for growth performance later in life. Hence, breeding strategies to improve the growth performance of meat-type chickens may focus on the first few weeks after hatching. Further research is needed to clarify this point.

The asymptotic weight estimated in this study by the Gompertz model was consistent with the findings of Zhao *et al*. (2015) and Al-Samarai (2015) on some improved indigenous chickens of China and meat-type chickens of Iraq respectively. However, lower values were obtained by Aggrey (2002), Osei-Amponsah *et al*. (2014) and Ngeno *et al*. (2010) for Athens-Canadian chickens and local chickens in Ghana and Kenya, respectively. The values of parameter A (or asymptotic weight) obtained for Logistic model is consistent with the values reported by Aggrey (2002) and Al-Samarai (2015) but lower than the values reported by Eleroglu *et al*. (2014) for Turkish indigenous chickens. Further, estimates of Parameter A (asymptotic weight) obtained in this study for Richards' model are consistent with the findings of Aggrey (2002) but higher than those reported by Rizzi *et al*. (2013) and Osei-Amponsah *et al*. (2014) for chickens in Italy and Ghana respectively. Variations in the asymptotic weight of these chickens could be attributable to a combination of factors including genetic differences, system of management, the prevailing climatic conditions of the environment in which these chickens were raised, as well as various possible interactions among these factors, which would ultimately influence the growth trajectory.

Overall, there seem to be a better fit to the data as the number of knots reduces. Stone (1986) concluded that fewer knots should be used unless the sample size is large enough and there is a theoretical background to assume that the relationship being studied changes rapidly over time. For both sexes, the Bertalanffy model had the lowest AIC and BIC as goodness-of-fit criteria, and was adjudged the best fit model. This was followed by the Gompertz, Richards' and Logistic models in that order. This was in agreement with the conclusions of several authors [Aworetan and Oseni, (2018), Eleroglu *et al*., (2014), Ngeno *et al*., (2010) and Osei-Amponsah *et al*. (2014)] who reported Bertalanffy as the best fit non-linear model

for evaluating the growth of indigenous chickens of Nigeria, Turkey, Kenya and Ghana, respectively. The lesser fit or inadequacy of Richards' model observed in this study might be due to the extra parameter in the model, for which it was penalized by the model selection criteria. Meng *et al*., (1997) reported that the Richards' model was inadequate in providing good fit to data patterns and observations. Aggrey (2002) suggested that the addition of the fourth parameter in the Richards' model may represent an over-parameterization of the growth model.

The high negative correlation coefficients between parameters A and K indicated that the higher the value of the maturity index, the lower is the value of the asymptotic weight. This might be due to the fact that chickens with higher maturity index reached the point of inflection faster as observed with the Logistic model with the highest maturity index value. As noted by Aggrey (2002), the position of the inflection point strongly influences the growth rate and the mature body weight, meaning that the faster the inflection point was reached the lower the value of the mature body weight. This is in agreement with the findings of Al-Samarai (2015) and Ngeno *et al*. (2010) who reported pronounced negative correlation coefficients between parameters A and K.

The relative growth rate patterns estimated by the non-linear models were in consonance with the findings of Eleroglu *et al*., (2014) that asserted that the relative growth rate was always highest at day old and decreased steadily until maturity. Further, there were no disparities in the relative growth rates estimated irrespective of feather morphology and distribution patterns of FAC, indicating that the manifestations of feather-reducing genes of frizzling and naked neck did not significantly influence relative growth rate.

CONCLUSION

This study generated regression coefficients to describe growth performance of FUNAAB-Alpha chickens using 3, 4, 5 and 6 knots. These coefficients can serve as specific breed descriptors for FUNAAB-Alpha chickens during selection and performance testing, or as part of a breed characterization process. The highest value of regression coefficients were estimated for the period of 3-10 weeks implying that growth rate at early stage of life might be a key response to selection for later growth performance and that selection for improved body weight could be done at these ages for further breeding of the breed. Spline models with 3 and 4 knots were found to be the best fit spline models for describing the growth trajectories of FUNAAB-Alpha chickens. Further, Bertalanffy and Gompertz models were found to be the best fit non-linear models for describing the growth performance of FUNAAB-Alpha chickens.

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SEUROP BEEF AND PIG CARCASS CLASSIFICATION IN SLOVAKIA: A REVIEW

Martina GONDEKOVÁ[1]*, Peter DEMO[1], Zuzana SALAGOVÁ[2], Ivan PAVLÍK[1], Ján HUBA[1]

[1]NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[2]Ministry of Agriculture and Rural Development of the Slovak Republic, Department of Animal Production, Bratislava, Slovak Republic

ABSTRACT

Carcass classification plays an important role in Europe, as a marketing aid within and among countries and as a mean of increasing the precision of price reporting for administrative purposes. It also provides important information about the quality of the slaughter population. Since carcass classification systems were first introduced over fifty years ago, EU support schemes and the red meat industry have changed considerably. Changes in the SEUROP classification system of beef and pig carcasses also took place in Slovakia. This paper reviews actual system and legislation, as well as definition, categorization, presentation, conformation and fat cover of beef and pig carcasses at the slaughterhouses in Slovakia.

Key words: SEUROP; classification; carcass; beef; pig

INTRODUCTION

Livestock production ranks among the most important sectors in agricultural production from the point of view of preserving and developing agrarian and rural employment. The most crucial in the field of livestock production is pig and cattle farming (Věžník *et al*., 2017). According to Chrastinová *et al*. (2014) livestock production in Slovakia has been showing a decreasing tendency over the long term, which is connected with the economic conditions such as higher cost of livestock breeding, for example. The drop in production is further strongly linked also to the import of products to the local market.

Nowadays, Slovakia is no longer self-sufficient in the production of any kind of meat (Věžník *et al*., 2017). The current consumption of meat (figures of the year 2018) is 64.5 kg per capita, of which beef presents 5.3 kg and pork 35.8 kg (ŠÚ SR, 2019). It is estimated that less than half of this consumption comes from the Slovak Republic.

Nowadays cows represent the most numerous category of slaughtered cattle at abattoirs in Slovakia. In fact, about 43 % of slaughtered cattle are culled cows (31 % dairy cows, 12 % beef cows). Cows are culled for various reasons including age, poor performance, and failure to reproduce (Bahelka *et al*., 2016).

Cows in better body condition, with higher live weight and fatness, are usually selected for export abroad to the countries such as Austria, Hungary, Poland or Turkey. There our farmers make higher profit than in Slovakia. Juvenile male animals of all meat breeds are predominantly exported abroad (Italy, Croatia, Slovenia, Austria, Turkey etc.), therefore, the number of bulls slaughtered in the Slovak slaughterhouses is low. According to Huba *et al*. (2019), if all raised bovine animals were slaughtered at domestic slaughterhouses, this would exceed current domestic beef consumption.

***Correspondence:** E-mail: martina.gondekova@nppc.sk
Martina Gondeková, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 386

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In such case, Slovakia would be self-sufficient in beef production.

Pork meat is being imported to Slovakia from almost all Europe, but mainly from the neighbouring countries. More than a half of the slaughter pigs are being exported as a result of closing down of numerous important slaughterhouses, e.g. in Lučenec, Rimavská Sobota, Humenné, Zbrojníky or Sereď. At present, there are only smaller slaughterhouses in the towns Myjava, Tešedíkovo or Komárno that operate and which until recently were of local importance only. Most slaughterhouses in Slovakia process meat from foreign production, which is economically more profitable for them (Věžník *et al*., 2017).

Legislation of the classification system for bovine and pig carcasses in Slovakia

In the Slovak Republic the classification of bovine and pig carcasses is currently carried out according to the SEUROP system that has been applied since 2001. Main principles are laid down in the European Parliament and Council Regulation (EU) No 1308/2013 while the Commission Delegated Regulation (EU) 2017/1182 and Commission Implementing Regulation (EU) 2017/1184 provide for more detailed provisions. The last two regulations have brought more flexibility to the system for the member states of the European Union by repealing and replacing the previous Commission Regulation (EC) No 1249/2008.

Currently, member states may decide that the requirements on classification of carcasses of bovine animals, laid down in points A.V and B.II of Annex IV to Regulation (EU) No 1308/2013, shall not be compulsory for slaughterhouses which slaughter less than 150 bovine animals aged eight months or more per week as an annual average. A similar rule may be applied to slaughterhouses slaughtering less than 500 pigs weekly as an annual average.

Member states may decide to apply lower limits for both beef and pig carcass classification, in particular to ensure the representativeness of price recording (Article 2 of the Regulation (EU) 2017/1182) as the rules on meat price recording and informing Commission on prices are essentially linked to the rules on carcass classification (Commission Implementing Regulation (EU) 2017/1185).

In accordance with national Slovak legislation principally represented by the basic Act No 491/2001 Coll. all slaughterhouses slaughtering bovines and/or pigs are obliged to classify carcasses of bovine animals aged eight months or more and pig carcasses. It is not mandatory to classify carcasses of bovine animals and pigs which are intended for own consumption in Slovakia. The detailed rules providing for the functioning of the system are laid down in the Decrees No 205/2007 Coll. and No 206/2007 Coll. The mentioned national legislation is currently a subject of an amendment preparation aimed at adapting the rules to the objective situation in Slovakia.

As regards involved authorities and institutions, the national rules on beef and pig carcass classification are laid down by the Ministry of Agriculture and Rural Development of the Slovak Republic, in addition to adopting legislation, also responsible for designation of the inspection body and issuing classifier's licences.

The State Veterinary and Food Administration of the Slovak Republic is designated by the Ministry as a competent authority in charge of inspections in slaughterhouses including the application of penalties.

The Agrarian Market Information of Slovakia (AMIS) is a part of the Agricultural Paying Agency and is responsible for price collection, analysis and notifications to European Commission. As a part of its activity, the Agricultural Paying Agency conducts inspections on price reporting on-the-spot.

Member States shall ensure that classification is carried out by qualified classifiers, who have obtained a licence for this purpose. The licence may be replaced by an approval granted by the Member State, where such approval corresponds to recognition of a qualification.

In the Slovak Republic, classification is currently performed by slaughterhouse operators and by one independent company.

The National Agricultural and Food Centre – Research Institute for Animal Production in Nitra is responsible for classifiers' training and examination. The classifier course takes two days. One day is reserved for the theoretical part at the Institute followed by a written test. The next day is dedicated to practical training followed by an exam at a slaughterhouse.

The results of the examination are transmitted to the Ministry that issues licences for classifiers. The validity of the licence is 5 years, renewable after passing a new examination. A refresh workshop on carcass classification is held on a yearly basis.

The National Agricultural and Food Centre – Research Institute for Animal Production in Nitra also provides scientific support to the Ministry on carcass classification issues.

Beef carcass classification

Cattle breeding in Slovakia has a long tradition. According to the data provided by The Breeding Services of Slovak Republic, s.e., the total bovine population in Slovakia was 453 133 heads in 2018 (191 975 cows). Approximately 65 % of the total cow's population was represented by dairy cows (the rest by suckler cows). The most numerous cattle breed in Slovakia is Holstein, followed by Simmental-Fleckvieh, Charolais and Limousine. First two breeds are considered to be dairy or dual-purpose, while Charolais and Limousine are typical beef breeds.

Cattle reared in temperate climates are of a type distinct from the humped cattle of the tropics, which were previously considered to be a different species. But even within the temperate zone, cattle are inherently very variable, their obvious phenotypic variation encompassing size, shape and coat colour. This is primarily due to the variety of breeds that have originated are also important factors although gender and husbandry. These, plus variation in age at slaughter, confer much variability on their carcasses, which not only vary in size and shape but also, quite obviously, in fatness. So, when animals or carcasses began to be traded for meat (as opposed to being slaughtered consumed at home), there was a need to discriminate between them (Lazzaroni *et al*., 2007).

Also the works of some domestic authors (Páleník *et al*., 1984; Chrenek, 1987) point to certain differences among the categories of cattle bred in Slovakia (Nosáľ *et al*., 1997).

In the past, the purchase of bovine animals took place in a live state, based on weight and subjective determination of the forage. If the animals for slaughter (except calves) were not fasted 12 hours prior to delivery, a feed rate of up to 5 % and for excessively fed animals up to 8 % of the live weight of the animal were applied. To estimate tissue representation in the carcass, classifiers had to take into account the development of the individual lots with respect to their breeding, gender, age and others. The advantage of this method of buying slaughtered animals was the modesty of the technical equipment and the speed of sale. The disadvantage was the lower accuracy of the determination of the purchase weight and the class of quality, which led to disputes between the trading parties. Disputes between supplier and customer were solved by control slaughters (Guzmická, 2010).

Definitions of terms

"Carcass" means the whole body of a slaughtered bovine animal as presented after bleeding, evisceration and skinning. Half-carcass means the product obtained by separating the carcass symmetrically through the middle of each cervical, dorsal, lumbar and sacral vertebra and through the middle of the sternum and the ischiopubic symphysis.

Carcass presentation

Carcass presentation means preparing the carcasses for classification and further marketing. Without prejudice to points A. IV, B.III and C.IV of Annex IV to Regulation (EU) No 1308/2013, no fat, muscle or other tissue may be removed from the carcass before weighing, classifying and marking, except for cases when veterinary requirements are applied.

Carcasses of bovine animals aged less than eight months shall be presented in accordance with point A.IV of Annex IV to Regulation (EU) No 1308/2013 and without:

a) thin skirt
b) thick skirt.

Carcasses of bovine animals aged eight months or more shall be presented without:

a) kidneys
b) kidney fat
c) pelvic fat
d) thin skirt
e) thick skirt
f) the tail
g) the spinal cord
h) cod fat
i) fat on the inside of topside
j) jugular vein and the adjacent fat.

Carcass categories

Determination of the carcass categories is defined by Regulation No 1308/2013 Annex IV A. The bovine carcasses shall be divided into the six categories (Table 1). Classification of category V (calves) is not mandatory in the Slovak Republic.

Carcass classification

The classification system is defined as that involving the identification of the animal, the dressing specification, the weighing, the assessment on conformation and fatness (under the SEUROP grid), the documentation of the attributes and related price reporting issues.

Carcass classification gives an estimation of the quantity of meat in the important parts such as round, back, shoulder and fat in/on the carcass. Figure 1 shows three major carcass parts (rump, back, shoulder).

Classification and weighing of a carcass shall take place not later than one hour after the animal has been struck.

Table 1. Identification and description of the bovine carcass categories

Identification	Description
V	carcasses of animals aged to less than 8 months
Z	carcasses of animals aged from 8 months to less than 12 months
A	carcasses of uncastrated male animals aged from 12 months to less than 24 months
B	carcasses of uncastrated male animals aged from 24 months
C	carcasses of castrated male animals aged from 12 months
D	carcasses of female animals that have calved
E	carcasses of other female animals aged from 12 months

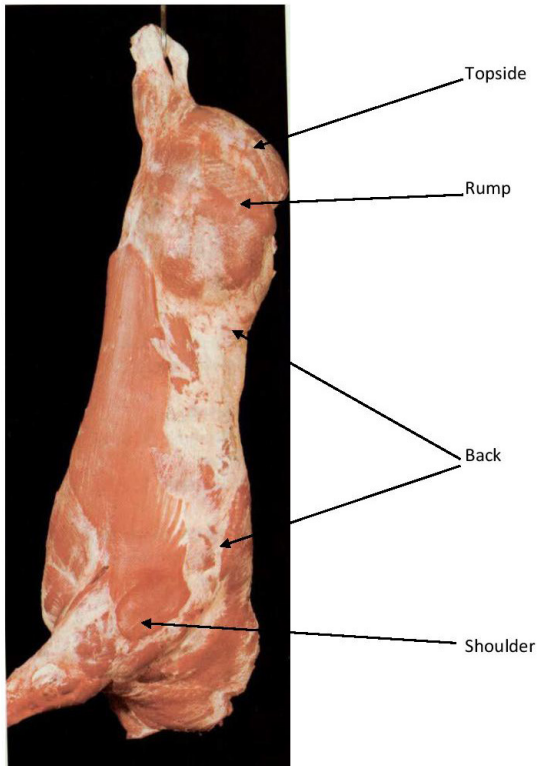

Figure 1. The major carcass parts

As already mentioned, Union scales for the classification of carcasses shall apply in accordance with, respectively, points A and B of Annex IV in the beef and veal sector as regards carcasses of bovine animals aged eight months or more (Regulation (EU) No 1308/2013, Part II, Title I, Chapter I, Section I, Article 10).

Carcass conformation

Table 2 and Figure 2 show criteria for classification of bovine carcasses in regard to conformation into the six classes (S, E, U, R, O, P). Member States of the EU may use subclasses for conformation: S+, S-, E+, E-, U+, U-, R+, R-, O+, O-, P+, P-. In the Slovak Republic the subclasses for conformation are not used.

Fat cover

Table 3 and Figure 3 show criteria for classification of bovine carcasses in regard to fat cover of the major carcass parts on the outside and fat cover of the thoracis cavity into the five classes (1, 2, 3, 4, 5). Member States of the EU may use subclasses for fat cover (1+, 1-, 2+, 2-, 3+, 3-, 4+, 4-, 5+, 5-). In the Slovak Republic the subclasses for fat cover are not used.

Table 2. Classification of bovine carcasses in regard to conformation

Class	Description	Additional provisions	
S (Superior)	• all profiles extremely convex • exceptional muscle development • double muscled carcass type	Round Back Shoulder	• very highly rounded double-muscled • visibly separated seams • topside spreads very markedly over the symphysis pelvis • very wide and very thick up to the shoulder • rump very rounded • very highly rounded
E (Excellent)	• all profiles convex to superconvex • exceptional muscle development	Round Back Shoulder	• very rounded topside spreads markedly over the symphysis pelvis • wide and very thick up to the shoulder • rump very rounded • very rounded
U (Very good)	• profiles on the whole convex • very good muscle development	Round Back Shoulder	• topside spreads over the symphysis pelvis • wide and thick up to the shoulder • rump rounded • very rounded
R (Good)	• profiles on the whole straight good muscle development	Round Back Shoulder	• well developed • topside and rump slightly rounded • still thick, but less wide at the shoulder • fairly well developed
O (Fair)	• profiles straight to concave • average muscle development	Round Back Shoulder	• average development to lacking development • average thickness to lacking thickness; rump straight profile • average development to almost flat
P (Poor)	• all profiles concave to very concave • poor muscle development	Round Back Shoulder	• poorly developed • narrow with bones visible • flat with bones visible

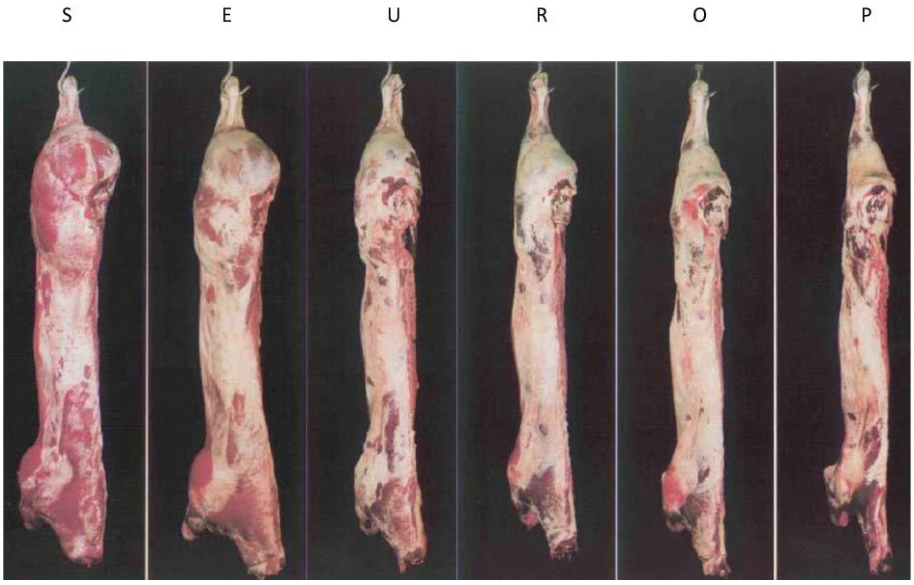

Figure 2. Degree of conformation

Table 3. Classification of bovine carcasses in regard to fat cover

Class	Description	Additional provisions
1 (Low)	• no up to low fat cover	• no fat within the thoracic cavity
2 (Slight)	• slight fat cover, flesh visible almost everywhere	• within the thoracic cavity the muscle is clearly visible between the ribs
3 (Average)	• flesh with the exception of the round and shoulder, almost everywhere covered with fat • slight deposits of fat in the thoracic cavity	• within the thoracic cavity the muscle is still visible between the ribs
4 (High)	• flesh covered with fat, but on the round and shoulder still partly visible, some distinctive fat deposits in the thoracic cavity	• he seams of fat on the round are prominent • within the thoracic cavity the muscle between the ribs may be infiltrated with fat
5 (Very high)	• entire carcass covered with fat • heavy deposits in the thoracic cavity	• the round is almost covered with fat, so that the seams of fat are no longer clearly visible • within the thoracic cavity the muscle between the ribs infiltrated with fat

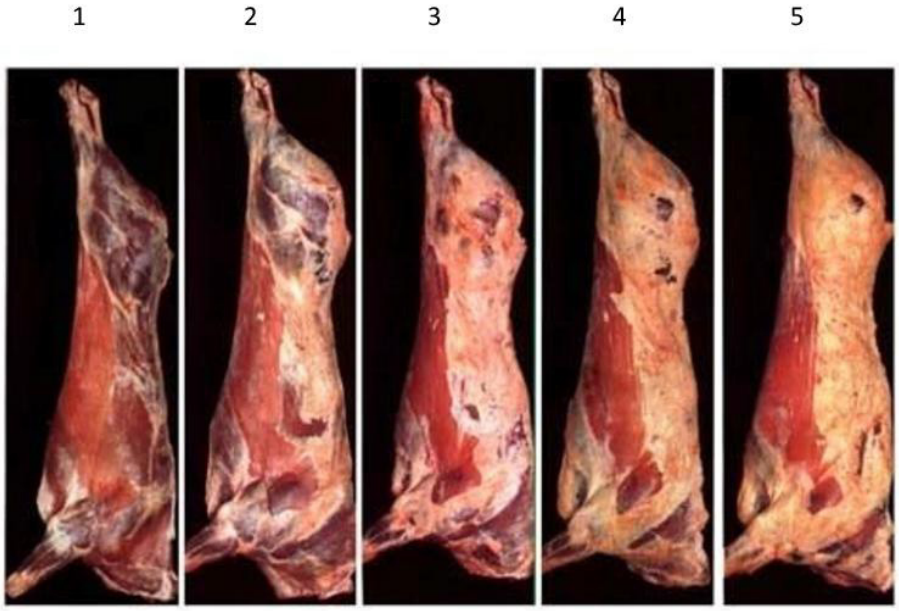

Figure 3. Degree of fat cover

Carcass identification (marking)

Identification and marking of the carcass shall take place no later than one hour after the animal has been struck. It is not allowed to remove marking before boning. In the Slovak Republic the marking is obligatory for all slaughterhouses. The identification of the carcasses shall be carried out by means of a mark indicating the category and the class of conformation and fat cover. This marking shall be carried out by stamping on the outside surface of the carcass using an indelible and non-toxic ink following a method approved by the competent authorities. The letters and figures must be no less than two centimetres in height. The marks shall

be applied on the hindquarters on the striploin at the level of the fourth lumbar vertebra and on the forequarters, on the brisket between 10 and 30 centimetres from the cut edge of the sternum. Marking can be also carried out by the use of labels under the following conditions:

a) the labels may be kept and attached only in the approved establishments which slaughter the animals; they must be of a size no less than 50 cm^2;
b) the labels must indicate an approval number of the slaughterhouse, the identification or slaughter number of the animal, the date of slaughter, the weight of the carcass;
c) all the indications must be perfectly legible and no alterations shall be permitted except if they are clearly marked on the label and carried out under the supervision of and the practical conditions determined by the competent authorities;
d) the labels must be tamper-proof, tear-resistant and firmly attached to each quarter in the places defined above.

Pig carcass classification

Definitions of terms

"Carcass" means the body of a slaughtered pig, bled and eviscerated, whole or divided down the mid-line.

Carcass presentation

Carcasses shall be presented without tongue, bristles, hooves, genital organs, flare fat, kidneys and diaphragm.

Carcass classification

In the process of breeding and production of high performing types of pigs (daily gain above 1000 g, reaching slaughter weight at 150 days of age), determination of the lean meat content in the pig's carcass is important. Precise determination of the lean meat content then enables fair monetization of the fattening pigs and remuneration of the breeders for their production of meat line pigs with low production costs (Demo *et al*., 2013).

Currently, multiple methods are utilized to estimate the lean meat content and the development of evaluation system progress at a dynamic rate with the equipment constantly improving. Objective classification determines the lean meat content in a carcass utilizing auxiliary indicators, which are the anatomic dimensions of the carcass. These are primarily the muscle thickness, resp. last rib fat thickness or lumbar region fat thickness, which display sufficiently close relation to the lean meat content. These parameters are easy and quick to measure and they do not require a dissection of the carcass, which would determine the lean meat content most accurately. However, a detailed dissection is demanding for time, manpower and finances as it requires high level of skill and compliance to a strict procedure.

In the EU countries, slaughter pigs are sorted into classes of quality based on the estimated total lean meat content using the SEUROP system, which uses information input (fat thickness, resp. the thickness of the muscle in the designated measuring sites). These are measured 6-8 cm (depending on the carcass weight) to the side of the central cut. Systems used to determine the lean meat content are categorized as follows (from the simplest to the most complex):

- mechanic, optic and optoelectronic rulers
- incision probes (e.g. Fat-O-Meater, Hennesy Grading Probe, Pork Grader, etc.)
- ultrasound probes (e.g. CSB Ultra Meater, US-Porkitron, Ultra-FOM)
- apparatuses on the base of electromagnetic resonance (EMS), resp. tomography (CT)
- fully automatic apparatuses on the base of digital imaging, which create ultrasound images (e.g. AUTOFOM)

As mentioned above, in EU legislation the detailed rules on classification of pig carcasses are laid down in the Delegated Commission Regulation (EU) 2017/1182 and Implementing Commission Regulation (EU) 2017/1184. In the conditions of Slovakia, the evaluation of quality and structure of pig carcass is additionally regulated by the Commission Decision No 2009/622/EC and Decree No. 205/2007 Coll. Since 2001 SEUROP has been the required classification system and permitted are two methods of lean meat content determination.

At slaughterhouses that slaughter less than 100 pigs per week on average, classification is done using two points – manual method using ruler (so-called ZP method from German 'Zwei Punkte'). Fat thickness, resp. muscle thickness in the lumbar region of the pig carcass is measured. Muscle thickness (M) is measured as the minimal muscle depth between the anterior extremity of the *musculus*

gluteus medius and the dorsal part of the medullar canal. Fat thickness is measured at the site of the thinnest fat layer over the *musculus gluteus medius*. The location and method of measuring is presented in Figure 4.

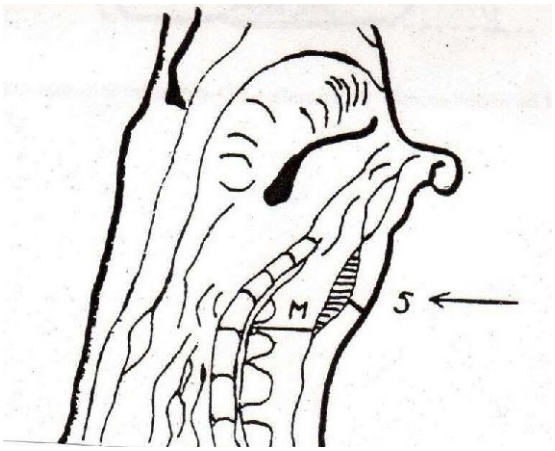

Figure 4. The location and method of measuring fat thickness

At slaughterhouses that slaughter more than 100 pigs per week on average, the lean meat content is in the Slovak Republic determined using machine equipment. In particular, it is the incision apparatus FOM, or ultrasound ULTRAFOM. The scheme of measurement locations is presented in Figure 5.

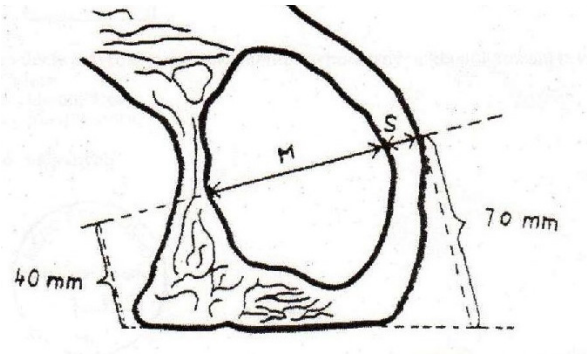

Figure 5. The scheme of measurement locations using ultrasound ULTRAFOM

The muscle depth (M) and fat thickness (S) are measured 70 mm from the central cut between the second and third to last ribs, both in a single incision. The FOM apparatus records values of each tissue's thickness during the removal of the probe; ultrasound apparatus ULTRAFOM records when the ultrasound transducer is put in contact with the carcass.

All methods for prediction of lean meat percentage use regression equations according to the methodology developed by Walstra *et al*. (1996) and Causeur *et al*. (2003). On each apparatus, a regression equation is installed, based on which the total lean meat content is calculated – the slaughter carcasses are sorted into quality classes based on weight, sex and lean meat content (Table 4, Table 5).

Table 4. Quality classes of slaughter pigs with taking-over weight between 60 and 120 kg, which are subject to classification

Class	Lean Meat Content (%)
S	60 and above
E	55 – 59.9
U	50 – 54.9
R	45 – 49.9
O	40 – 44.9
P	under 40

Table 5. Quality classes of slaughter pigs not subject to classification according to Point 1

Class	Characteristic
N	Carcasses with take-over weight through 59.9 kg
T	Carcass with take-over weight above 120 kg
Z	Meaty sows and incisors
H	Thin sows and incisors
K	Boars and cryptorchids

Carcass identification (marking)

The marking of pig carcasses shall be carried out on the skin of the hind leg or the front of the thigh of each carcass, the letters and numbers being at least 20 mm in height. The pig carcass shall be indicated immediately after classification with a harmless, non-washable and indelible colour.

The marking of pig carcasses may be replaced by a label of at least 5 x 10 cm. In addition to the quality and weight classes, the label shall include the classifier identification number, slaughterhouse number, animal identification number, breeder number and date of slaughter.

CONCLUSION

In the Slovak Republic SEUROP system has been used since 2001. Over the years it has undergone several legislative and practical changes and is currently under preparation to amend the above national legislation in relation to the EU rules in force.

In the sector of the beef carcass classification, consideration should be given to the use of subclasses for the most commonly occurring major conformation classes (O, P). Only main classes are used for conformation and fat cover classes. Due to the productive structure of the country, mainly small slaughterhouses are involved in the system. No slaughterhouses out of 62 active approved bovine slaughterhouses in Slovakia have, on average, weekly performance above 150 bovines aged 8 months or more per week. The small number of carcasses is also a problem when considering training of classifiers and the final practical examination. It depends on the carcass availability for the specific training day. Culled dairy cows are a prevailing category of slaughter bovines in the slaughterhouses.

In the sector of pig carcass classification, there is a trend to support diversification of production and utilization of automatized technologies to estimate the lean meat content. Attention will be focused not only on the total lean meat content of the carcass but also the share of meat on particular parts of the carcass and included in the evaluation of pigs will be also meat quality parameters. Breeding strategies and methods of classification will target uniformity of slaughter pigs, in which the lean meat content of the carcases will be high and within a narrow range. Already growing in popularity are the opinions that classification will have to take into account not only the total lean meat content but also meat quality parameters. It will be necessary to develop standardized methods to measure drip losses, pH, tenderness, colour and other parameters. In Slovakia, we are hearing also calls for less pressure to breed for high lean meat content as this is often accompanied by occurrences of abnormalities in the meat quality and worse parameters of culinary utilization of pork (juiciness, delicacy, tenderness). These topical questions will also need to be sufficiently answered in evaluating quality of pig carcasses and their monetisation.

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USE OF PROBIOTIC BACTERIA IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AQUACULTURE: SHORT COMMUNICATION

Peter POPELKA*, Dagmar MUDROŇOVÁ, Jana KOŠČOVÁ, Adriana FEČKANINOVÁ, Ivana CINGEĽOVÁ MARUŠČÁKOVÁ

University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic

ABSTRACT

In recent years, there has been a growing interest in controlling disease-related problems through alternative methods, since the use of chemotherapeutic agents may lead to occurrence of resistant bacteria. This short communication summarizes the current understanding of probiotic use in aquaculture of rainbow trout to prevent pathogenic bacteria, including the definition and mechanism of probiotics action, and describes their application, prospects and difficulties associated with their use in aquaculture. Our contribution to the use of probiotic bacteria in aquaculture represents isolation of lactic acid bacteria (LAB) from the intestinal content of rainbow trout (*Oncorhynchus mykiss*), subsequently potentially used as probiotics in order to improve health status of fish during fish farming. An effective probiotic must comply with criteria which determine its effect. Selection criteria are used to obtain suitable probiotic candidates for aquaculture including antimicrobial susceptibility test, determination of *in vitro* and *in vivo* survival conditions in the gastrointestinal tract of rainbow trout, and tolerance to different pH values, bile, temperature and the best growth properties.

Key words: aquaculture; *Oncorhynchus mykiss*; probiotics; selection criteria

INTRODUCTION

The fact that pathogenic strains, such as *Aeromonas* species, are resistant to a number of antimicrobial agents, suggests caution in the treatment of aquatic animals with antibiotics (Aravena-Román *et al*., 2012). A serious problem of multi-resistance has been demonstrated in several farms (Balta *et al*., 2016). For these reasons, we are looking for new safe solutions using mainly substances of natural origin, which would not reduce the quality of aquaculture products and, at the same time, would not burden the environment. Such an alternative is represented by probiotic microorganisms, by means of which it is possible to modulate not only the intestinal microbiota of aquatic animals in aquaculture, but also the microbiota of the aquatic environment (Newaj-Fyzul *et al*., 2014). In terms of safety for the use of probiotics in practice, the fact that probiotic microorganisms will not increase the already existing risks of antibiotic resistance associated with normal microbiota in the intestine or in food must be confirmed. In the European Union, all microorganisms are subject of antimicrobial susceptibility testing before being used as a feed additive (Bories *et al*., 2008; EFSA, 2012; EFSA, 2013).

Previously, probiotics were used to improve water quality and control bacterial infections. However, there is also documented evidence that probiotics can improve nutrient digestibility, increase stress tolerance and promote reproduction. In the past, aquaculture research has focused on

***Correspondence:** E-mail: peter.popelka@uvlf.sk
Peter Popelka, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

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known probiotic strains derived from terrestrial animals, ignoring the fundamental differences in the physiology of cultivated species – mammals or birds versus fish – as well as the differences in the environment, where the relevant microbial communities have been developed - aquatic versus ground environment. Therefore, probiotic bacteria isolated from the respective host (fish) are expected to perform better in their natural environment than those originating from terrestrial hosts (Van Doan *et al*., 2018).

The selection of probiotics for aquaculture and their development for commercial use in aquaculture is a multistage and multidisciplinary process requiring first basic and later applied research and assessment of its use in practice (Edun and Akinrotimi, 2011). Anyway, microorganisms used as probiotics must be safe not only for aquatic hosts, but also for their surrounding environment and humans (Muñoz-Atienza *et al*., 2013). Merrifield *et al*. (2010) identified a list of properties that potential probiotic bacteria for aquaculture should meet. The isolates should be subjected to microbiological, biochemical and genetic testing to identify and select the most suitable candidates for further assessment as probiotics for sustainable aquaculture. It is unlikely to find a candidate that meets all the criteria and, therefore, it is necessary to focus research on the concomitant use of several probiotic strains or a symbiotic combination of a probiotic with a prebiotic (Ibrahem, 2015).

An important selection criterion that a candidate probiotic for aquaculture must meet is a survival in the digestive tract. If the probiotics tolerate acidic environment and bile, they will survive the passage through the gastrointestinal tract more easily and may colonize the intestine (Sica *et al*., 2012). Another selection criterion is the determination of antagonistic activity of potential probiotic strains against key fish pathogens. For salmonids, the main pathogens are *Aeromonas salmonicida* subsp. *salmonicida* and *Yersinia rückeri*. The lactobacilli that we have isolated, showed inhibitory activity against both tested pathogens of *Aeromonas salmonicida* subsp. *salmonicida* CCM 1307 and *Yersinia rückeri* CCM 6093. Antagonist activity was determined on the basis of the inhibition zones obtained by performing a combination of the disc diffusion and pouring method (Fečkaninová *et al*., 2019).

In order to confirm the results obtained *in vitro*, these results must be verified in a clinical trial. The results of clinical trials with probiotics are very contradictory. Several authors have described positive preventive or therapeutic effects of probiotics in various diseases of salmonids (Irianto and Austin, 2002; Brunt *et al*., 2007; Maricchiolo *et al*., 2015). However, other reports have not detected any, or significant, effects of probiotic microorganisms on the health status of aquatic animals (Gomez-Gil *et al*., 2000; Marques *et al*., 2004). Scientific studies have shown that probiotics are most effective in animals during the development of their microbiota or when their stability is impaired (Fečkaninová *et al*., 2017). Differences in results are due to many factors, such as probiotic strain selection, survival and stability, species specificity of the strain in relation to the host, the dose, the frequency and route of application of the probiotic, fish health and nutritional status, species and age, drug or microbiota interactions, the stress, the overall organization of the experiment etc. (Fečkaninová *et al*., 2019).

Currently, fish farms are facing a reduced amount of available water and deteriorating water quality in rivers. This can lead to oxygen reduction and accumulation of fish metabolites, ammonia, CO_2, NO_2^- in the water. Impairment of water quality creates stress, increases susceptibility to disease, affects feed intake, growth, and induces a decrease in fish welfare (Ellis *et al*., 2002). Research on the interaction of water quality and welfare should be encouraged, particularly in commercial fish farming conditions (EFSA, 2008). In rainbow trout aquaculture, good water quality and its sufficient quantity are important (Sener, 2012). The decrease in dissolved oxygen concentration in water is a consequence of its consumption by fish and decomposing organic and inorganic substances. Decomposing waste materials are subject to biochemical oxidation associated with the evacuation of oxygen, the intensity of which depends largely on the temperature of the water and the qualitative composition of the decomposed organic matter and its quantity (Pokorný *et al*., 2003).

Feed is considered to be the most important polluting factor in fish farming. The effect of feeding on changes in water quality flowing through a fish farm depends primarily on the composition and

physical properties of the feed, the technology of its production, the digestibility and quality of the components of the present feed and the feeding technique (Pokorný *et al*., 2003). For the production of rainbow trout, an energy-rich extruded feed is used, which has a significant effect on the quality of run-off water (Viadero *et al*., 2005). On the other hand, the quality of the inflow water also has a considerable influence on the effluent parameters. Fish feeding is the only factor affecting all measured water parameters (Bergheim and Asgard, 1996). Organic pollution is considered to be a significant negative factor in salmonid fish farming, and the requirements for the lowest possible organic load on water are among the most important. The decomposition of organic matter leads to the depletion of oxygen and the formation of toxic substances (ammonia, hydrogen sulphide, methane and others). There is also an increased risk of serious fish diseases (e.g. bacterial gill disease).

Feeding experiments are costly- and time-consuming, therefore, it is necessary to establish rapid *in vitro* screening strategies to select the most promising isolates. Ideally, *in vitro* screening would allow identification of the beneficial effects and reduce the risk of negative effects. In addition, such research can provide new insights into the biology and ecology of autochthonous bacteria and improve knowledge on microbial host interactions (Wong and Rawls, 2012).

CONCLUSION

It is assumed that probiotic strains can significantly contribute to improving fish health and also improve the immune status of fish. They can also contribute to improving fish production parameters (feed conversion and increasing weight gains), thus contributing to improving the economic viability of farming. The strains are expected to demonstrate a dominant ability to colonize intestinal mucosa of fish even after *in vivo* application. The presence of a dominant bacterial strain at high densities in an aqueous environment indicates its ability to grow successfully under given conditions and it can be expected that this strain will compete effectively for nutrients with possible undesirable microorganisms. In order to confirm this assumption in further research, it will be necessary to prepare a dosage form of selected strains of probiotic bacteria and subsequently verify the *in vitro* properties of the bacteria under *in vivo* conditions. It will be necessary to test the interactions of the strains with the feed components, the aquatic environment and the real digestive tract conditions of the fish.

There is a wealth of information regarding the microbial modulating effects of dietary modifications and the presence of LAB in the gastrointestinal tract (GIT). However, in the screening of the GIT, there is a concern that most studies evaluating microbiota in the intestine focus on characterizing the communities in the GIT (allochthonic microbiota), while those bacteria that have the ability to adhere to the mucosal surface (autochthonous microbiota), which are important for specialized physiological functions, remain uncharacterized. Therefore, we recommend paying more attention to the autochthonic intestinal microbiota.

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THE USE OF HOMA-IR AND QUICKI IN RODENT DIABETIC MODEL: SHORT COMMUNICATION

Marcela CAPCAROVA*, Anna KALAFOVA, Rudolf DUPAK, Peter KISSKA, Monika SCHNEIDGENOVA

Slovak University of Agriculture in Nitra, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Nitra, Slovak Republic

ABSTRACT

Regarding *diabetes mellitus* type 2 (DMT2) in animal research, a non-invasive and less traumatic method, such as the mathematic calculation of indexes expressing the insulin sensitivity and resistance, is required. There are some methods and formulas for calculation and estimation of insulin resistance. The most well-known validated methods are the homeostatic model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI), which are suitable for clinical and research purposes. The goal of this study was to calculate HOMA-IR and QUICKI indexes in an experiment with Zucker diabetic fatty (ZDF) rats fed normal or high-energy diet. Additionally, the correlations between both models were inquired. Animals were divided into three groups: lean untreated control rats (C, n = 10) fed a complete feed mixture for rats and mouse (10 MJ.kg^{-1}), diabetic rats fed the same chow (E1, 10 MJ.kg^{-1}) and diabetic rats fed high energy diet (E2, enriched KKZ-P/M, 20 MJ.kg^{-1}). After overnight fasting, the rats were monitored for blood glucose level by a FreeStyle Optium Neo Glucose and Ketone Monitoring System (Abbott Diabetes Care Ltd., UK) using test strips. An ELISA commercial kit (Biotech, Bratislava, Slovak Republic) was used to measure the serum content of insulin. Values of fasting plasma insulin and serum glucose were used to calculate HOMA-IR and QUICKI indexes. HOMA-IR and QUICKI significantly differed among the groups. Strong negative correlations were found in dependence on the diet. This study indicated that the calculation of HOMA-IR and QUICKI can potentially be an effective tool in determination, evaluation, onset and progress of DMT2.

Key words: HOMA-IR; QUICKI; diabetes; Zucker diabetic fatty rats; diet

INTRODUCTION

Insulin resistance is considered a major risk in the ethology of diabetes mellitus second type (DMT2; Bray, 2004). It is a predictor for the onset and development of DMT2 even in patients with normal level of serum glucose. Therefore, it is very useful to determine insulin resistance in the pre-diabetic stage because at this point the treatment and therapy of DM is more successful than in the developed disease (Boden, 2001). Generally, insulin resistance refers to a state in which cells of peripheral tissues have a reduced level of response to insulin (Choi and Kim, 2010).

HOMA and QUICKI models

Both models are the most widely applied in the case of assessing insulin sensitivity. They are based on fasting glucose and insulin values. These two models mainly differ by the log transformation of the variables in QUICKI, and the constant denominator in HOMA (Antuna-Puente *et al.*, 2008).

***Correspondence:** E-mail: marcela.capcarova@uniag.sk
Slovak University of Agriculture in Nitra, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

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Homeostatic Model Assessment of Insulin Resistance

Homeostatic model assessment of insulin resistance (HOMA-IR) was developed in 1985 by Matthews *et al.* (1985). It is a method for quantification of insulin resistance and β-cell function from fasting serum glucose and insulin concentrations (Gutch *et al*., 2015). The model has been generally used since it was first published (Wallace *et al.*, 2004). It is induced from the use of the insulin-glucose product, divided by a constant according to the formula (Haffner *et al.*, 1997):

$$HOMA\text{-}IR = \frac{\text{serum insulin (mmol.L}^{-1}\text{)} * \text{blood glucose (mmol.L}^{-1}\text{)}}{22.5}$$

Quantitative Insulin Sensitivity Check Index

Quantitative insulin sensitivity check index (QUICKI) is an empirically-derived mathematical transformation of fasting serum glucose and insulin concentrations that gives consistent information about insulin sensitivity with a predictive possibility. It can be calculated from fasting serum glucose and insulin concentrations (Chen *et al*., 2003; Gutch *et al.*, 2015) QUICKI is a variation of HOMA equations, as it transforms the data by using both the logarithm and the reciprocal of the glucose-insulin product, so slightly canting the distribution of fasting insulin values (Chen *et al*., 2003).

$QUICKI = 1/(\log I_0 + \log G_0)$,

where I_0 means fasting insulin and G_0 – fasting glucose.

Some studies showed that HOMA is less reproducible than QUICKI (Sarafidis *et al.*, 2007). This is probably due to the normalization by logarithmic transformation of the data. But, log HOMA did not increase its reproducibility (Antuna-Puente *et al*., 2008). Alternatively, HOMA could be more sensitive to variations in insulin values (Antuna-Puente *et al*., 2008), what occurs mainly in individuals suffering from DM2T. In this case HOMA index appears to be a very suitable method. The given biological variability of insulin values is the main source of variation.

The applicability of HOMA-IR and QUICKI in experimental research is questioned due to the lack of data for validation in most animal species (Wallace *et al*., 2004). Therefore, the aim of this report was to determine HOMA-IR and QUICKI index in Zucker diabetic fatty (ZDF) rats fed a normal or high-energy diet. Additionally, the relationships between both models were inquired.

MATERIAL AND METHODS

Animals

Male Zucker diabetic fatty (ZDF) rats (a fatty *fa*/*fa* mutation (-/-); n = 20) and their healthy lean controls (lean, non-diabetic, +/+ or +/-, not display expression of *fa* phenotype, n = 10) of the same strain at the age of 3 months (12 weeks of age) were involved in the experiment. The animals were purchased from Breeding Facility of the Institute of Experimental Pharmacology and Toxicology (Dobra Voda, Slovak Republic, SK CH 24016). All animals were housed in number of two rats per plastic cage (80 cm^2) and under specific pathogen-free conditions at 23 ± 2 °C and 55 ± 10 % relative humidity with a 12 h light-dark cycle. Rats were provided with water and diet on *ad libitum* base.

Experimental design

Rats were divided into three groups (n = 10 each) as follows: lean rats (C) fed KKZ-P/M (a complete feed mixture for rats and mouse, reg. no 6147, Dobra Voda, Slovak Republic, 10 $MJ.kg^{-1}$), diabetic rats fed by KKZ-P/M (E1, 10 $MJ.kg^{-1}$) and diabetic rats fed high energy diet (E2, enriched KKZ-P/M, 20 $MJ.kg^{-1}$, 30 % saturated fatty acids, 5 % starch and 15 % disaccharides). The initial body weigh did not differ between rats within the same genotype (E1, E2). The experiment lasted 3 months.

Glucose analysis

At the end of the experiment after overnight fasting the rats were monitored for blood glucose level by a FreeStyle Optium Neo Glucose and Ketone Monitoring System (Abbott Diabetes Care Ltd., UK, measurable extent 1.1 – 27.8 $mmol.l^{-1}$ (20 - 500 $mg.dl^{-1}$) using test stripes (FreeStyle, Abbott Diabetes Care Ltd., UK). One drop of blood was collected from the tail vein in the morning between 7:00 to 9:00 a.m. and directly used for glucose value measurement.

Insulin analysis

At the end of the experiment, after glucose measurement the animals were anesthetized by

intraperitoneal injection with chloral hydrate (40 mg.100 g^{-1} body weight). Blood samples were collected into EDTA-treated tubes. ELISA commercial kit (Biotech, Bratislava, Slovak Republic) was used to measure the serum content of insulin according to the instruction of the manufacturer.

HOMA-IR and QUICKI determination

Values of fasting plasma insulin and serum glucose were used to calculate HOMA-IR and QUICKI indexes, as a mathematical model that includes interactions between fasting serum insulin and blood glucose concentration.

Statistical analysis

Data are expressed as mean ± SD (standard deviation). One-way ANOVA test was performed to calculate basic statistical characteristics and to determine significant differences. A SAS Release 9.1 statistical software (SAS Institute Inc. Cara, USA, 2002-2003) was used. Pearson correlation coefficient was used to determine correlations between the methods. Differences were compared for statistical significance at the levels $P < 0.001$, 0.01, and 0.05.

RESULTS AND DISCUSSION

A proper estimation of insulin resistance is needed due to its key role in the pathophysiology of DMT2 (Choi and Kim, 2010). Various tools for quantifying insulin sensitivity and resistance directly and indirectly were reported (Mari *et al.*, 2001). Animal model of ZDF rat is a relevant tool that mirrors the pathogenesis of DMT2 in humans (Capcarova e*t al*., 2018a; Capcarova *et al*., 2018b). In our study we calculated HOMA-IR and QUICKI indexes. HOMA represents the glucose-insulin homeostasis by means of a set of elementary, mathematically-derived nonlinear equations (Matthews *et al*., 1985). QUICKI was determined from fasting serum glucose and insulin values (Table 1). The HOMA model is frequently used as a useful tool in clinical and epidemiological studies for descriptions of the pathophysiology of DMT2. It facilitates determination of inherent β-cell function and insulin sensitivity and can explain the pathophysiology in those with abnormal glucose tolerance (Wallace *et al*., 2004). Values less than 2.5 are reported as normal values (Gutch *et al.*, 2015). Mean HOMA-IR in this study (Figure 1) was the lowest in the lean group (1.37 ± 0.08), followed by the group on a normal diet (2.24 ± 0.29) and the highest values were measured in the group fed high-energy diet (5.31 ± 0.46). Significant differences were noted between lean and energy diet, between normal and energy diet ($P < 0.001$) and between lean and normal diet ($P < 0.05$).

Very similar results were obtained by Antunes *et al.* (2016) in the study with a model of insulin-resistance induced by high-fat diet in Wistar rats (2.32 ± 0.75 normal diet and 4.58 ± 1.85 high-fat diet) with significant differences between both groups. The authors confirmed that HOMA-IR has a strong correlation with the insulin tolerance test and may be used as a surrogate marker of insulin resistance in rats. Appleton *et al.* (2002) considered HOMA-IR for the most useful predictor of insulin resistance.

The values of QUICKI reported by Gutch *et al.* (2015) were 0.382 ± 0.007 for non-obese, 0.331 ± 0.010 for obese individuals and 0.304 ± 0.007 for diabetic patients. In our study we found similar

Table 1. Fasting glucose and insulin values in ZDF rats

	Group		
Parameter	Lean	Diabetic normal diet	Diabetic high-energy diet
Glucose (mmol.l^{-1})	3.89 ± 0.08^{a}	9.56 ± 1.08^{b,A}	15.54 ± 1.16^{b,B}
Insulin (µg. l^{-1})	7.72 ± 0.477^{a}	5.24 ± 0.40^{b}	5.13 ± 0.19^{b}

$^{a-b}$ or $^{A-B}$ mean significant difference ($P < 0.001$) in rows

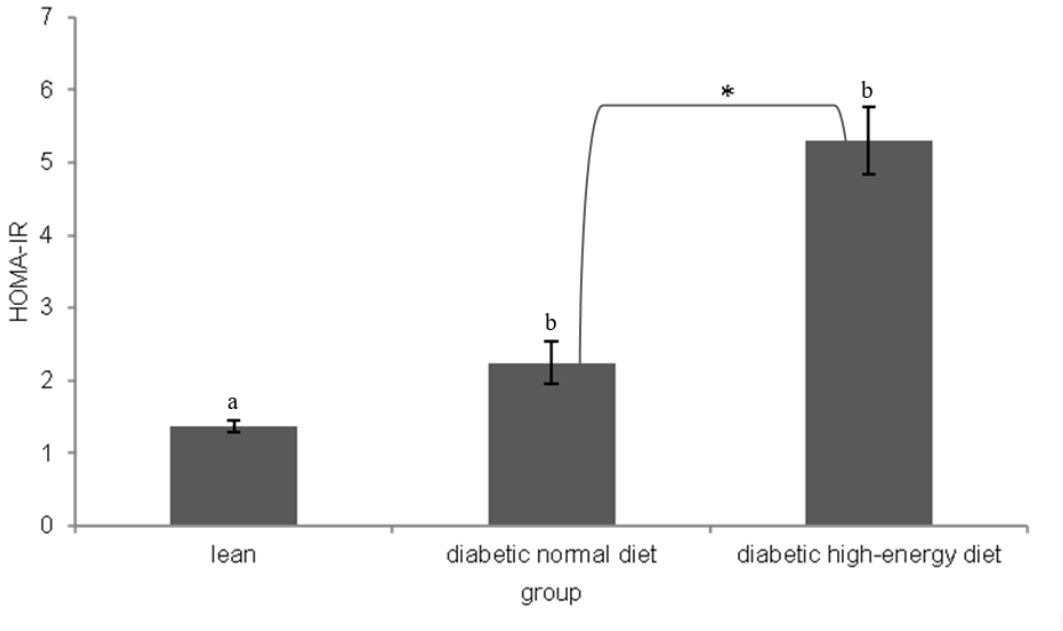

Figure 1. Homeostatic model assessment of insulin resistance (HOMA-IR) in ZDF rats
[a-b] mean significant difference (P < 0.05; P < 0.001) among the groups
[*] means significant differences (P < 0.001) between diabetic groups

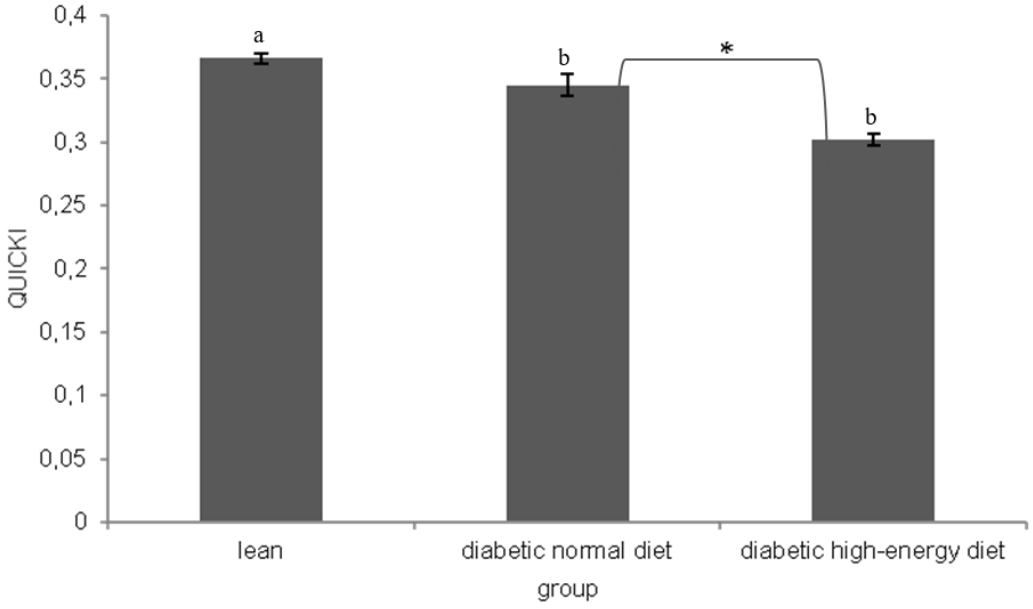

Figure 2. Quantitative insulin sensitivity check index (QUICKI) in ZDF rats
[a-b] mean significant difference (P < 0.05; P < 0.001) among the groups
[*] means significant differences (P < 0.001) between diabetic groups

Table 2. Pearson correlation coefficients between HOMA-IR and QUICKI model in ZDF rats

	Normal diet QUICKI	Energy diet QUICKI	Lean HOMA-IR	Normal diet HOMA-IR	Energy diet HOMA-IR
Lean QUICKI	0.559	-0.394	**-0.967**	-0.564	0.349
Normal diet QUICKI		-0.432	-0.508	**-0.955**	0.366
Energy diet QUICKI			0.384	0.446	**-0.985**
Lean HOMA-IR				0.537	-0.344
Normal diet HOMA-IR					-0.362
Energy diet HOMA-IR					1

0-0.33 – weak correlation, 0.34-0.66 – medium correlation, **0.67-1 – strong correlation**

values for non-obese and non-diabetic lean rats 0.366 ± 0.004 (Figure 2). In diabetic rats, the values were: 0.345 ± 0.009 – in the diabetic group and 0.302 ± 0.005 – in the diabetic group with high-energy diet. The differences among the groups were significant ($P < 0.001$ and $P < 0.05$). Similar data (0.30 ± 0.02 and 0.31 ± 0.02) were reported by Sarafidis *et al.* (2007) in study with patients suffering DMT2. The values less than 0.4 are considered normal and within the physiological range (Gutch *et al*., 2015).

In this study we found significantly high correlation ($r = -0.97$; $r = -0.96$; and $r = -0.99$; $P < 0.0001$) between HOMA-IR and QUICKI in all groups of animals (Table 2) depending on the diet. QUICKI is recognized as simply being log HOMA-IR, which interprets the high correlation with HOMA (Wallace *et al*., 2004). QUICKI was strongly correlated with insulin resistance index determined by euglycemic-hyperinsulinemic clamp, which is the most used standard method in the type 2 diabetic patients (Yokoyama *et al*., 2003).

The eventuality of evaluating insulin sensitivity and resistance in animals using a simpler and less traumatic method is important for experimental research (Antunes *et al*., 2016). Both, HOMA and QUICKI have been fully validated in human studies. In animal research it is important to focus and improve insulin and glucose measurement in order to use these methods. Generally, the determination by both methods in animal studies has advantages. They are simple methods with no need in special expertise, causing minimal stress to individuals, and practically free from the risk of hypoglycaemia (Antunes *et al.*, 2016). HOMA-IR and QUICKI indexes of insulin resistance founded on fasting measurements of insulin and glucose can serve as practical and useful surrogate of more complicated and time-consuming clamp-based measurements (Mather, 2019).

To conclude, this study indicated that the calculation of HOMA-IR and QUICKI can potentially be an effective tool in determination, evaluation, onset and progress of DMT2. Further studies are still needed to standardize the method and for better understanding and interpretation of the data in rodent experiments.

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