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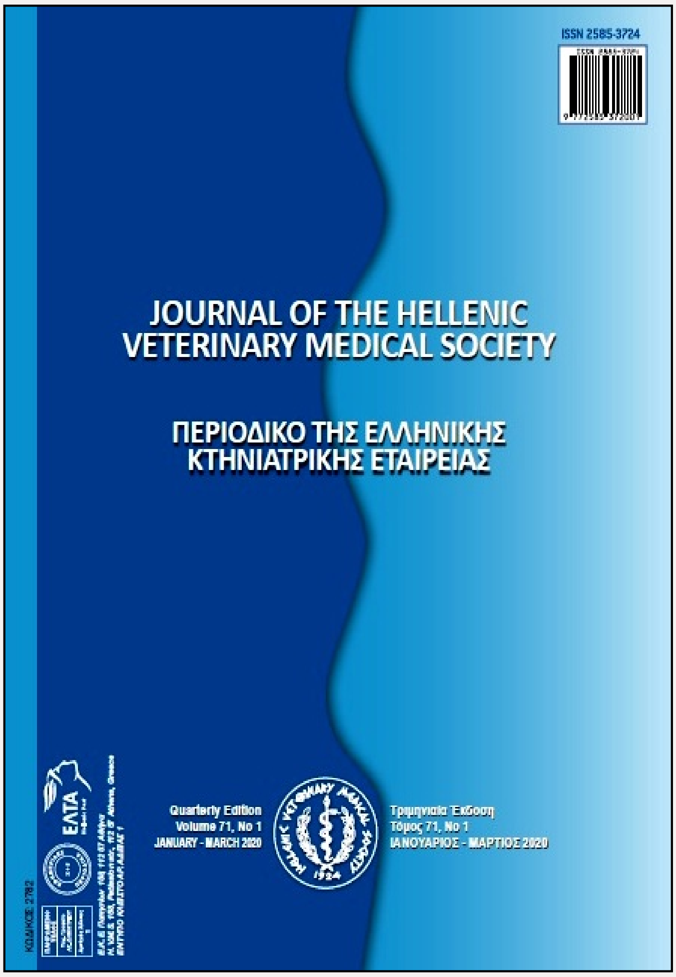

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Research article
Ερευνητικό άρθρο

Carcass characteristics and meat quality of broilers fed on earthworm (*Lumbricus rubellus*) meal

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ABSTRACT. The aim of the research was to evaluate the carcass characteristics and meat quality from chickens fed on diets in which fish meal was substituted with raw earthworms (*Lumbricus rubellus*) or earthworm meal. In the trial which lasted 42-days, 100 one-day-old *Hybro* broilers were divided into the control and three experimental groups. The control group was fed on standard broiler feed, the first (E-I) and the second experimental groups (E-II) were fed a diet in which 50% or 100% of fish meal was substituted with earthworm meal, respectively, whilst the third group (E-III) consumed feed without fish meal, but was given raw chopped earthworms *ad libitum* from day 1 to day 42. The replacement of fish meal with fresh earthworms resulted in significantly lower carcass weights in the E-III group in comparison with the control group ($p<0.05$). The differences in drumstick, thigh and breast meat share relative to the carcass mass were not significant ($p>0.05$). The lowest fat content in thigh and breast meat was in the group in which fish meal was replaced with earthworm meal. The lightness (L*) of thigh and breast meat was highest in the broilers fed fresh earthworms *ad libitum.* No significant differences in pH value were detected between the experimental groups ($p>0.05$). The most consumer acceptable were drumstick samples from E-II group and the least acceptable samples from the control group. Earthworm meal may be considered an adequate substitute for fish meal in broiler chickens' diet since it does not impair the production performance, carcass yield and meat quality.

Keywords: broilers, earthworm meal, carcass, meat quality, test panel

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INTRODUCTION

Besides reducing environmental pollution and production of quality fertiliser - biohumus, treatment of manure with various earthworm species provides significant quantities of a newly emerging feed i.e. worms (Ncobela and Chimonyo, 2015; Jacob, 2015). The production and demand for poultry meat is supposed to continue growing worldwide. This increase in meat production will require huge quantities of protein feed such as fish and soybean meals, which are expensive. For these reasons, there is a continuous research on the introduction of alternative protein feeds, which will help to solve the problem of deficient high-value proteins for animal nutrition (Khan et al., 2016; Djordjević et al., 2008; Radulović et al., 2018; Tiroesele and Moreki, 2012). Nowadays, with the existing methods of extraction and meal preparation 25-50% of proteins can be obtained from earthworm meal. The meal produced from red Californian earthworms (*Eisenia fetida*) is a tasty feed characterised by a high protein content (60-70%) of favourable amino acid composition that can replace fish and soybean meals in every phase of broiler production (Istiqomah et al., 2009). No negative effects on production performance in broiler chickens fed earthworm meal (obtained from *Perionyx excavatus*), as well as no detrimental impact on their health and food conversion rate were observed in previous studies (Vu and Quang, 2010).

Nutrition can significantly influence the poultry meat quality. The nutritive value of meat it is primarily determined by the content and composition of proteins and fats. Physical parameters for meat assessment are its colour, pH and water binding capacity. Colour is one of the first characteristics noted by the consumers, especially in the fillet products, and is also an indicator of meat quality (Fanatico et al., 2007). Factors determining sensory quality have the major influence on the consumer acceptability and are decisive in meat consumption. Parameters which determine carcass quality are carcass yield, breast meat yield, and the meat fat content (Araujo et al., 2004; Suchy et al., 2002). Nutrition is a factor which is most frequently used to improve the desired nutritional, sensory and technological quality of meat (Džinić et al., 2012).

Research has shown that red earthworms *(Lumbricus rubellus)* either dried or fresh, can successfully be used as an alternative protein feed component in broiler chickens fattening (Vu and Quang, 2010). However, to our knowledge not much is known about the influence of these feed components on carcass parameters and broiler meat quality. Thus, our research targeted their influence on carcass parameters (processed carcass yield and relative weight of main body parts - thighs, drumsticks and breasts), meat quality (basic chemical composition of red and white meats), physical characteristics of thigh and breast meat (pH and colour) and sensory characteristics of broiler drumsticks. The present study was part of a bigger research project and the data on the effects on the production performance in broilers, their health and food conversion have been reported earlier (Janković at al., 2015).

MATERIAL AND METHODS

Birds, menagement and experimental diets

The study was performed on a total of 100 one-days-old *Hybro G* female chickens (mean initial body weight 44.90±3.86 g). The chickens were equally divided into a control and three experimental groups. Throughout the experiment the preventive measures, housing and nursing were adjusted to the floor rearing system of *Hybro G* broilers. Feed and water were provided *ad libitum*. The ambient conditions were identical for all the groups and in agreement with the recommended technological standards for *Hybro G* broilers. Research protocols concerning animal handling were in accordance with the guidelines proposed by the Ethical Committee of the University of Belgrade, Faculty of Veterinary Medicine, and with the EU Directive 2010/63/EU for animal experiments.

Broiler feed

The control group (C) was fed with standard feed mixture (NRC, 1994). In the first (E-I) and second (E-II) experimental groups 50% and 100% of fish meal (FM) was replaced with earthworm meal respectively, whereas the third experimental group (E-III) was fed on diets with no fish meal, but was given fresh chopped earthworms from day 1 to day 42 of the experiment *ad libitum.* The composition of the diets in each experimental phase is given in Table 1.

Table 1. Composition of broiler diets in each experimental phase [%]

Ingredients [%]	**Experimental phase[1]**											
	Days 1-21				**Days 22-35**				**Days 36-42**			
	C	E-I	E-II	E-III	C	E-I	E-II	E-III	C*	E-I*	E-II*	E-III*
Maize	55.7	53.8	51.9	60.7	62.4	61.2	60.0	65.4	66.0	66.0	66.0	66.0
Soybean meal 44%	26.0	26.0	26.0	26.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0
Sunflower meal 33%	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Dicalcium phosphate	1.0	1.2	1.6	1.6	1.0	1.1	1.2	1.2	1.1	1.1	1.1	1.1
[2]Limestone	0.6	0.8	0.9	0.6	0.8	0.8	0.8	0.8	1.1	1.1	1.1	1.1
Brewers Yeast	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0	2.0	2.0
Fish meal	5.0	2.5	-	-	3.0	1.5	-	-	-	-	-	-
Earthworm meal	-	4.0	8.0	-	-	2.5	5.0	-	-	-	-	-
Fresh earthworm *(ad libitum)*	-	-	-	FE	-	-	-	FE	-	-	-	FE
Soybeane oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
[3]VMP	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100	100	100	100	100	100	100	100

[1]Experimental phases: I experimental phase (days 1-21); II experimental phase (days 22-35); III experimental phase (days 36-42); C - Control group; E-I - first experimental group; E-II-second experimental group; E-III - third experimental group;
*- In the final phase of fattening (36th - 42nd day), all experimental groups and the control group were fed on the same mixture: fish and worm meal were excluded from diet in the experimental group1 and 2, respectively; the experimental group 3 was fed on the same mixture, but was given fresh chopped worms during the entire fattening period. This is why all three groups have same numerical values of chemical content of the mixture.
[2]Limestone - Calcium carbonate;
[3]VMP - Vitamin mineral premix
[4]FE - Fresh earthworm

The earthworms (*Lumbricus rubellus)* used in the experiment were obtained by vermicomposting cattle manure ("Lumbri bio agrar", Leštane, Belgrade, Serbia). They were washed with water to remove dirt from the surface and afterwards kept in cold water (at 14°C) for 24 hours to free them from faecal mud (Edwards, 1985). The earthworms were dried in an oven at 50^{0}C for 10 hours following the method described by Istiqomah et al. (2009). After drying, the worms were weighed and ground with a hammer milling machine into powder. The mixtures intended for feeding the experimental animals were mixed (25 min.) in the Vrieco-Nauta conical screw mixer with a capacity of 50 kg (Hosokawa Micron Group, Osaka, Japan). Broilers were fed on starter feed from day 1 to 21, grower from day 22 to 35, and finisher from day 36 to 42 (all groups).

Chemical analysis of feedstuff

Standard methods of AOAC (2006) were followed for measuring dry matter, fat, cellulose, proteins and crude ash. Calcium was determined by colorimetric method SRPS ISO 6490-1:2001 (2001) and phosphorus contents by method SRPS ISO 6491:2002 (2002). The concentrations of lysine and methionine, as well as the dietary gross energy (MJ/kg) were calculated according to Sauvant at al. (2004).

Slaughtering parameters of poultry meat

The broilers were transported to a slaughterhouse at the end of the experiment, on day 42. Having been measured individually, the birds were electrically stunned, sacrificed by manual exsanguination, plucked and eviscerated. The measurement of body weight before slaughter, and carcass mass after slaughtering was done by the usual procedure for an industrial slaughter house. Carcass yield was calculated from the ratio between the mass of chilled carcass and body mass before slaughter. Chilled carcasses were cut in accordance with legislations, Rules on the quality of poultry meat (Sl. paper SFRJ 1/81, 51/88) (1988) into main commercial cuts (thighs, drumsticks and breasts) and measured on automatic scales with a precision of ±0.05 g.

Chemical analysis of the meat

In order to assess the meat quality certain chemical analyses were performed. By means of random sampling 10 carcasses were chosen from each group and from these 7 random samples of following tissues were taken: breasts, thighs and drumsticks. The preparation of these samples was done according to AOAC procedure (2006). The chemical analyses of meat samples (breast and thighs) were performed after 24 hours of storage at 4^0C following standard procedures: water content was measured by means of loss of mass while drying homogenised samples at 105±1°C until constant mass was achieved; fat content was determined by the Soxhlet method i.e. extracted from samples extracted with petrol ether and dried at 105±1°C to constant mass; proteins were quantified by the method described by Kjeldahl; ash was determined by sample incineration at 550°C until constant mass.

Physical characteristics of the meat

To assess the meat quality certain physical and sensory analyses were carried out. Ten carcasses randomly chosen from each group were used as a source of 7 samples of thigh and breast muscles taken at random. The detection of meat colour was done 24h post mortem using the colorimeter Konica Minolta CR-400 Chroma Meter (Ramsey, NJ, USA) calibrated on white panel (L*=93.30; a*=0.32 and 1.8; b*=0.33). Measurement area Φ 8.0 mm, illumination D_{65}, viewing angle 0°. The colour was measured in terms of the CIE system (Commission International de L'éclairage, 1976), where L* value defines lightness, a* redness and b* yellowness in meat samples prepared according to Warriss (2000). The colour of each sample resulted from three consecutive measurements and is expressed as the averages for each of the parameters assessed.

The pH values were measured 24h after slaughtering and cooling of carcasses with a pH meter "Testo 205" (Testo Ltd., Hampshire, UK) by direct insertion of the probe into the thigh and breast muscles (with ± 0.01 accuracy). Before and during its use the pH meter was calibrated with standard phosphate buffers (pH of the calibration buffer was 7.00 and 4.00 at 20°C). The mean of two pH values measured at the same point was considered to be the result (Korkeala et al.,1986).

Sensory evaluation of broiler meat samples

The sensory evaluation of broiler meat was performed by trained analysts. The panel consisted of twelve assessors who were chosen according to the defined standard, which is identical to the international one ISO 8586:2012 (2012). The means of preparation of all the samples were identical. The skin was removed from the drumstick meat before thermal treatment. Thermal processing was performed on an electric grill and lasted for about 20 minutes, until the temperature of 80^0C was achieved in the core of each piece of meat. After the roasting the samples were presented to the assessors on identical plastic saucers. They were asked to inspect the smell and taste, and rank the samples so that the first place would take the most acceptable, followed by less acceptable and the least acceptable one. The differences in the acceptability of broiler meat were detected with Rang test ISO 8587/2006 (2006). The significance of the differences in the acceptability at levels 95% and 99% was determined having in mind the number of samples, number of rankings and the difference in the sums of ranks between the samples.

Statistical analysis

Data were statistically processed and analysed by the Graph Pad Prism 5.0 software. Study results were statistically analysed and presented using mean value and standard deviation. The one-way analysis of variance (ANOVA) was applied and followed by Tukey HSD-test.

RESULTS

The chemical composition of fresh earthworms and dehydrated earthworm meal is presented in Table 2. These results represent the mean values of the chemical analyses of the earthworms sampled at regular intervals.

The chemical compositions of complete broiler feeds in the experiment are given in Table 3. The results showed that they fully satisfied the animals' requirements NRC (1994) as well as those of the experiment.

The average body mass before slaughter was lowest in E-II broilers (Figure 1), the group fed on the diet without fish meal which was completely replaced with earthworm meal, whilst the highest was in the control. However, the differences between the groups were not significant ($p>0.05$). Carcass weight was lowest in E-III group and the highest in the control, and differed significantly ($p<0.05$). Carcass yield broiler was lowest in E-III group and the highest in the E-II group but the differences between the groups were not significant.

Table 2. Chemical composition of fish meal, fresh worms and earthworm meal [100% DM]

Chemical composition	Fish meal	Fresh worms	Redworm meal
Moisture, %	10.00	84.76	11.44
Ash, %	19.57	1.32	9.20
Crude Protein, %	67.39	6.89	41.42
Crude Fat, %	7.17	2.25	9.20
Crude Fibre, %	0.65	0.55	1.77
NFE[1], %	5.22	4.14	25.00
Ca, %	7.28	0.20	1.46
P, %	3.48	0.14	0.80
Lysine, %	5.11	0.51	3.33
Methionine, %	1.96	0.14	0.96

[1] NFE - nitrogen free extract;

Table 3. Chemical composition of feed mixture for broilers per experimental phase [%]

Chemical composition [%]	Feed mixture I (01-21 days)				Feed mixture II (22-35 days)				Feed mixture III (36-42 days for all groups)			
	C	E-I	E-II	E-III	C	E-I	E-II	E-III	C*	E-I*	E-II*	E-III*
Moisture	11.11	11.13	11.15	11.36	11.33	11.34	11.36	11.48	11.50	11.50	11.50	11.50
Ash	5.40	5.68	5.60	4.56	5.13	5.27	5.41	4.62	4.94	4.94	4.94	4.94
Crude protein	22.28	22.28	22.27	19.58	19.38	19.41	19.45	17.76	17.32	17.32	17.32	17.32
Crude fat	5.47	5.60	5.73	5.34	5.56	5.64	5.72	5.48	5.48	5.48	5.48	5.48
Crude fibre	4.02	4.03	4.05	4.09	3.85	3.87	3.87	3.90	3.88	3.88	3.88	3.88
NFE	51.71	51.26	50.82	55.05	54.74	54.46	54.17	56.75	56.86	56.86	56.86	56.86
Ca	0.98	1.00	1.00	0.65	0.90	0.88	0.87	0.7	0.82	0.82	0.82	0.82
P	0.81	0.80	0.80	0.66	0.74	0.74	0.75	0.65	0.66	0.66	0.66	0.66
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
ME (MJ/kg)	12.79	12.61	12.43	12.96	13.03	12.93	12.82	13.10	13.09	13.09	13.09	13.09

C - Control group; E-I - first experimental group; E-II-second experimental group; E-III - third experimental group
*- In the final phase of fattening (36th - 42nd day), all experimental groups and the control group were fed on the same mixture: fish and worm meal were excluded from diet in the experimental group1 and 2, respectively; the experimental group 3 was fed on the same mixture, but was given fresh chopped worms during the entire fattening period. This is why all three groups have same numerical values of chemical content of the mixture.

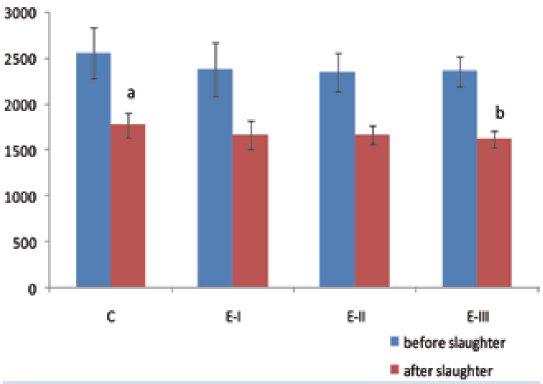

Figure 1. Broiler body mass (before slaughter) and carcass mass (g); Value expressed as $X \pm$ Sd; ^{a,b,c,d} - small letters indicate significant difference (p<0.05)

The average mass of thighs and drumsticks (Figure 2) was the lowest in broilers of the E-III group and the highest in the control, and differed significantly (p<0.05).

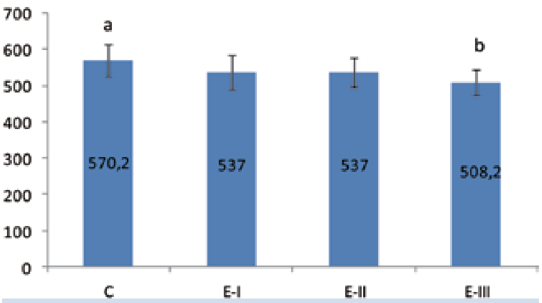

Figure 2. Mass of whole legs (thigh and drumstick) (g); Value expressed as $X \pm$ Sd; ^{a,b,c,d} - small letters indicate significant difference (p<0.05)

The mass share of the thigh and drumstick was lowest in broilers of the E-III group and the highest in E-I group. Nevertheless, the differences between the groups were insignificant (p>0.05).

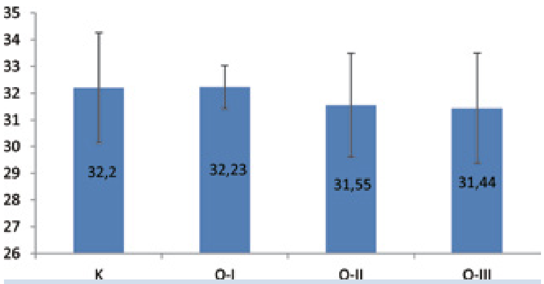

Figure 3. Mass share of breast meat in the carcass mass (%); Value expressed as X ± Sd

The average mass of breast meat (Figure 4) was lowest in the E-III group and the highest in the control, but the difference was not assessed as significant (p>0.05).

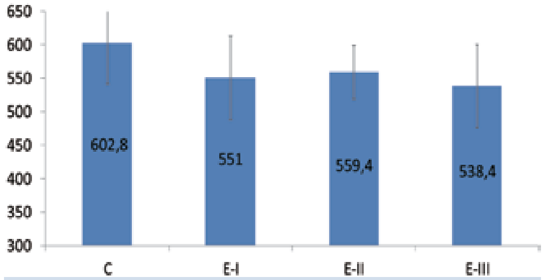

Figure 4. Mass breast meat (g); Value expressed as *X* ± Sd

In Figure 5 it can be seen that the relative mass share of breast meat in the carcass was highest in broilers of E-II group and the least in E-III. However, the difference between them was not significant (p>0.05).

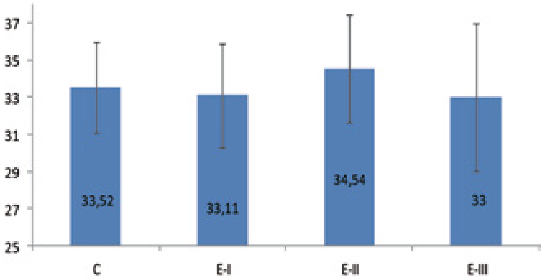

Figure 5. Relative average weight of breast meat (%)

The results of chemical analyses of the meat are presented in Table 4.

From the results of the analysis of the thigh meat it is noticeable that the mean water content in the broilers of E-I group was significantly lower than in the other groups (p<0.01). The lowest average fat content in thigh meat was detected in samples taken from E-II group whilst the highest was in E-I group. All the intergroup differences in fat content were significant (p<0.01), with the exception of the one between the control and E-I (p>0.05). Protein levels were significantly higher (p<0.01) in E-I group in comparison to the control. A significant difference in protein content was recorded for groups E-I and E-III (p<0.05). Different diets did not influence the concentrations of minerals in thigh meat (p>0.05).

According to the results of chemical analysis, in breast muscles (Table 4) the water content was highest in E-I group significantly higher than in all the others experimental groups (p<0.01). The average fat content was lowest in E-II group. Differences in fat content between the groups were significant (p<0.01). Proteins were detected in lowest concentrations in the breast meat in group E-I, highest in breast meat in E-III group. The differences in protein content were significant (p<0.01) between the control and E-I on one side and E-I other groups on the other side. Ash content was highest in meat E-I group, significant differences were observed between the control and E-1 group (p<0.05), as well as between groups E-I and E-III.

The results of instrumental assessment of the colour of thigh meat (Table 5) showed that its lightness (L*) was significantly higher (p<0.01) in the group which was given feed without fish meal, but had access to fresh earthworms *ad libitum* in comparison to all other groups. The lightness in the control was significantly higher (p<0.01) in comparison with E-I and E-II. Redness (a*) was significantly more intensive in the group in which fish meal was completely replaced with earthworm meal in comparison with the others groups. The yellowness (b*) in E-III was significantly higher (p<0.01) in comparison with the control, E-I and E-II groups. Moreover, in the control yellowness was significantly higher (p<0.01) in comparison with E-II.

Breast meat was lightest in group E-III and darkest in E-II (Table 5). Significant differences (p<0.01) were detected between all the groups tested. The redness of breast meat in group E-III was significantly higher (p<0.01) in comparison with all other groups. The yellowness in the control and E-III was significantly higher (p<0.01) in comparison with the two other groups.

The results measurements of pH in thigh and drumstick meat (Table 5) were found to be with no significant differences between groups (p>0.05). The results of electrochemical measurements of pH in breast meat (Table 5) were found to be without differences between the groups (p>0.05).

In Table 6 the differences in the assessment of acceptability of drumstick meat samples can be seen. Ranking meat samples resulted in the notion that the most acceptable were those from broilers of the E-II group, significantly more acceptable than any other. The samples from the control group were assessed as the least acceptable by the panel of jurors.

Table 4. Chemical composition* of thigh and breast meat (%)

Chemical composition	C	E-I	E-II	E-III
Thigh meat				
Water	72.29±0.38^{x}	70.79± 0.32^{y}	71.95±0.13^{x}	72.29±0.21^{x}
Fat	6.74±0.27^{x}	6.80 ±0.12$^{a, x}$	6.24±0.14^{y}	6.54±0.13^{c}
Protein	20.10±0.50$^{a, x}$	21.38±0.24^{a}	20.91± 0.38^{c}	20.58±0.75^{a}
Ash	1.03±0.00	1.03±0.00	1.02±0.00	1.03±0.00
Breast meat				
Water	74.36±0.38^{a,x}	75.38±0.23^{y}	74.82±0.21^{b,x}	74.18±0.30^{y}
Fat	1.88±0.06^{x}	1.34±0.05^{x}	1.31±0.04^{x}	1.89±0.07^{y}
Protein	22.50±0.33^{x}	21.82±0.18^{y}	22.45±0.20^{x}	22.51±0.30^{x}
Ash	1.42±0.02^{a}	1.45±0.03^{b}	1.42±0.01	1.42±0.02^{a}

*X ± Sd results are presented as mean ± standard deviation (SD); small letters indicate significant difference: a, b, c, d (p<0.05); x,y,z,w (p<0.01) within the same row

Table 5. pH and colour values of thing and breast meat

	C	E-I	E-II	E-III
Thigh meat				
pH	6.45±0.03	6.45±0.03	6.45±0.02	6.46±0.03
L*	50.43±0.24^{a}	49.52±0.20^{b}	49.15±0.24^{b}	52.15±0.41^{c}
a*	6.92±0.11^{A}	6.76±0.11^{a}	7.15± 0.19^{B,b}	6.89±0.12^{a}
b*	5.87±0.12^{a}	5.74±0.13^{a,b}	5.58±0.14^{b}	6.14±0.15^{c}
Breast meat				
pH	5.85±0.04	5.85±0.03	5.86±0.03	5.86±0.03
L*	47.30±0.29^{a}	46.36±0.26^{b}	45.39±0.29^{c}	48.08±0.24^{d}
a*	4.69±0.08^{a}	4.33±0.12^{b}	4.51±0.16^{a,b}	4.89±0.12^{c}
b*	8.13±0.16^{a}	7.43±0.13^{b}	7.25±0.20^{b}	8.16±0.17^{a}

L* = lightness; a*=redness; b*=yellowness; values in the same row marked with different upper case (A,B,C,D) or lower case letters (a,b,c,d) differ significantly (p<0.05 and p<0.01, respectively)

Table 6. Significance of differences between acceptability assessment of broiler **drumstick meat samples**

Groups	E- III	E-II	E-I	C
Sum of rank	**27**	**48**	**30**	**25**
C	2^{ns}	23**	5^{ns}	-
E-I	2^{ns}	18*	-	
E-II	21**	-		
E-III	-			

ns=not significant (p>0.05); *p<0.05; **p<0.01

DISCUSSION

Chemical analysis of feed

The protein content (41.42%) in the earthworm meal is lower in comparison to some literature data, which ranged between 46.57% and 63.0% (Khan et al., 2016; Jacob, 2015), but also more than reported by Mahmoud et al. (2015), who claimed that the content was 38.87%. Similarly, the amino acid content was somewhat lower compared to relevant published data, but still satisfactory. The results of the absolute content of amino acids are in agreement with the levels obtained by other authors (Sogbesan and Ugwumba, 2008). Fat contents were higher than the ones reported in literature according to Dynes (2003). Due to high fat contents in comparison with other feed components, earthworms are a rich source of energy with regard to dry matter. The ash content in earthworms is in agreement with some previously published data obtained by Sogbesan and Ugwumba (2008). The results of chemical analyses of feed consumed by broiler chickens in this research confirmed that it satisfied the norms required by the management guide for *Hybro* broilers and regulations NRC.

Slaughtering parameters of broiler meat

The analysis revealed that various diets did not affect significantly the average broiler body mass measured immediately before slaughter. These results are in agreement with the report of Bahadori et al. (2015), who, having studied the nutritive value of earthworms, found no significant differences in the body mass between the control and experimental groups. By contrast, Ton et al. (2009) claimed that the mean body mass of the broilers fed on diets containing earthworms (*Perionyx excavatus*) was higher than in the control.

Lower mass of chilled carcasses in comparison to the control was registered in the group which consumed feed with chopped raw earthworms instead of fishmeal. Resnawati (2002) conducted the research in order to clarify the effect of earthworm meal in the diet on carcass parameters of broiler chickens and observed no significant differences between the treatments, on that occasion he concluded that earthworm meal could be used up to 15 percent in broiler diets with no impact on carcass parameters or feed conversion. Results by Mahmoud et al. (2015) indicated that good results of broiler performance and carcass characteristics were obtained when 3.5% of earthworm meal was included in the broiler diet.

There were no significant differences in the carcasses yield of the broilers in the experiment. However, the lowest carcass yield was in the third experimental group i.e. 1.18% lower than in the control. The second group achieved 2.58% and the first one 0.92% higher carcass yields than the control. Ton et al. (2009) suggested that feeding with various quantities (1%, 1.5% and 2% on a dry matter basis) of red worms (*Perionyx excavatus*) resulted in higher yield of carcass.

Significantly lower weight of thighs and drumsticks in comparison to the control were detected in the group fed on feed in which the whole content of fish meal was replaced with chopped fresh earthworms. The mass share of thigh and drumstick meat in the carcass mass was lowest in the third group and the highest in the first one (32.23%). These data on the relative weight of whole legs are in accordance with those published by Bogosavljević-Bošković et al. (2010).

Breast meat is the carcass component with the highest economic value. The average chicken breast mass was lowest in the third experimental group and highest in the control, but they did not differ significantly. The yield of breast meat results from the satisfactory quality of feed which was used in the experiment: had there been a shortage of limiting amino acids in broiler feed, the primary indicator would have been a poor breast meat yield because the most intensive protein synthesis takes place in breast muscles (Araujo et al., 2004). However, the highest share of breast meat in chilled carcasses was detected in the second experimental group (34.54%) and the lowest in the third group (33.00%), but the differences were insignificant. Ton et al. (2009) reported about relative weights of deboned breast meat from 25.26% to 27.75% in broilers fed earthworms (1%, 1.5% and 2% on a dry matter basis). Having considered the slaughtering parameters for the experiment as a whole one may conclude that broilers fed on diets in which the fish meal was replaced with earthworm meal achieved satisfactory production results. There were no significant differences in carcass yield between the chicken groups. In addition, the relative weight of more valuable main commercial cuts were highly satisfactory, especially the one of breast meat.

Chemical composition of thigh and breast meat

Besides carcass yield, chemical composition is a main criterion for broiler meat quality assessment. The analysis of thigh meat revealed that its average water content ranged from 70.79% to 72.29% and that there were some highly significant differences between the experimental groups. The percentage of water in thigh meat measured in the current research was lower than reported previously by Ristić et al. (2008).

The intergroup difference in fat content was significant. The lowest average level in thigh meat (6.24%) was measured in the group consuming the feed with completely substituted fish meal with earthworm meal and the highest (6.80%) in chicken fed on partially substituted diet. Fat content in thigh meat in the experimental groups was lower than claimed for *Hybro* broilers (8.9%-9.3%) by other researchers (Suchy et al., 2002; Ristić et al., 2008). These lower fat percentages in poultry meat are of utmost importance for the consumers, which may be concluded from increasing demands of the market for lean meat.

The average protein levels in thigh meat ranged from 20.10% to 21.38%, and varied significantly between groups. The highest level was detected in the meat of chickens which were fed on diets with 50% of fish meal and 50% of earthworm meal. The protein content in thigh meat obtained in the research is somewhat higher than for *Hybro G* broilers (18.3%-19.1%) claimed by Bogosavljević-Bošković et al. (2010) and Suchy et al. (2002). The ash percentages in thigh meat were roughly the same in all chickens and similar to previously published data obtained by other authors (Bogosavljević-Bošković et al*.,* 2010; Suchy et al*.*, 2002).

The percentage of water in breast meat varied from 74.18% to 75.38%, with significant differences between the chicken groups. These results correspond exactly to those obtained by Ristić et al. (2008), who measured a water content of 75%. The average fat content in breast meat varied significantly, and ranged from 1.31% in the second experimental group to 1.89% in the third one. These results are much lower than previously published data (2.1 to 2.5%) claimed by Suchy et al. (2002). Partial or complete replacement of fish meal with earthworm meal or raw earthworms leads to significantly lower percentages of fat in broiler breast meat. This is of outstanding nutritional importance since low fat content, especially in white meat, is one of the reasons for the increasing demand for poultry meat. The average protein levels in breast meat ranged from 21.82% to 22.51%, with significant differences between groups. Having analysed the results obtained in the current experiment, one can conclude that the complete replacement of fish meal with earthworm meal or raw earthworms in the broiler diet exerts highly positive effects on protein contents in breast meat. These protein percentages in breast meat correspond to data for *Hybro G* broilers (22.5% to 22.7%) claimed by Suchy et al. (2002), but are somewhat lower (22.5% to 23.7%) than those presented by Bogosavljević-Bošković et al. (2010). Ash content varied slightly from 1.42% to 1.45%, which is higher than the one (1.1%) published by Ristić et al. (2013).

Physical characteristics of chicken meat

The results of the measurements of meat colour in thigh samples revealed significant differences in colour. Highest L* and b* values were detected in E-III group, which consumed the feed without fish meal but had chopped worms *ad libitum*. Thigh meat was reddest in E-II group. The lowest L* value, indicating the darkest meat, was in the broiler group which was fed on the feed with completely replaced fish meal with earthworm meal.

Breast meat of the chickens in E-III had significantly higher L* and b* values than those in other groups. The lowest was the L* value in broiler meat from E-II group. Qiao et al. (2002) have classified chicken breast muscle into three groups according to its colour: lighter-than-normal or light (L*>53), normal (48<L*<51), and darker-than-normal or dark (L*<46). Comparison of our results with these criteria leads to the conclusion that breast muscles of all broiler groups had normal colour and differed only slightly and were all visually acceptable.

Measurements of the electrochemical reaction of thigh meat failed to detect significant differences between inspected meat samples. These results are comparable with the data on the average pH (which is claimed to be 6.4) of fresh poultry meat (Ristić and Damme, 2013). The shelf-life of breast meat, its smell, colour, water adsorption during marination, water-holding capacity and loss during thermal processing depend mainly on pH. The results showed that there were no significant differences in pH values in breast meat between the groups, which is in accordance with the claims of Ton et al. (2009).

By means of sensory assessment it was discovered that the most acceptable were the drumsticks and breast meat originating from broilers fed on the diet without any fish meal, which was completely substituted with earthworm meal, and the least acceptable those from the control group and E-III group. The differences in the acceptability were based on the complete impression about the samples assessed - the colour, smell, juiciness, tenderness, texture, taste, aftertaste, fullness of flavour - on all traits perceptible by senses, rather than on one characteristic only (Baltić, 1994; Font-I-Furnols and Guerrero, 2014).

Conclusion is that earthworm meal may be considered an adequate substitute for fish meal in broiler chickens' diet since it does not impair the production performance, carcass yield and meat quality.

REFERENCES

AOAC (2006) International: Official Methods of Analysis of AOAC International. 18th Edition. Gaithersburg, MD (USA).

Araujo LF, Junqueira OM, Araujo CS, Faria DE, Andreatti MO (2004) Different criteria of feed formulation for broilers aged 43 to 49 days. Braz J Poult Sci 6:61-64.

Bahadori Z, Esmaeilzadeh L, Torshizi MAK (2015) The Effect of Earthworm (*Eisenia fetida*) and vermihumus meal in diet on broilers chicken efficiency and carcass components. Biol Forum Int J 7:998-1005.

Baltić ŽM (1994) Kontrola namirnica. Rang test. Fakultet veterinarske medicine Univerziteta u Beogradu 83-105.

Bogosavljević - Bošković S, Mitrović S, Djoković R, Dosković V, Djermanović V (2010) Chemical composition of chicken meat produced in extensive indoor and free range rearing systems. Afr J Biotechnol 9:9069-9075.

Djordjević M, Radenković-Damnjanović B, Vučinić M, Baltić M, Teodorović R, Janković Lj, Vukašinović M, Rajković M (2008) Effects of substitution of fish meal with fresh and dehydrated larvae of the house fly (*Musca domestica L)* on productive performance and health of broilers. Acta Vet - Beograd 58:357-368.

Dynes RA (2003) Earthworms. Technology information to enable the development of earthworm production. A report for the rural industries research and development corporation. RIRDC Publication. 03/085: 1-39.

Džinić N, Petrović Lj, Jokanović M, Tomović V, Filipović S, Tasić T, Ikonić P, Okanović Đ (2012) Quality of breast meat of broilers fed with extruded rapeseed meal. J edible oil industry 43(1-2):7-13.

Edwards CA (1985) Production of feed protein from animal waste by earthworms. Phil. Trans R Soc Lond 310:299-307.

Fanatico AC, Pillai PB, Emmert JL, Owens CM (2007) Meat quality of slow-and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. Poult Sci 86:2245-2255.

Font -I-Furnols M, Guerrero L (2014) Consumer preference, behavior and perception about meat and meat products: An overview. Meat Sci 98 (3):361-371.

ISO 8586-2012 (2012) Sensory analysis - General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. https://www.iso.org/standard/45352.html [2012-12].

ISO 8587:2006 (2006) Sensory analysis - Methodology - Ranking. https://www.iso.org/standard/36172.html [2006-11].

Istiqomah L, Sofyan A, Damayanti E, Julendra H (2009) Amino acid profile of earthworm and earthworm meal (*Lumbricus rubellus*) for animal feedstuff. JITAA 34:253-257.

Jacob J (2015) Including earthworms in organic poultry diets. Organic agriculture. http://www.extension.org/pages/69709/including-earthworms-in-organic-poultry-diets [october 02. 2013].

Janković Lj, Radenković-Damnjanović B, Vučinić M, Šefer D, Teodorović R, Đorđević M, Radisavljević K (2015) Effects of fish meal replacement by red earthworm (*Lumbricus rubellus*) meal on broilers' performance and health. Acta Veterinaria-Beograd 65 (2): 271-286.

Khan S, Naz S, Sultan A, Alhidary IA, Abdelrahman MM, Khan RU, Khan NA, Khan MA, Ahmad S (2016) Worm meal: a potential source of alternative protein in poultry feed. World's Poult Sci J 72: 93-101.

Korkeala H, Maki-Petais O, Alanko T, Sorventula O (1986) Determination of pH in meat. Meat Sci 18: 121-125.

Mahmoud M, Musa A, Ahmed AE, Ismail M, Hassan H, Yagoub I, Salih S (2015) Effect of dietary inclusion of earthworm meal replacing super-concentrate on broiler performance and carcass characteristics. International research on food fecurity, natural resource management and rural development management of land use systems for enhanced food security: conflicts, controversies and resolutions. Book of abstracts. Tropentag 2015. http://www.tropentag.de/2015/proceedings/proceedings.pdf.

Ncobela CN, Chimonyo M (2015) Potential of using non-conventional animal protein sources for sustainable intensification of scavenging village chickens: A review. Animal Feed Sci Techn 208:1-11.

NRC (1994) Nutrient Requirements of Poultry. Ninth revised edition. Washington, DC. The National Academies Press. (https://doi.org/10.17226/2114).

Qiao M, Fletcher DL, Smith DP, Northcutt JK (2002) Effects of raw broiler breast meat color variation on marination and cooked meat quality. Poult Sci 81:276-280.

Radulović S, Pavlović M, Šefer D, Katoch Š, Hadži - Milić M, Jovanović D, Grdović S, Marković R (2018) Effects of housefly larvae (*Musca domestica*) dehydrated meal on production performances and sensory properties of broiler meat. Thai J Vet Med 48(1): 63-70.

Resnawati H (2002) Carcass production and internal organs of broiler fed earthworm *Lumbricus rubellus* meal. Nasional Teknologi Peternakan dan Veteriner 294-297.

Ristić M, Damme K (2013) Significance of pH - value for meat quality of broilers - influence of breed lineas. Vet glasnik 67: 67-73.

Ristić M, Freudenreich P, Damme K (2008) Chemical composition of poultry meat- comparison of broiler, hen, turkey, duck and goose meat. Tehnologija mesa 49:82-89.

Rules on the quality of poultry meat (Službeni list SFRJ, 1/81 and 51/88). Serbian.

Sauvant D, Perez JM, Tran G (2004) Tables of composition and nutritional value of feed materials; pigs, poultry, cattle, sheep, goats, rabbits, horses, fish. Wageningen Academic Publishers, the Netherlands: pp 13-304.

Sogbesan AO, Ugwumba AA (2008) Nutritional values of some non-conventional animal protein feedstuffs used as fishmeal supplement in aquaculture practices in Nigeria. Turk J Fish Aquat Sci 1:59-164.

SRPS EN 15621:2017 (2017) Animal feeding stuffs - Determination of phosphorus content - Spectrometric method. Institute for Standardization of Serbia, Belgrade, Serbia. https://www.iss.rs/en/standard/?natstandard_document_id=58768 [Accessed 30 Nov 2017].

SRPS ISO 6490-1:2001 (2001) Animal feeding stuffs - Determination of calcium content - Part 1: Titrimetric method. Institute for Standardization of Serbia, Belgrade, Serbia. https://www.iss.rs/rs/standard/?natstandard_document_id=15234 [Accessed 15 Dec 2001].

Suchy P, Jelinek P, Strakova E, Hucl J (2002) Chemical composition of muscles of hybrid broiler chickens during prolonged feeding. Czech J Animal Sci 47:511-518.

Tiroesele B, Moreki JC (2012) Termites and earthworms as potential alternative sources of protein for poultry. Int J Agro Vet Med Sci 6:368-376.

Ton DV, Hanh QH, Linh DN, Duy VN (2009) Use of earthworms (*Perionyx excavatus*) to manage agricultural wastes and supply valuable feed for poultry. Livestock Research Rural Development 21:1-8.

Vu DT, Quang HH (2010) Determining appropriate amount of redworm meal supplemented in the diet of broiler (Ho x Luong Phuong) in the open air production system. J Sci Development 8 (6): 949-958.

Warris PD (2000) Measuring the composition and physical characteristics of meat in: Meat Science: An introductory text (2nd Ed.). CAB International, New York 179-193.