

EFFECTS OF FISH MEAL REPLACEMENT BY RED EARTHWORM (*Lumbricus rubellus*) MEAL ON BROILERS' PERFORMANCE AND HEALTH

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The research was performed to determine the nutritive value of raw earthworms (*Lumbricus rubellus*) and dried earthworm powder, or earthworm meal (EM). In addition, the effects of a diet in which fish meal was substituted with EM or fresh earthworms on the health and productive performance of broilers were monitored. The experiment, which lasted 42 days, was conducted on one hundred *Hybro G* broilers divided into four equal groups. The control group was fed a standard feed, whereas, the first and the second one were given diets in which 50% or 100% of fish meal had been substituted with EM. The third experimental group received no fish meal but was given fresh earthworms *ad libitum*. The results of chemical analyses showed that earthworm meal contained 41.42% proteins (in dry matter) and satisfactory amounts of amino acids. Microbiological examination and tests for heavy metals suggest that earthworm meal and fresh earthworms did not contain heavy metals and harmful bacteria. Therefore, these feeds may be considered suitable for chickens. In the experiment as a whole no statistically significant difference ($p > 0.05$) was observed in the productive performance between the experimental groups of broiler chickens. These results suggest that dehydrated earthworm meal can be an adequate substitute for fish meal in the broilers' feed.

Key words: broilers, earthworm meal, nutrition, performance

INTRODUCTION

Organic waste processing with earthworms - vermicomposting - has become very popular lately. During this process, besides a high-quality organic fertilizer (humus), a great amount of earthworm biomass is obtained. Either fresh or dried, this can serve as an alternative source of high-quality proteins for non-ruminants (fattening poultry,

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pigs and fish) [1-6]. By means of extraction as much as 25 to 50% of their need in proteins can be satisfied with EM.

The red earthworm, *Lumbricus rubellus*, contains more protein per unit of dry matter than soy bean or sunflower pellets, but less than fish meal [1,7], dehydrated housefly larvae [8] and maggot meal [9]. The dominant essential amino acid in fresh earthworms was histidin and in earthworm meal isoleucine [10]. Among the non-essential amino acids the most common was glutamic acid both in fresh earthworms and earthworm meal.

Due to their relatively high fat content compared to other feeds, red earthworms represent a rich source of energy if dry matter content is taken into consideration [4,7,11,12]. Research revealed that the lipid content in earthworms varies between 1% and 20% in dry matter and is of a composition similar to that of fish oil. More than 70 fatty acids can be extracted from earthworm meal [13]. Earthworms lipid fatty acid composition depends on their nutrition and season when they were collected.

Fresh earthworms and EM are readily consumed by broilers [2,6,7]. Feeding experiments confirmed that EM is a good source of protein for chickens [4,7,10]. Moreover, Ulep [14] states that EM can replace fish meal, meat meal or soy meal at any production stage, resulting in somewhat lower body mass in broilers. Feeding experiments [11,15,16] proved that weight gain in broilers fed fresh earthworms was significantly higher than in the control. Other authors by contrast, [6,17] point to higher daily weight gains in the first fattening phase and somewhat lower in the second phase, whilst food conversion and feed consumption remained roughly similar in all groups.

However, some authors [18] suggested that the ingestion of growing earthworms poses a risk to animal health due the fact that heavy metals and other pollutants are taken up by the worms.

The purpose of this experiment was to study the quality of earthworm biomass and EM (*Lumbricus rubellus*), as well as the possibility of their use as an alternative protein source in broilers, by means of monitoring the productive performance and health status of the chickens.

MATERIAL AND METHODS

Experiment design

The experiment was performed on 100 one-day-old *Hybro G* chickens of both sexes, divided into four equal groups. Clinical examination showed that all the birds were healthy and in good condition. The experiment lasted 42 days and was carried out in three phases: the first lasted three weeks, the second one two, and the third one week only. Throughout the experiment the preventive measures, housing, nursing, feeding and watering were adjusted to the floor rearing system of *Hybro* broilers. The ambient

conditions were identical for all the groups and in agreement with the technological standards for *Hybro* broilers. The experimental animals were fed and watered *ad libitum*.

Broiler feed

The control group was fed complete feed for fattening chickens, made from standard ingredients which fully satisfied the requirements of different categories of broilers. In the first and second experimental group fish meal was replaced with EM 50% and 100% respectively, whereas the third experimental group was fed a diet with no fish meal, but supplemented with fresh chopped earthworms from the first day of the experiment *ad libitum* (Table 1).

In order to provide adequate feed for the experiment, earthworms (*Lumbricus rubellus*) were collected from the earthworm manufacturer “Lumbri bio agrar”. Based on Edwards [19] method, the earthworms were washed using water in order to separate the manure from the outside skin and the fecal (fecal mud) thereon were kept in cold water (14°C) for 24 hours. The earthworms were dried using an oven set at 50°C 10 hours according to the method described by Istiqomah *et al.* [10]. After drying, the worms were weighed, then milled with a hammer milling machine into a powdered form. The obtained meal was a light brown color with a pleasant aroma.

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

| Ingredients [%] | Experimental phase ¹ | | | | | | | | | | | |
|---------------------|---------------------------------|------|------|-------|------------|------|------|-------|------------|------|------|-------|
| | Days 01-21 | | | | 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 | 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 Pellets | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| 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 | - | - | - | - | - |
| Vegetable 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 |
| 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 Chalk | 0.6 | 0.8 | 0.9 | 0.6 | 0.8 | 0.8 | 0.8 | 0.8 | 1.1 | 1.1 | 1.1 | 1.1 |
| 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 |

¹Experimental 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; ²Chalk - Calcium carbonate; ³VMP - Vitamin mineral premix

The mixtures intended for feeding experimental animals were mixed in the Vrieco-Nauta conical screw mixer with a capacity of 50 kg (Hosokawa Micron Group, Osaka, Japan). In order to make the mixtures homogeneous, they were mixed for 25 min.

Broilers were fed a starter from day one to 21, grower from day 22 to 35, and finisher from day 36 to 42 (the end of the experiment) (Table 1).

Standard procedures and methods were used for sampling of fresh earthworms, EM and complete feed.

Chemical analysis of feed

Contents of crude ash, crude protein, crude fat, crude cellulose and nitrogen free extract (NFE) of fresh earthworms and EM were determined in relation to air dry matter. Preparation of the samples was done according to AOAC procedure (1990) [20]. Determination of basic nutritive matter was done according to the following procedures: crude moisture SRPS ISO 6496:2001 [21] crude ash SRPS ISO 5984:2002 [22] crude protein SRPS ISO 5983-2:2010 [23]; crude fat SRPS ISO 6492:2000, [24] and the content of crude cellulose by accredited laboratory method (DM1); while the content of nitrogen free extract (NFE) was determined by mathematical calculation. The colorimetric method was used to detect calcium SRPS ISO 6490-1:2001 [25] and phosphorus contents by method SRPS ISO 6491: 2002 [26]. The calculation of metabolic energy was based on the results obtained. Content of amino acids in the feed was determined by SRPS EN ISO 13903:2011 [27]. The fatty acid content was analysed by gas-chromatography using the standards of fatty acid methyl esters on the gas chromatograph (Hewlet Packard HP 6890) according method SRPS CEN ISO/ TS 17764-2:2009 [28]. The heavy metal content (Pb, Cd) in EM was determined by flame atomic absorption spectrophotometry (Varian Spectra 220/880, Varian Inc.) according method SRPS EN 14084:2008 [29]. Mercury was detected by cold vapour atomic absorption spectrometry according method SRPS EN 13806:2008 [30] and arsenic by hydride generation flame atomic absorption (VGA 77, Agilent Technologies Inc.) according method SRPS EN 14627:2008 [31].

Microbiological analysis

The microbiological analysis of fresh earthworms and EM was performed with conventional bacteriological methods [33]. Sampling of fresh earthworms and meal made of dehydrated earthworms was carried out following standard procedures [32]. After the samples were collected, they were minced and macerated in a sterile mortar. After that, samples were diluted 10 to 100.000 times with sterile 0,85% physiological saline. For the isolation and determination of the total number of bacteria and fungi, different sample dilutions were cultured on conventional bacteriological and mycological media: MacConkey agar (Torlak), Columbia agar with 5% sheep blood (bioMeriux), nutrient agar (Torlak) and Sabouraud dextrose agar with chloramphenicol (BioLab). For the cultivation of bacteria, incubation was performed at 37°C during

24 hours, and for the fungi and yeasts incubation was performed at 37°C during the first 24 hours and after that at 25°C during the following 72 hours. Identification of bacteria was performed using conventional microbiological methods and also using BBL Crystal Gram positive ID kit and BBL Crystal Enteric/nonfermenter ID Kit (Becton Dickinson). Identification of fungi was performed using standard microbiological methods – microscopy and morphological characteristics of the colonies.

Production results

In order to monitor their productive performance, the broilers were measured individually at the beginning of the experiment and at the end of each of its phases (on day 21, 35 and 42). The body weight was measured with electronic scales to a precision of 10⁻²g. The mean body mass of broilers was calculated at the end of each phase, and at the end of the research. The total and daily weight gains were determined for each experimental phase and the experiment as a whole.

In addition, throughout the experiment the total amount of food consumed by each group was calculated, as well as the amount of fresh earthworms ingested by the third group of broilers. Feed conversion was determined for each experimental phase, and for the experiment as a whole.

The following formula was used to calculate the production number, which depends on the body mass, feed conversion, duration of fattening process and mortality rate:

$$PN = \text{Average live weight} \times \% \text{ survivability} / \text{days (duration of fattening)} \times FCR \div 10$$

Statistical analysis

The results are expressed as means \pm standard deviations (SD). One-way analysis of variance (ANOVA) was applied and followed by Tukey HSD-test. All calculations were performed with the statistical software package Vassar Stats [34].

RESULTS

The chemical composition of fresh earthworms and dehydrated earthworm meal was analysed with standard methods [20] and is presented in Table 2. These results represent the mean values of the chemical analysis of the red earthworms sampled at regular intervals.

The fatty acid contents in dehydrated earthworm meal are given in Table 3. The following acids were found in EM: palmitic (C16:0), palmitoleic (C 16:1), stearic (C 18:0), oleic acid (C 18: 1), linoleic (C 18:2), linolenic (C 18:3) and arachidic acid (C 20:0).

Heavy metal contents are given in Table 4. The results show that lead, cadmium and mercury were not detected, whereas, the quantities of arsenic (0.59 mg/kg) were within the permissible limits (2 mg/kg) prescribed by the Regulation.

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

| Chemical composition | Fresh worms | Earthworm meal | Fish meal |
|-------------------------|-------------|----------------|-----------|
| Moisture | 84.76 | 11.44 | 10.00 |
| Ash | 1.32 | 9.20 | 19.57 |
| Protein | 6.89 | 41.42 | 67.39 |
| Fat | 2.25 | 9.20 | 7.17 |
| Fibre | 0.55 | 1.77 | 0.65 |
| ¹ FNE | 4.14 | 25.00 | 5.22 |
| ² ME (MJ/kg) | | 10.64 | 14.18 |
| Ca | 0.20 | 1.46 | 7.28 |
| P | 0.14 | 0.80 | 3.48 |
| Lysine | 0.51 | 3.33 | 5.11 |
| Methionine | 0.14 | 0.96 | 1.96 |

¹FNE – free nitrogen extract; ²ME – metabolic energy

Table 3. Fatty acid contents in red earthworm powder [% of total fatty acids]

| Fatty acids | $\bar{x} \pm SD$ | Range | SE | CV |
|---------------------|------------------|-------------|------|------|
| Palmitic (C16:0) | 11.52±0.09 | 11.39-11.65 | 0.04 | 0.82 |
| Palmitoleic (C16:1) | 6.24±0.04 | 6.18-6.29 | 0.02 | 0.67 |
| Stearic (C18:0) | 6.26±0.04 | 6.20-6.31 | 0.02 | 0.60 |
| Oleic (C18:1) | 12.87±0.37 | 12.14-13.14 | 0.15 | 2.85 |
| Linoleic (C18:2) | 4.83±0.06 | 4.77-4.91 | 0.02 | 1.15 |
| Linolenic (C18:3) | 0.55±0.02 | 0.52-0.58 | 0.01 | 3.64 |
| Arachidic (C20:0) | 0.15±0.01 | 0.14-0.17 | 0.00 | 7.71 |

Table 4. Heavy metal contents in earthworm meal [mg/kg]

| Heavy metals | Content [mg/kg] | Maximum allowable concentration (MAC) |
|--------------|-----------------|---------------------------------------|
| Pb | 0.00 | 10.00 |
| Cd | 0.00 | 0.50 |
| Hg | 0.00 | 0.20 |
| As | 0.59 | 2.00 |

Microbiological analysis confirmed that both fresh earthworms and EM samples met the strict hygienic requirements required by law (Table 5).

The chemical composition of complete feeds for the experimental broilers is given in Table 6.

Table 5. Microbiological analysis of samples of fresh earthworms and earthworm meal

| Microorganisms | Samples of fresh earthworms | Samples of earthworm meal |
|------------------------------|-----------------------------|---------------------------|
| <i>Bacillus spp.</i> | + | + |
| <i>Staphylococcus spp.</i> | + | + |
| <i>Pseudomonas spp.</i> | + | + |
| <i>Coliform bacteria</i> | + | + |
| Total no. of bacteria | 9.0 x 10 ⁶ /g | 1.8 x 10 ⁶ /g |
| <i>Mucor spp.</i> | + | + |
| <i>Aspergillus spp.</i> | + | + |
| <i>Penicillium spp.</i> | + | + |
| Total no. of fungi | 36 x 10 ³ /g | 1 x 10 ³ /g |

Legend: + microorganisms and fungi present in the sample

Table 6. 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) | | | |
|--------------------------|-----------------------------|-------|-------|-------|------------------------------|-------|-------|-------|-------------------------------|-------|-------|-------|
| | 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 |
| FNE | 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

The results of the chemical composition analysis of complete broiler feeds show that they fully satisfy the animals' requirements [23] as well as those of the experiment.

Figure 1. shows the broilers' body mass changes in the experiment. In the beginning the body mass of all the experimental groups was standardized (44.9 ± 3.86 g), without significant differences between the groups ($p > 0.05$). At the end of the first phase a significant difference ($p < 0.01$) was observed between the control (837.60 ± 84.21) and third group (750.20 ± 80.82). However, at the end of the second and third phase, as well as regarding the whole experiment, the body mass of the control did not differ from other experimental groups ($p > 0.05$).

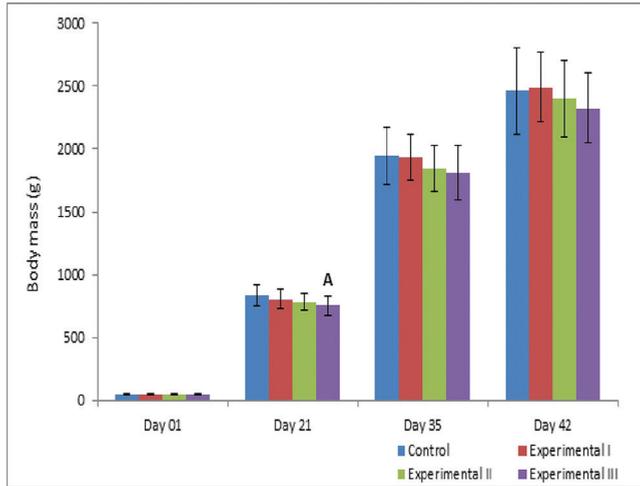


Figure 1. Broilers' body mass in the experiment [g] (All values are given as the means \pm SD, n=25 animals per group; A - $p < 0.01$ compared to the control group)

The daily weight gain of the chickens is presented in Figure 2. During the first phase, the third group of broilers had significantly ($p < 0.01$) lower weight gain (33.58 ± 3.76) in comparison to the control (37.75 ± 3.84) and the first group (36.28 ± 3.47 , $p < 0.05$). During the second and the third phase, as well as if the whole experiment is taken into account, the differences between the groups were not significant ($p > 0.05$).

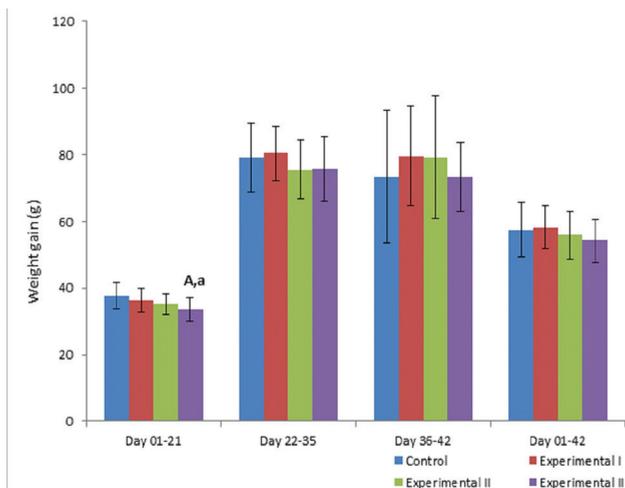


Figure 2. Weight gain during the experiment [g] (All values are given as the means \pm SD, n=25 animals per group; A- $p < 0.01$ compared to the control group; a- $p < 0.05$ compared with experiment-I group)

Figure 3. contains the changes in feed consumption in each fattening phase. It is noticeable that the control group consumed usual amounts of feed. In addition, the daily feed consumption varied from group to group in all experimental phases, as well as in the experiment as a whole.

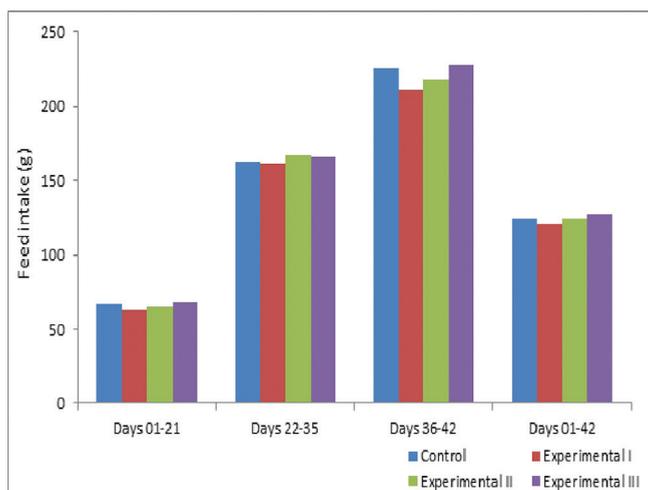


Figure 3. Daily feed intake during the experiment [g]

Regarding the experiment as a whole, the third group, given feed without fish meal, but supplemented with fresh earthworms, had somewhat higher feed intake in comparison to the control, the first and the second experimental group. The best feed gain ratio was achieved in the first experimental group, whilst the broilers in the control and second group it was the same (Figure 3).

Feed conversion ratio, a measure of animals' efficiency in converting feed into weight gain, is demonstrated both per phase and for the whole experiment in Figure 4.

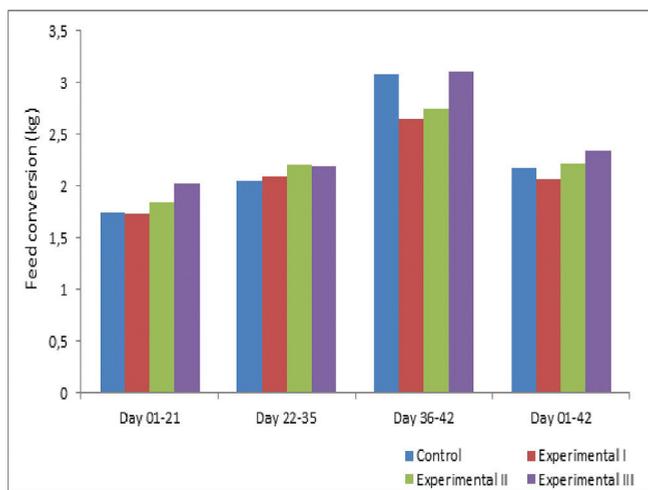


Figure 4. Feed conversion during the experiment [kg]

The average feed conversion ratio ranged from 2.07 to 2.34 kg and varied during all the fattening periods. Taking into consideration the results for the entire experiment, the first group had the best conversion (2.07 kg). By contrast, the highest feed intake per kg of weight gain was observed in the third group (2.34 kg).

Table 7. Production number of broilers in experiment

| Groups | Production numbers | Index |
|------------------|--------------------|--------|
| Control | 269.91 | 100.00 |
| Experimental I | 286.40 | 106.11 |
| Experimental II | 256.28 | 95.06 |
| Experimental III | 236.06 | 87.46 |

C – control group; E-I – first experimental group; E-II – second experimental group; E-III – third experimental group

The control group reached a satisfactory production number (Table 7). The substitution of fish meal with earthworm meal in the first and second experimental group did not have any significant influence on this parameter. By contrast, the production number of broilers fed without fish meal with added fresh worms was lower than in the control.

DISCUSSION

The chemical composition of earthworm meal used in this research proved adequate for animal nutrition. The results of the chemical analysis of fresh red earthworms (*Lumbricus rubellus*) confirmed that they had a high water content (84.76%). The crude protein content in the earthworm meal was 41.42%, much higher than in sunflower pellets (37.78%), but lower than in fish meal (67.39%). These results referring to the protein content are lower in comparison to literature data, which ranged between 46.57% and 62.0% for earthworm meal [1,4,6,7,36] and reached 59.48% in dehydrated housefly larvae meal [8].

Similarly, the amino acid content was somewhat lower compared to relevant literature data, but still satisfactory. The contents of lysine and methionine in fresh earthworms was 0.51% and 0.14% (air-dry matter), respectively, that is 7.40% and 2.10% per protein unit. The lysine and methionine contents in EM were 3.33% and 0.96% (air-dry matter), respectively, that is 8.03% and 2.32% per protein unit. The results of the absolute content of amino acids are in agreement with Sogbesan and Ugwumba [7]. The results of chemical analysis of the meal lead to the conclusion that it is a satisfactory source of proteins and essential amino acids.

On the other hand, fat contents (9.20% in dry matter, DM) were found to be higher than previously reported (5.15% in DM). According to [13], EM with the highest fat content originated from earthworms with less agility and lower feed consumption. Due to high fat contents in comparison with other feed red earthworms are a rich source of energy with regard to dry matter [17].

The ash content of 9.20% in DM (1.46% Ca and 0.80% P in DM) in EM is in agreement with previously reported data [7].

By chemical analysis of the fatty-acids in EM myristic and myristoleic acids were not detected. The palmitic acid content (11.52±0.09%) was markedly higher, but the stearic

and linoleic acids levels ($6.26 \pm 0.04\%$ and $4.83 \pm 0.06\%$, respectively) were somewhat lower than reported by Dynes [13] (palmitic acid $0.74 \pm 0.11\%$, stearic $7.61 \pm 0.41\%$ and linoleic $6.11 \pm 0.39\%$). The average oleic acid concentration in EM, $12.87 \pm 0.37\%$, was lower than in fish meal, where levels as high as 22.7% were detected [7]. By contrast, linoleic acid was present in considerably higher concentrations, $4.83 \pm 0.06\%$, than reported for fish meal (from 1.15% to 1.50%). It is supposed that fatty acid composition in earthworms depend on the amount of ingested feed and the season [4,7,11,12,13].

By means of heavy metal content analysis lead, cadmium and mercury were not detected, whereas, the quantities of arsenic (0.59 mg/kg) were within the permissible limits were within the permissible limits (2 mg/kg) prescribed by the Regulation [33].

The results of the microbiological investigations showed that fresh earthworms as well as EM samples met the strict hygienic requirements required by law. Analysis of the results referring to the chemical composition and hygiene requirements confirmed that EM is a quality nutrient and can be successfully used in broiler nutrition.

The results of the chemical analyses of feed consumed by control broiler chickens in this research confirmed that it satisfied the norms required by the management guide for *Hybro* broilers and legislation [35].

In comparison with the chemical analysis, the biological trial and the monitoring of production performance provide more reliable data on the feed quality. At the beginning of the experiment the body mass in all experimental groups was roughly similar ($44.9 \pm 3.86 \text{ g}$) with insignificant differences between the groups ($p > 0.05$). The control group, during all fattening phases, achieved the results expected for *Hybro G* broilers [37]. At the end of the first phase a significant difference ($p < 0.01$) was observed between the control (837.60 ± 84.21) and third group (750.20 ± 80.82). However, at the end of the second and third phase, as well as regarding the whole experiment, the body mass of the control did not differ from other experimental groups ($p > 0.05$). The research performed by Banaszekiewicz [38], Katoch *et al.* [39] and Živkov-Baloš *et al.* [40] indicated that low concentrations of Ca and P in broiler feed resulted in significant body weight loss. However, in our experiment this could not be confirmed: although earthworm meal contained somewhat less Ca and P (1.46% and 0.80% in DM, respectively) than fishmeal (3.53% and 0.96% in DM, respectively) the decrease in body weight was insignificant in comparison to the control. The obtained results are in agreement with those of Julendra *et Supadmoz* [15] and Prayogi [11], who, having studied the nutritive value of earthworms found no significant differences in the body mass between the control and experimental groups. On the other hand, Das *et Dach* [16] claimed that the mean body mass of the broilers fed diets containing EM was higher compared to the control.

Although body mass at the end of the fattening period is a good indicator of consumed feed quality, it is considered that daily weight gain is much more reliable. The daily weight gain of the chickens in the current research ranged between the limits required

from this broiler chicken breed. During the first phase, the third group of broilers, fed diets without fish meal but substituted with fresh earthworms, had significantly ($p < 0.01$) lower weight gain (33.58 ± 3.76) in comparison to the control (37.75 ± 3.84) and the first group (36.28 ± 3.47 , $p < 0.05$). During the second and the third phase, as well as if the whole experiment is taken into account, the differences between the groups were not significant ($p > 0.05$). Very similar data on somewhat lower weight gain during the first phase and improvement during the second one were provided by Radovanović et al. [17]. Favourable results in weight gain following the addition of EM to poultry rations were described by Das et Dach [16], Zhen Jun [6] and Julendra et Supadmoz [15].

On the whole, it can be concluded that EM supplied in broiler rations can secure weight gain identical to that of chickens fed diets with fish meal in compliance with NCR recommendations [35]. During the experiment no effects of anti-nutritive factors were observed in the third experimental group, which is in accordance with the data supplied by Julendra et Supadmoz [15] and Prayogi [11]. The average feed intake varied among the groups. Considering the experiment as a whole, in comparison to the control (average feed intake 124.62 g), the first (120.59 g) and the second experimental group (124.43 g), the third group had somewhat higher feed intake (127.23 g), as it was fed diets without fish meal, but supplemented with fresh earthworms. It was observed that the third group of broilers, willingly ingested fresh earthworms throughout the experiment. The analysis of the obtained results suggest that diets in which fish meal was replaced by EM, or feed without fish meal but with the addition of fresh earthworms, do not affect significantly feed consumption. These results are in accordance with the claims of Prayogi [11], Das et Dach [16] and Radovanović et al. [17] that earthworms, either fresh or in the form of meal, are a tasty feed to chickens.

Feed conversion, a measure of broilers' efficiency in converting feed into weight gain, is one of the best indicators of feed quality in the experiment. Feed conversion rate ranged from 2.07 to 2.22 kg. Differences in feed conversion were observed during all fattening periods, and the first group was the best if the results of the entire experiment were taken into consideration. However, the highest feed intake per kg of weight gain was observed in the third experimental group. The obtained results do not comply with the data on standardized feed conversion among the groups cited by Radovanović [17], but are in compliance with the results expected for Hybro broilers [37].

The production number offers much better insight into the effect of the applied treatment on the broilers productive results as a whole, as it incorporates all important production indicators. In this research the control group had a satisfactory production number, which did not differ significantly from all other groups, which justifies the replacement of fish meal with EM and its use in practice. These results confirmed some of the others published previously [6,11,15,16,17].

CONCLUSION

Having analysed the results of the experiment performed, conclusion can be drawn that raw earthworms consist of a high percentage of water and little nutritional elements.

Earthworm meal is characterised by 41.42% of proteins (DM) and satisfactory quantities of lysine (3.33% DM) and methionine (0.96% DM).

The fat content in earthworm meal (9.20% DM) is somewhat lower than in fish meal and is of a more desirable fatty-acid composition.

Considering the analysis of heavy metal contents and bacteriological assessment it can be concluded that both earthworm meal and raw earthworms (*Lumbricus rubellus*) are hygienic feed which can be given to broilers without negative effects on the health

Broilers readily consume raw biomass and, given the satisfactory contents of amino acids, the addition of raw earthworms to their feed in organic production is justifiable.

Having considered the absence of negative effects on production performance in broilers, their health, food conversion rate and meat quality, earthworm meal is a suitable substitute for fish meal in broiler chickens' diet.

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UTICAJ SUPSTITUCIJE RIBLJEG BRAŠNA BRAŠNOM KALIFORNIJSKE GLISTE (*Lumbricus rubellus*) NA PROIZVODNE REZULTATE I ZDRAVSTVENO STANJE BROJLERA

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Ispitivana je hranljiva vrednost glista (*Lumbricus rubellus*) i brašna dobijenog od njih, kao i uticaj ishrane smešama u kojima je izvršena zamena ribljeg brašna brašnom od glista ili svežim glistama, na zdravstveno stanje i proizvodne rezultate brojlera. Ogled izveden na četiri grupe brojlera *Hybro G* provenijence trajao je 42 dana. Brojleri kontrolne

grupe dobijali su hranu standardnog sirovinskog i hemijskog sastava. U prvoj i drugoj eksperimentalnoj grupi riblje brašno u smeši za ishranu bilo je zamenjeno brašnom od glista, i to u količini od 50% u prvoj i 100% u drugoj. Treća grupa hranjena je smešama iz kojih je potpuno isključeno riblje brašno, uz dodatak svežih glista *ad libitum*. Rezultati hemijskih analiza pokazali su da brašno od glista sadrži 41,42% proteina (računato na SM) i zadovoljavajuću količinu amino kiselina. Na osnovu ispitivanja sadržaja teških metala i bakterioloških analiza, utvrđeno je da brašno dehidrovanih glista i sveže gliste predstavljaju higijenski ispravno hranivo i da ne sadrži teške metale i štetne mikroorganizme. Analizom proizvodnih rezultata dobijenih u ogledu kao celini nije utvrđena statistički značajna razlika između grupa ($p > 0.05$). Dobijeni rezultati su pokazali da zamena ribljeg brašna u ishrani brojlera brašnom dobijenim od dehidrovanih glista predstavlja realnu mogućnost.