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Original Article

Growth Performance, Blood Metabolites and Jejunum Morphology of Broiler Chickens Fed Diets Containing Earthworm (*Eisenia foetida*) Meal as a Source of Protein

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ABSTRACT

Objective: The protein quality of earthworm meal was evaluated and compared to that of soybean meal (SBM) in two experiments using broiler chicks. **Methods:** The protein quality of earthworm meal was evaluated and compared to that of soybean meal (SBM) in two experiments using broiler chicks. **Results:** The results of experiment 1 indicated that there were no significant differences between earthworm meal and soybean meal in PER or NPR, but the breast muscle weight was increased in broilers fed earthworm meal diets, significantly ($p<0.05$). The birds fed with earthworm meal diets had a higher crude protein digestibility ($p<0.05$). In experiment 2, feed efficiency ratio was decreased in broilers fed diets supplemented with earthworm meal ($p<0.05$). The results of carcass characteristics showed that dietary treatments had a significant effect on breast percentage ($p<0.05$). The crypt depth of jejunum was higher in birds fed with maximum inclusion rate of earthworm meal ($p<0.05$). In experiment 2, the blood biochemical parameters of broilers were not affected by treatments. In regards with these results, it is suggested that use of earthworm meal in broiler diets may be suitable.

1.INTRODUCTION

Earthworms have been widely used in traditional medicine for a long time. However, in the past few decades with the development of biochemical technologies, the research on the pharmaceutical effects of earthworms has been studied for its use in veterinary and medical field. It has dense nutritional contents because of their soil based origin (Balamurugan et al., 2008). Also, earthworms have an important role in waste management by recovering organic materials that are transformed into vermicompost (Ignacio et al., 1993). The vermicompost is used as a biological soil biological fertilizer. The utilization of vermicompost has been increased in developing country at recent decades. In addition to direct use in agricultural and soil and plants fertilizing, it can also have byproducts. Earthworm meal is one of these byproducts which can be obtained in the production of vermicompost. In this way the appropriate volume of earthworms can be placed in a suitable temperature in the oven and after drying use as meal.

The earthworm *Eisenia foetida* belongs to the family of *lumbericidae* and genus *eisenia*, which in known by other names such as the tiger worm and flatworm. The food consumption and reproduction rate in these worms is high and have life, nutrition and reproduction ability in environments are rich in organic matter so that these

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worms are able to use the daily equivalent of half its weight of organic waste (fadaee, 2012).

Soybean meal is a conventional feedstuff which has been used as the main source of protein for poultry diets in Iran. There are some alternatives that can be used as a substitute for soybean meal in poultry diets such as fish meal and meat meal. Many scientists have reported the possible use of some alternative animal protein feedstuffs to fish meal such as earthworm meal (Edwards, 1998; Sogbesan and Ugwumba, 2006; Reinecke et al., 1991; Ignacio et al., 1993; Prayogi, 2011). Reinecke (1990) reported that worm meal has a potential as protein source in diets of broilers. In this regards, Ignacio et al. (1993) indicated that earthworm (*Eisenia foetida*) meal has 65.2% crude protein, approximately. In addition, earthworm meal is a good source of essential amino acids which are needed in broiler diets (Reinecke et al., 1993). Suitable compounds of fatty acids and essential amino acids and high amount of omega 3 in earthworm meal distinguished it from plant resources (fadaee, 2012). As a feed supplement, it has been found that earthworm is equal to or surpasses fish meal and meat meal as an animal protein source for poultry (Istiqomah, et al., 2009). Edwards (1985) reported that inclusion of earthworm meal improved feed conversion ratio and muscle tissue forming in broilers. But, there is a dearth of information about protein quality of earthworm (*Eiseina foetida*) meal and comparison of this supplement to soybean meal in broiler diets. So, the aim of this study was to determine the protein quality of earthworm meal as a protein supplement compare to soybean meal and its effects on growth performance, blood metabolites and jejunum morphology of broiler chickens.

2. MATERIALS AND METHODS

2.1. Experiment 1

The first experiment was conducted to evaluate the protein quality of earthworm meal using protein efficiency ratio and (PER) and net protein ratio (NPR) values. Earthworm (Eisenia foetida) meal was purchased from a local vermicompost company at the north part of Iran. Chemical composition of earthworm meal was determined in our nutrition laboratory, except for metabolizable energy and essential amino acids content. The earthworm meal samples were found to contain approximately 93.7% dry matter, 64.8% crude protein, 0.5% calcium and 0.81% phosphorous. The metabolizable energy and amino acids content of earthworm meal was extracted from the report of Sogbesan and Ugwumba (2008). A nitrogen-free diet was formulated with earthworm meal replacing the cornstarch/dextrose mixture to provide 0, 5 and 10% crude protein. Soybean meal also replaced cornstarch/dextrose in the nitrogen-free diet to provide 0, 5 and 10% crude protein, resulting in five dietary treatments (table 2). Diets 1 to 3 contained 0, 7.69 and 15.39% earthworm meal, respectively, while, diets 4 to 5 contained 11.1 and 22.23% soybean meal. The five dietary treatments were fed to four replicate groups of five male chicks from 7 to 18 d post-hatch. Individual chick weights and feeder weights were recorded at the beginning and end of the experimental period. Chick weight gain, feed intake, protein intake, PER, NPR and breast muscle weight was calculated on a per pen basis. Protein efficiency ratio (PER) and net protein ratio (NPR) were calculated by theses equations:

PER = weight gain/protein intake

NPR = (weight gain – weight gain of chicks fed N-free diet)/protein intake

Chromic oxide was added to all diets at a level of 3 gr/kg of diet as an indigestible marker at d 13. On day 18, the birds were killed by cervical dislocation and the small intestine was immediately exposed and contents of the lower half of ileum were expressed by gentle flushing with distilled water into plastic containers. The ileum was defined as that portion of the small intestine extending from the vitelline diverticulum to a point 35mm proximal to the ileo–caecal junction. Digesta were oven dried and ground to pass through a 0.5mm sieve and stored in airtight plastic containers at −4◦C for chemical analyses. Samples of diets and ileal digesta were analyzed for chromic oxide, dry matter and crude protein. Apparent ileal digestibility coefficients of dry matter and crude protein were calculated, using the ratio of these nutrients to chromic oxide in the diet and digesta. Also, the weight of breast muscle of broilers was recorded at the end of the first experiment.

2.2. Experiment 2

The second experiment was done to investigate the effects of different inclusion rates of earthworm (Eisenia foetida) meal on growth performance, blood parameters, jejunum morphology and carcass characteristics of broiler chickens. A total of one hundred and eighty ROSS broiler chickens (male and female) were obtained from a local hatchery and allotted to 3 treatments with 4 replicates per each. Maize-soybean meal based diets (for the starter and grower periods) were formulated to meet or exceed the nutrient requirement for chickens recommended by the National Research Council (1994). The experiment was carried out in a completely randomized design with 3 treatments, including 3 levels of earthworm meal (0, 3 and 6% for starter and 0, 2 and 4% for grower). The ingredients and chemical

composition of dietary treatments are shown in table 3. Feed intake and body weight gain of each pen was measured at the end of each week. Feed conversion ratio for each pen was calculated by dividing feed intake by body weight gain. Mortality was recorded daily and feed consumption data were corrected for body weight of mortality. Two birds per replicate were bled through the wing vein at 35 d of age to determine the blood biochemical parameters. The blood sample (3 mL) was drawn and allowed to clot at room temperature (18 ºC) for 2 h prior to serum collection. Serum was separated by centrifugation and stored at -20 ºC for further analysis. Serum concentrations of glucose, low-density lipoproteins (LDL), triglycerides (TG) and cholesterol were determined using commercial kits. Also, sera samples were used to measure the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as the indicators of liver health. At 42 days of age, eight randomly selected birds from each treatment were weighed and sacrificed. The weight of intestinal tract (after digesta removal), breast, thigh, liver (without gallbladder), heart and pancreas were recorded and their weights (%) relative to body weight were measured.

Segment of jejunum was excised for morphological evaluation. The intestinal segment was flushed clean with PBS (phosphate buffered saline) to avoid damage to the tissues. Then, samples were fixed in Clark solution for one hour. Samples were then transferred into 50% ethanol solution. A 0.5-cm section was then processed and embedded in paraffin, stained with eosin blue and examined with a light microscope. The fifteen longest and straightest villi and associated crypts were measured in each segment. Statistical analysis was carried out using general linear model (GLM Procedure) to evaluate the effects of treatments on protein quality and nutrient digestibility in experiment 1 and performance, carcass characteristics, intestinal morphology and blood metabolites of broilers in experiment 2 using SAS (1990) software. Pen was the experimental unit. Statistical significance of differences among treatments was done using the Duncan's multiple range test at ($P < 0.05$).

3. RESULTS

3.1. Experiment 1

Data on the earthworm meal protein quality, breast muscle weight and nutrient digestibility of broilers are shown in table 4. The results showed that weight gain, breast muscle weight and protein intake increased in broilers fed diets with 10% earthworm meal and soybean meal ($P<0.05$). However, protein efficiency ratio (PER) and net protein ratio (NPR) were not influenced by dietary treatments ($P>0.05$).

3.2. Experiment 2

Effects of dietary treatments on growth performance and carcass traits of broiler chickens are presented in table 5. The results indicated that feed conversion ratio of broilers was improved by adding earthworm meal in diets at grower (21-42 d) and total (1-42 d) period ($P<0.05$). Feed intake and weight gain of broilers were not statistically affected by dietary treatment ($P>0.05$). The results of table 5 showed that except for breast muscle weight, the other carcass traits were not influenced by adding earthworm meal in broiler diets ($P>0.05$). In this regard, the breast muscle weight increased in broilers fed diets with highest inclusion rate of earthworm meal in diets. The results of blood metabolites and jejunum morphology of broilers are shown in table 6. The results showed that in contrast with villi width and height, crypt depth of jejunum was statistically affected by dietary treatments ($P<0.05$). The birds fed diets containing high levels of earthworm meal had the biggest jejunum crypt depth. The results of table 6 indicated that supplemental earthworm meal did not affect all blood biochemical parameters in broilers ($P>0.05$).

4. DISCUSSION

In general, the nutritional quality of proteins depends on the proportion and availability of the amino acid that they contain. It is cleared that a high quality protein with balanced amino acid will promote more weight gain per unit of protein consumed than low quality protein. Although present analytical methods approximate the amino acid content of proteins, they reveal very little information concerning their bioavailability. Therefore growth assay is needed for comparing relative values of various proteins based on qualitative growth response of the chicks. The protein efficiency ratio (PER) and net protein ratio (NPR) have been previously examined to assess different sources of protein in chicks. Ignacio et al. (1993) reported that there is a relative deficiency in tryptophan and sulfur amino acids in earthworm (*Eisenia foetida*) meal. However, they found that the biological indices of PER and NPR for earthworm meal and casein in rats were not statistically significant. Taboga (1980) fed broilers (1 d to 8 weeks) with protein-free diet supplemented with earthworm and found that the growth rate of experimental chickens was not significantly different from that of control birds fed on a complete grower diet. Nguyen and Ulfert, (2009) indicated that fish fed on diets containing earthworm powder had similar or higher growth rate, protein efficiency and energy retention than the control group. The net protein utilization (NPU) of broilers fed diets containing three species of earthworm meals were equal to fish meal and broiler starter diets (Reinecke et al., 1991). Also, in this report concluded that earthworm meal was better than plant protein supplements in broiler diets. In this regard, Taboga (1980) indicated that

the amino acid composition of earthworm was closed to amino acid requirements of chickens. The results of PER and NPR were in accordance with the weight beast muscle and nutrient digestibility data in the present study. Briefly, nutrient digestibility and breast muscle weight was the highest in birds that had the better protein quality values. Data about nutrient digestibility of earthworm meal in broilers was not found in the literature review. So, the direct comparison was not performed.

The results of growth performance are in accordance with other studies (Julendra et al., 2012; Reinecke et al., 1991). It is revealed that the earthworm (*Eisenia foetida*) meal had no anti nutrient factor which decline growth performance of broilers (Reinecke et al., 1991). In this regard, Prayogi (2011) reported that weight gain and feed conversion ratio was improved in quails fed diets supplemented with 10% earthworm meal. Also, it is indicated that growth performance of broilers fed diets containing earthworm meal was equal to those fed fish meal diets (Reinecke et al., 1991). In contrast, Supplementation of earthworm meal in feed until 5% level has no significant effect on broiler body weight (Resnawati, 2004). Popovic et al. (2005) reported that earthworm (*Eisenia foetida*) has glycolipoprotein (G-90), a mixture of homogeneous tissue containing antibacterial activity against *Staphylococcus* sp. higher than commercial antibiotics such as gentamicin and enrofloxacin-5. Earthworm (*E. foetida*) also has lumbricin I which contain antimicrobial activity (Liu et al., 2004). So, Antibiotic characteristics of earthworm meal could thin the intestinal mucosa and increase the nutrient absorption and feed efficiency. In addition, Julendra et al. (2012) indicted that improvement of body weight gain in broilers fed diets containing earthworm meal may be due to the antibacterial characteristics of earthworm meal. Previous earthworm studies have shown its antimicrobial and anticancer (Cooper et al., 2004), and hepatoprotective (Balamurugan et al., 2008) characteristics. The anti-inflammatory activity together with antioxidant properties seems to be due to the high polyphenolic content in earthworm tissues (Cooper et al., 2004). The transaminases (ALT and AST) are useful enzymes as biomarkers predicting possible toxicity. Any damage to the parenchymal liver cells will results in elevations in both these transaminases. Our study was in accordance with Anitha and Jayraaj (2012) who reported that no significant changes observed in the hematological indices (HB, RBC and WBC), hepatic serum markers (AST, ALP, ALT and protein) in the wistar male rats treated orally with earthworm (*Eudrillus euginae*) powder. In contrast, the dried earthworm powder when administered orally to rats and human beings has an antihyperlipemic effect, a blood sugar lowering effect and a blood pressure regulating effect (Adil ansari and Sitaram, 2010). However, the reason for this has not been fully understood. In spite of that, Mihara et al. (1996) indicated that the effects of earthworm powder on blood metabolites may be due to the action of proteolytic enzymes contained in the dried earthworm powder, precursors of these enzymes, other proteins, lipids or unknown compounds. Balamurugan et al., 2008 studied the hepatoprotective effects of earthworm extract in rats and reported that earthworm extract prevents the formation of the reactive oxygen groups or scavenges these groups, and simultaneously modulates the genes responsible for synthesis of antioxidant enzymes in liver tissue. In regard with other blood metabolites (glucose, cholesterol and triglycerides) no reports was found. So, direct comparison was not made. The efficiency of utilization of dietary nutrients partly depends on the development of the gastrointestinal tract. Such development can be assessed through measurements of the crypt, a region in which new intestinal cells are formed; villus height and surface area, to determine the area available for digestion/absorption, and the activities of membrane-bound digestive enzymes of the small intestine. The role of protein quality and amino acid profile of diets on broilers intestinal morphology have been studied by some researchers. It is cleared that glutamine, glutamic acid (Ebadiasl, 2011) and threonine (Rezaeipour et al, 2012) had an important role in intestine histology of broilers. Ignacio et al. (1993) indicated that earthworm meal was a good source of glutamic acid (glutamate) for broilers. Glutamine can be produced by the combination of an amino group and glutamate by the action of Glutamine synthetase. Soltan (2009) observed that broilers fed diet containing glutamine had significantly longer villi in duodenum and jejunum compared to the control group. Interestingly, Murakami et al. (2007) showed that on day 14 post-hatch, broilers fed a diet supplemented with 10 mg vitamin E along with 1% glutamine had longer villi and deeper crypts in duodenum and deeper crypts in jejunum than broilers fed a diet containing vitamin E without glutamine. So, increasing of crypt depth in this study may be due to the effect of the content of glutamic acid in earthworm meal. There is a dearth of reports linking the effects of earthworm (*Eisenia foetida*) meal on gastrointestinal morphology of broilers.

CONCLUSION

In conclusion, the earthworm powder having high protein content along with the antimicrobial and antioxidant could be used as a feed supplement in broiler diets.

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Table 1.

The composition of experimental diets containing earthworm meal and soybean meal (Experiment 1)

Ingredients	N-free diet	5% CP	10% CP	5% CP	10% CP
Soybean meal	-	11.10	22.23	-	-
Earthworm meal	-	-	-	7.69	15.39
Dextrose	59.77	46.50	33.53	53.65	46.05
Cornstarch	35.00	34.60	34.00	33.66	33.78
Soybean oil	-	2.75	5.30	-	0.05
DCP	2.50	2.30	2.20	2.20	1.83
Salt	0.50	0.50	0.50	0.50	0.50
Vit-premix[1]	0.50	0.50	0.50	0.50	0.50
Min-premix[1]	0.50	0.50	0.50	0.50	0.50
Oyster shell	1.23	1.25	1.24	1.30	1.40
Chemical composition					
AME (kcal/kg)	3450	3450	3450	3450	3450
CP (%)	-	5	10	5	10

[1]Provides per kg of diet: 9000 I.U. vitamin A; 2000 I.U. vitamin D3; 18 I.U. vitamin E; 2 mg menadion; 1.8 mg thiamine; 6.6 mg riboflavin; 30 mg niacin; 3 mg pyridoxine; 15 mg vitamin B12; 100 mg D-pantothenic acid; 1 mg folic acid; 0.1 mg biotin; 500 mg choline chloride; 100 mg antioxidant; 100 mg manganese; 84.7 mg zinc; 50 mg iron; 10 mg copper; 1 mg iodine; 0.2 mg selenium.

Table 2.

Ingredients and chemical composition of experimental diets (Experiment 2)

Ingredients (%)	Starter (1-21d)			Grower (21-42d)		
Corn	60.00	60.41	61.27	64.47	64.64	64.53
Earthworm meal	-	3.00	6.00	-	2.00	4.00
Wheat bran	1.00	2.62	4.00	1.00	2.20	3.72
Soybean meal	33.10	28.18	23.21	28.00	24.72	21.35
Soybean oil	2.00	1.67	1.20	3.00	2.80	2.65
Oyster shell	1.44	1.48	1.53	1.44	1.46	1.51
DCP	1.36	1.30	1.20	1.11	1.05	0.95
Salt	0.43	0.43	0.43	0.33	0.33	0.33
Vit-premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Min-premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met	0.17	0.23	0.30	0.08	0.12	0.16
L-Lys	-	0.12	0.25	-	0.08	0.16
L-Thr	-	0.06	0.11	0.02	0.05	0.09
Anticoccidial	-	-	-	0.05	0.05	0.05
AME (kcal/kg)	3000	3000	3000	3100	3100	3100
CP	21.59	21.59	21.59	19.46	19.46	19.46
Ca	0.95	0.95	0.95	0.87	0.87	0.87
AP	0.40	0.40	0.40	0.34	0.34	0.34
Na	0.19	0.19	0.19	0.15	0.15	0.15
Met+Cys	0.85	0.85	0.85	0.70	0.70	0.70
Lys	1.14	1.14	1.14	1.00	1.00	1.00
Thr	0.80	0.80	0.80	0.73	0.73	0.73

[1]Provides per kg of diet: 9000 I.U. vitamin A; 2000 I.U. vitamin D3; 18 I.U. vitamin E; 2 mg menadion; 1.8 mg thiamine; 6.6 mg riboflavin; 30 mg niacin; 3 mg pyridoxine; 15 mg vitamin B12; 100 mg D-pantothenic acid; 1 mg folic acid; 0.1 mg biotin; 500 mg choline chloride; 100 mg antioxidant; 100 mg manganese; 84.7 mg zinc; 50 mg iron; 10 mg copper; 1 mg iodine; 0.2 mg selenium.

Table 3.

The effects of dietary treatments on growth performance (g/bird) and nutrient digestibility (%) in broiler chicks (Experiment 1)

	Dietary treatments					
Traits	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Weight gain	-215^{c}	200^{b}	548^{a}	226^{b}	560^{a}	30.91
Protein intake	-	100.8^{b}	213.4^{a}	88.9^{b}	199.8^{a}	8.51
Breast weight	23.18^{c}	33.10^{b}	40.63^{a}	34.02^{b}	42.50^{a}	1.21
PER	-	2.07	2.59	2.54	2.80	0.25
NPR	-	4.14	5.19	5.08	5.20	0.50
Nutrient digestibility						
Dry matter	-	78.37^{ab}	76.42^{b}	77.86^{ab}	79.80^{a}	0.61
Crude protein	-	74.53^{c}	77.32^{b}	79.43^{ab}	80.58^{a}	0.75

Means within rows followed by different superscript are significantly different (P<0.05).

T1:N-free diet, T2: earthworm meal (5%), T3: earthworm meal (10%), T4: soybean meal (5%) and T5: soybean meal (10%).

Table 4.

Growth performance (g/d) of broiler chickens fed diets containing earthworm meal

Traits	T1	T2	T3	SEM
1-21d				
Weight gain	31.04	30.27	29.57	1.05
Feed intake	51.74	49.28	47.19	1.64
FCR	1.67	1.63	1.60	0.02
21-42d				
Weight gain	62.05	68.16	67.34	2.23
Feed intake	137.34	146.01	135.38	3.31
FCR	2.22[a]	2.14[ab]	2.01[b]	0.05
1-42d				
Weight gain	46.54	49.22	48.43	1.87
Feed intake	94.55	97.65	91.28	2.95
FCR	2.03[a]	1.98[ab]	1.88[b]	0.03

Means within rows followed by different superscript are significantly different (P<0.05).

T1: 0% earthworm meal, T2: starter and grower diets with 3 and 2% earthworm meal and T3: starter and grower diets with 6 and 4% earthworm meal.

Table 5.

The effects of diets containing earthworm meal on carcass characteristics (%) of broiler chickens

Traits	T1	T2	T3	SEM
Breast	21.41[ab]	20.51[b]	23.04[a]	0.71
Thigh	18.43	18.37	18.54	0.63
Heart	0.51	0.46	0.44	0.23
Liver	2.38	2.25	2.08	0.20
Pancreas	0.25	0.23	0.22	0.01
Intestine	4.10	3.77	3.68	0.27

Means within rows followed by different superscript are significantly different (P<0.05).

T1: 0% earthworm meal, T2: starter and grower diets with 3 and 2% earthworm meal and T3: starter and grower diets with 6 and 4% earthworm meal.

Table 6.

The effects of diets containing earthworm meal jejunum morphology and blood biochemical parameters of broiler chickens

Traits	T1	T2	T3	SEM
Jejunum morphology				
Villi height (µm)	1155	1168	1178	7.17
Villi width (µm)	135.0	137.7	147.5	5.33
Crypt depth (µm)	182.2[b]	186.5[b]	202.5[a]	4.65
Blood metabolites				
Glucose (mg/dl)	229.0	231.1	222.2	9.89
Cholesterol (mg/dl)	141.2	131.3	138.2	7.90
Triglycerides (mg/dl)	137.5	151.1	153.0	8.61
ALT (IU/L)	4.2	4.07	3.98	0.67
AST (IU/L)	280.4	291.3	286.7	12.51

Means within rows followed by different superscript are significantly different (P<0.05).

T1: 0% earthworm meal, T2: starter and grower diets with 3 and 2% earthworm meal and T3: starter and grower diets with 6 and 4% earthworm meal.