The Effect of Replacing Dried Meal Worm With Soybean Meal on Growth Performance and Carcass Characteristics of Broiler Chickens

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Abstract

An experiment was conducted on 240 one-day old male (Ross308) chickens to the effect of substitution of dried mealworm (Tenebrio molitor) with soybean meal on growth performance and carcass characteristics broiler chicks. Chicks fed in a completely randomized design with 4 replicate cages (12 birds per cage). Five dietary treatments used: 1- Control treatment: Basal diet based on corn-soybean meal without adding dried mealworm, 2- Replacement of 5% dried mealworm with soybean meal in the base diet, 3- Replacement of 10% dried mealworm with soybean meal in the base diet, 4- Replacement of 15% dried mealworm with soybean meal in the base diet and 5- Replacement of 20% dried mealworm with soybean meal in the base diet. The results of the experiment showed that 3rd and 4th treatments with a substitution of 10 and 15 percent showed a higher mean weight gain and a daily gain, but their feed intake was lower comparing other treatments, and finally, the feed conversion ratio showed a significant decrease with respect to control treatment using these replacement levels. At 21 and 42 days of age, carcass yield and relative weight of the breasts showed significant increases in treatments 3 and 4, and other characteristics of chicken carcasses were not affected by the test treatments. The experiment showed that replacing soybean meal with 10% and 15% worm powder significantly improved the performance of broiler chickens, but no significant difference was found between the two levels.

Keywords: Broiler chickens; Carcass characteristics; Dried mealworm; Soybean meal

Introduction

The International Feed Industry Federation (IFIF) announced that the world population maybe increased to more than 10 billion people by the year 2050 [1]. Moreover, it is expected that consumption of animal protein will be doubled for mentioned population [1]. For poultry meat, the expected growth of consumption from 2010 to 2050 is 173% [1]. Dietary sources of protein are one of the essential nutrients in poultry nutrition. These resources, in addition to being an important part of the poultry diet, are also considered the most expensive part of the diet [2]. Oily meal is the most important source among plant protein origin. Currently, the Soybean meal is the most important oily meal that is used in the formulation of diets of broilers and laying hens which is due to the quantity and quality of its protein as well as the appropriate amino acid composition of this meal [3]. However, in Asia, the shortage of availability of soybean meal for livestock and poultry feed is as the result of the significant amount of soybeans consumed as human food and the low soybean production capacity in the tropics due to insufficient agricultural facilities and
lack of suitable crops [4]. According to that, the most of the costs in the poultry industry are related to feed, it is important to try to find a suitable alternative to soybean meal [3]. Insects are the third generation of proteins in the world. More than 10 million of insect species have been identified, which account for a half of all creatures in earth [5]. Among of them, approximately 1,500 species of insects are known to be an edible protein source for humans and animals [5]. In between, Mealworm (Tenebrio molitor), is a good food source for small mammals and poultry. Nutritional value of mealworms varies depending on species and developmental stage. These worms are rich in protein, fats, essential aminoacids, and vitamins or minerals [6]. Insects, in general, contain a higher amount in lysine and threonine which is deficient in most commonly used cereal as wheat, rice, cassava and maize but have the lower amount of amino acids, methionine and cysteine [7]. The small size of the worms is ideal for small reptiles and young birds as a dietary supplement [8]. Because these worms feed on bran, barley, soy, are the best food supplements for raising chickens, turkeys, and quails and are the result of bird growth and production of completely natural and quality meat [9-11]. Some authors evaluated the effects of Tenebrio molitory (TM) meal utilization on growth performance, haematochemical profile [10], and carcass traits of broilers, while others focused their researches also on the evaluation of intestinal morphology and histological features in free-range chickens fed diets including TM meal. The economic production parameters of mealworms include their ability to convert organic matter into protein more efficiently compared with their animal counterparts, less space and water needed [12], and the lower environmental impact of insect-based products compared with animal-based products [13]; have a nutrient profile similar to fish meat, so they can contribute to more balanced human and animal diets convert to try to find a suitable alternative to soybean meal [3]. Each group was fed for 42 days with isocaloric and iso-nitrogenous diets (Table 1) that were formulated based on standard recommendation. Five dietary treatments used: 1- Control treatment: Basal diet based on corn-soybean meal without adding dried mealworm, 2- Replacement of 5% dried mealworm with soybean meal in the base diet, 3- Replacement of 10% dried mealworm with soybean meal in the base diet, 4- Replacement of 15% dried mealworm with soybean meal in the base diet and 5- Replacement of 20% dried mealworm with soybean meal in the base diet. Nutritional value in 100 g of dry mealworm, amino acids and fatty acids composition is shown in tables 2 and 3, respectively [16]. Birds were provided with known amounts of feed ad libitum. The chicks had free access to water. Birds were maintained at a brooding temperature of 32 °C for 5 day and then the environmental temperature was gradually reduced to 22 °C. During the experiment, light was provided continuously (24 hours). Routine vaccination and health care was given when it was necessary. All animal experiments were approved by the state committee on animal ethics, Karaj Branch, Islamic Azad University, Karaj, Iran (IACUC no:4687; 4/10/2018). The recommendations of the European Council Directive (2010/63/EU) of September 22, 2010, regarding the standards in the protection of animals used for experimental purposes were also followed.

Notes: Mineral mix supplied/kg diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg. Vitamins mix supplied/kg diet: vitamin A, 18,000 IU; vitamin D3, 4000 IU; vitamin E, 36 mg; vitamin K3, 4 mg; vitamin B12, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg.

Sample collection and measurements
The chickens in each pen were also weighed at the beginning of the experiment and tried to keep the average of each pen almost close to each other. At the end of each week of the trial, after 2 hours of starving, the chickens were weighed using a digital scale with an accuracy of 0.01 g. Then, the average body weight of a bird was calculated according to the number of birds in each unit. To calculate weight gain at each time point, the difference in body weight at the end and beginning of the period was determined. By dividing the weight by 7, the daily average weight of each bird was calculated according to the number of birds in each unit. To calculate weight gain at each time point, the difference in body weight at the end and beginning of the period was determined. By dividing the weight by 7, the daily weight gain (DGW) was calculated. Feed intake (FI) was calculated from the difference between feed given during the week and return feed. Feed conversion ratio (FCR) was calculated by dividing feed consumption by body weight gain. Two birds from each pen were randomly selected for estimation of carcass characteristics on day 21 and 42 of the trial. Chickens slaughtered by displacing the

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Table 1: Ingredients and chemical composition of used diets at (1-21 d and 22-42d).

| Experimental group | Control | 5% mealworm | 10% mealworm | 15% mealworm | 20% mealworm |
|--------------------|---------|-------------|--------------|--------------|--------------|
| 1-21 d             | 22-42 d | 1-21 d      | 22-42 d      | 1-21 d      | 22-42 d      |
| Ingredients (g/100g as-fed) |         |             |              |              |              |
| Corn               | 66.25   | 68.73       | 66.25        | 68.73        | 66.25        |
| Soybean meal       | 32.5    | 30          | 30.88        | 28.5         |              |
| Mealworm           | 0       | 0           | 1.62         | 1.5          |              |
| Dicalcium phosphate| 0.13    | 0.12        | 0.11         | 0.11         |              |
| Shell              | 0.18    | 0.18        | 0.18         | 0.18         |              |
| Lysine             | 0.1     | 0.1         | 0.1          | 0.1          |              |
| Methionine         | 0.18    | 0.18        | 0.18         | 0.18         |              |
| Vitamin mineral premix¹ | 0.5  | 0.5         | 0.5          | 0.5          |              |
| Na chloride        | 0.2     | 0.2         | 0.2          | 0.2          |              |
| Calculated analysis|         |             |              |              |              |
| Metabolizable energy (MJ/kg) | 3000   | 3100        | 3000         | 3100         | 3000         |
| Available phosphorus, g/kg | 5     | 4.5         | 5            | 4.5          | 5            |
| Lysine, g/kg       | 11      | 10.3        | 11           | 10.3         | 11           |
| Methionine, g/kg   | 3.5     | 3.65        | 3.65         | 3.65         | 3.65         |
| Analysed composition (g/kg) |       |             |              |              |              |
| Crude protein (N × 6.25) | 220   | 190         | 220          | 190          | 220          |
| Calcium            | 10.2    | 8.45        | 10.2         | 8.45         | 10.2         |
| Fiber              | 38.2    | 36.5        | 38.2         | 36.5         | 38.2         |
| Notes: Mineral mix supplied/kg diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg. Vitamins mix supplied/kg diet: vitamin A, 18,000 IU; vitamin D3, 4000 IU; vitamin E, 36 mg; vitamin K3, 4 mg; vitamin B12, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg.

Table 2: Nutritional value in 100 g dry mealworm

| Ingredients   | 0 (%) |
|---------------|-------|
| Moisture      | 5     |
| Protein       | 50    |
| Fat           | 28    |
| Fiber         | 6     |
| Carbohydrate  | 1.73  |
| Fe            | 2.06  |

Results

The results of the WG and DWG were shown in the tables 4 and 5. During The first weeks of testing, only the replacement of 10% and 15% of soybean meal with mealworm significantly (P<0.05) improved the WG and DWG gain of the tested chickens and Levels of 5 and 20% replacement did not have a significant effect on the WG and DWG gain (P>0.05). But in the other weeks of testing Replacement of 5% and 20% of soybean meal mealworm compared to the control diet caused a significant increase in the WG and DWG gain. But compared to the treatments where the replacement level was 10% and 15%, this increase was less. The results showed that replacement of 10% and 15% of soybean meal mealworm compared to control diet, improved (P<0.05) the WG and DWG gain. Due to the price difference between these two protein sources, it can have a significant effect on the cost of the ration. But no significant difference between the two alternative levels of 10% and 15% was observed (P>0.05). The results related to the average FI were shown in the table 6. Results showed that the highest FI numerically was observed in chickens fed with control treatment. Replacement of mealworm with soybean meal in the diet only at the level of 15% significantly decreased daily FI (P<0.05). Other replacement levels, however, numerically
reduced FI but the difference with control treatment was not significant (P <0.05). The results related to FCR were shown in the table 7. In the third week of the experiment, the highest FCR numerically was observed in the control treatment but the difference was significant with treatments with replacement of 15 and 10% of mealworm with soybean meal (P <0.05). In the fourth week, control and treatment 4 caused the highest and lowest FCR, respectively, and their differences with other experimental treatments were significant (P <0.05). At the fifth and sixth weeks of the experiment, the control treatment showed the highest FCR and its difference with other experimental treatments was significant (P <0.05). Treatment 4 with a replacement level of 15% also showed the lowest FCR. The difference with other experimental treatments except treatment 3 was significant (P <0.05). The results showed that level of replacement of 15% mealworm with soybean meal could improve performance in broilers and reduced the FCR. The results related to Carcass characteristics at 21 and 42 days were shown in the tables 8 and 9. At 21 and 42d, experimental diets had significant effect on carcass yield (P <0.05). Treatments 3 and 4 had higher carcass yields and their differences with other treatments were significant (P <0.05). Between experimental treatments in terms of ratio of thigh weight to live weight was not observed significant difference (P <0.05). The ratio of breast weight to live weight was affected by experimental treatments. Treatments 3 and 4 had higher relative breast weights compared to other treatments and their difference with other treatments was significant (P <0.05). at 21 and 42d the effect of experimental treatments on the relative weight of liver, spleen, heart, pancreas, ventricular adipose tissue and bursa of fabricius was not significant (P <0.05).

Discussion

High amount of protein, fats, amino acids and mineral content were found in T. molitor; consequently, it can easily be used in the poultry ration [18]. The existence of chitin in insects’ meal has the potential to improve poultry health as the result of decreasing populations of intestinal Escherichia coli and Salmonella spp., beside it could increase intestinal Lactobacillus spp. The larvae of a darkling beetle are also rich in fiber and fatty acids; thus, it improves digestion [19]. It can be used as an alternative to antibiotics in the poultry

Table 3: Composition of mealworm amino acids and fatty acids

| Item          | %   |
|---------------|-----|
| Aspartic acid | 3.07|
| Serine        | 1.86|
| Glutamic acid | 4.77|
| Glycine       | 2.04|
| Alanine       | 3.15|
| Valine        | 3.14|
| Isoleucine    | 1.39|
| Tyroine       | 2.65|
| Phenylalanine | 1.56|
| Lysine        | 1.56|
| Histamine     | 1.07|
| Arginine      | 2.03|
| Methionine    | 0.54|
| Cysteine      | 0.35|
| Total         | 35.67|

| Fatty acids composition | %   |
|-------------------------|-----|
| C14                     | 2.85|
| C15                     | 7.1 |
| C16                     | 9.33|
| C16:1                   | 2.12|
| C18                     | 2.4 |
| C18 1n9                 | 40.78|
| C18 12n6                | 33.58|
| C18 3n6                 | 1.85|
| Total                   | 100 |

Table 4: Effect of experimental diets on live weight of broiler chickens (g)

| Age (d) | 1    | 7    | 14   | 21   | 28   | 35   | 42   |
|---------|------|------|------|------|------|------|------|
| Experimental diet | Live weight (g) |
| 1       | 41.20* | 102.54* | 235.25* | 585.60* | 868.52* | 1303.50* | 1902.21* |
| 2       | 41.35* | 103.32* | 238.78* | 597.20* | 893.03* | 1345.82* | 1938.09* |
| 3       | 42.06* | 109.58* | 250.08* | 607.21* | 913.76* | 1363.86* | 1998.93* |
| 4       | 41.65* | 108.23* | 249.68* | 610.05* | 921.36* | 1370.14* | 2010.07* |
| 5       | 42.19* | 103.05* | 239.43* | 597.96* | 900.74* | 1335.24* | 1959.08* |
| ± SEM* | 0.02  | 1.05  | 2.15  | 6.45  | 7.28  | 12.07 | 17.09 |

1: 1- Control treatment: Basal diet based on corn-soybean meal without adding dried mealworm, 2- Replacement of 5% dried mealworm with Soy meal in the base diet, 3- Replacement of 10% dried mealworm with Soy meal in the base diet, 4- Replacement of 15 dried mealworm with Soy meal in the base diet and 5- Replacement of 20% dried mealworm with Soy meal in the base diet.

2: Mean error standard

Column means with common superscripts do not differ (P > 0.05).

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Table 5: Effect of experimental diets on daily weight gain of broiler chickens (g/d)

| Age (d) | Experimental diet | Daily weight gain (g/d) |
|---------|-------------------|------------------------|
|         | 1-7               | 8-14                   | 15-21                  | 22-28                  | 29-35                  | 36-42                  |
| 11      | 9.78 a            | 19.04 b                | 40.23 b                | 39.34 c                | 58.45 c                | 67.28 c                |
| 2       | 9.83 a            | 20.57 b                | 43.02 b                | 41.95 b                | 61.72 b                | 71.94 b                |
| 3       | 10.02 b           | 24.02 b                | 46.86 b                | 44.29 b                | 65.34 b                | 75.14 a                |
| 4       | 10.08 a           | 23.96 a                | 45.95 a                | 46.88 a                | 66.08 a                | 77.09 a                |
| 5       | 9.90 a            | 20.99 b                | 42.94 b                | 41.85 b                | 62.01 b                | 71.61 b                |

± SEM

P-value

NS 0.0018 0.0001 0.0188 0.024 0.0023

1: Control treatment: Basal diet based on corn-soybean meal without adding dried mealworm, 2- Replacement of 5% dried mealworm with Soy meal in the base diet, 3- Replacement of 10% dried mealworm with Soy meal in the base diet, 4- Replacement of 15% dried mealworm with Soy meal in the base diet, 5- Replacement of 20% dried mealworm with Soy meal in the base diet.

2: Mean error standard

Column means with common superscripts do not differ (P > .05).

Table 6: Effect of experimental diets on feed intake of broiler chickens (g/d)

| Age (d) | Experimental diet | Feed intake (g/d) |
|---------|-------------------|-------------------|
|         | 1-7               | 8-14              | 15-21            | 22-28            | 29-35            | 36-42            |
| 11      | 16.28 a           | 52.43 a           | 79.86 a          | 92.02 a          | 122.12 a         | 152.24 a         |
| 2       | 16.49 a           | 51.03 a           | 79.06 a          | 90.57 ab         | 120.02 a         | 150.62 a         |
| 3       | 16.37 a           | 48.04 b           | 78.53 c          | 89.62 a          | 119.43 ab        | 149.32 b         |
| 4       | 16.50 a           | 48.97 b           | 78.73 c          | 88.72 a          | 118.97 b         | 148.25 b         |
| 5       | 16.31 a           | 51.04 a           | 79.94 b          | 90.79 ab         | 121.71 b         | 149.25 b         |

± SEM

P-value

0.0001 0.0001 0.0016 0.001 0.0012 0.0001

1: Control treatment: Basal diet based on corn-soybean meal without adding dried mealworm, 2- Replacement of 5% dried mealworm with Soy meal in the base diet, 3- Replacement of 10% dried mealworm with Soy meal in the base diet, 4- Replacement of 15% dried mealworm with Soy meal in the base diet, 5- Replacement of 20% dried mealworm with Soy meal in the base diet.

2: Mean error standard

Column means with common superscripts do not differ (P > 0.05).

Table 7: Effect of experimental diets on feed: gain of broiler chickens.

| Age (d) | Experimental diet | Feed: gain |
|---------|-------------------|------------|
|         | 1-7               | 8-14       | 15-21      | 22-28      | 29-35      | 36-42      |
| 11      | 1.75 a            | 1.70 a     | 1.76 a     | 1.97 a     | 2.11 a     | 2.12 a     |
| 2       | 1.72 a            | 1.67 a     | 1.72 a     | 1.86 a     | 1.96 b     | 1.97 a     |
| 3       | 1.74 a            | 1.69 a     | 1.54 b     | 1.83 a     | 1.86 c     | 1.92 c     |
| 4       | 1.80 a            | 1.71 a     | 1.50 a     | 1.72 a     | 1.82 c     | 1.79 a     |
| 5       | 1.71 a            | 1.68 a     | 1.74 a     | 1.84 a     | 1.87 b     | 1.98 b     |

± SEM

P-value

NS NS 0.0001 0.0002 0.003 0.0001

1: Control treatment: Basal diet based on corn-soybean meal without adding dried mealworm, 2- Replacement of 5% dried mealworm with Soy meal in the base diet, 3- Replacement of 10% dried mealworm with Soy meal in the base diet, 4- Replacement of 15% dried mealworm with Soy meal in the base diet, 5- Replacement of 20% dried mealworm with Soy meal in the base diet.

2: Mean error standard

Column means with common superscripts do not differ (P > 0.05).
Table 8: Effect of experimental diets on carcass and some organs relative to the body weight of Ross 308 broilers at 21 days of age.

| Experimental diets | Carcass yield | Thighs | Breast | Liver | Heart | Spleen | Pancreas | Abdominal fat | Fabricius |
|--------------------|---------------|--------|--------|-------|-------|--------|----------|---------------|-----------|
| 11                 | 63.24<sup>c</sup> | 27.14  | 28.24<sup>c</sup> | 3.12  | 0.67  | 0.1    | 0.25     | 1.32          | 0.27      |
| 2                  | 65.37<sup>b</sup> | 28.52  | 28.02<sup>b</sup> | 2.25  | 0.65  | 0.11   | 0.32     | 1.52          | 0.26      |
| 3                  | 66.53<sup>a</sup> | 29.46  | 30.12<sup>a</sup> | 3.36  | 0.63  | 0.1    | 0.3      | 1.13          | 0.25      |
| 4                  | 66.86<sup>a</sup> | 28.03  | 30.28<sup>a</sup> | 3.16  | 0.65  | 0.09   | 0.29     | 1.72          | 0.26      |
| 5                  | 64.92<sup>b</sup> | 27.89  | 28.68<sup>b</sup> | 3.2   | 0.68  | 0.08   | 0.27     | 1.54          | 0.23      |
| ± SEM<sup>2</sup>  | 0.42          | 0.53   | 0.76   | 0.05  | 0.03  | 0.04   | 0.15     | 0.08          | 0.03      |

P value: 0.0001 NS 0.0012 NS NS NS NS NS NS

Table 9: Effect of experimental diets on carcass and some organs relative to the body weight of Ross 308 broilers at 42 days of age.

| Experimental diets | Carcass yield | Thighs | Breast | Liver | Heart | Spleen | Pancreas | Abdominal fat | Fabricius |
|--------------------|---------------|--------|--------|-------|-------|--------|----------|---------------|-----------|
| 11                 | 65.03B        | 30.64  | 30.43b | 4.25  | 1.05  | 0.17   | 0.52     | 1.13          | 0.26      |
| 2                  | 64.25b        | 31.86  | 31.01b | 4.19  | 1.12  | 0.18   | 0.57     | 1.25          | 0.27      |
| 3                  | 68.10a        | 32.23  | 33.26a | 4.54  | 1.02  | 0.16   | 0.59     | 1.33          | 0.25      |
| 4                  | 67.67a        | 31.57  | 32.67a | 4.36  | 1.06  | 0.15   | 0.6      | 1.28          | 0.25      |
| 5                  | 65.12b        | 30.53  | 30.24b | 5.86  | 1.07  | 0.18   | 0.56     | 1.17          | 0.24      |
| ± SEM<sup>2</sup>  | 2.26          | 2.02   | 1.12   | 0.31  | 0.06  | 0.03   | 0.05     | 0.3           | 0.03      |

P value: 0.0012 NS 0.0001 NS NS NS NS NS NS

1: 1- Control treatment: Basal diet based on corn-soybean meal without adding dried mealworm, 2- Replacement of 5% dried mealworm with Soy meal in the base diet, 3- Replacement of 10% dried mealworm with Soy meal in the base diet, 4- Replacement of 15% dried mealworm with Soy meal in the base diet and 5- Replacement of 20% dried mealworm with Soy meal in the base diet.
2: Mean error standard
Column means with common superscripts do not differ (P > 0.05).

The use of mealworm in the diet when fermented with probiotics. Also, chickens that used mealworm in their diet had softer, and to be juicy meat [20,21]. The results of some researchers also showed the positive effects of the use of mealworm in the diet of broilers. Biasatu et al. [3] used 50, 100 and 150 g / kg of yellow swarm larvae in broiler diets. Results showed that mean body weight and daily body weight gain linearly increased with increasing yellow mealworm in the diet. Ballitoc and Sun and Bovera et al. reported improved growth performance of broilers using levels of 0 to 100 g / kg or 296 g / kg mealworm, respectively. Jane et al. attributed the positive effects of the use of mealworm in the diet to the digestibility of nutrients. They stated that animal protein sources are better accessible than plant sources and their amino acid composition is more balanced. On the other hand, Fink [22,23] reported that the amount of unsaturated fatty acids in mealworm is high and when replacing soybean meal with mealworm, the ability to digest fat is maintained. Therefore, the positive effects of replacing mealworm with soybean meal to 15% can be justified but a lack of significant impact at the 20% level may be related to the presence of certain factors in soy or mealworm which has not been reviewed yet. Some researchers have also reported a lack of significant effect of mealworm on broiler performance [22,24]. The difference between the results of these tests and the results of the present test may be in the form of usage or the amount of mealworm supplementation. In this experiment, replacing 15% of mealworm with soybean meal significantly reduced feed intake (P>0.05). Poultry consume enough feed to meet their needs [25]. According to supplementation of mealworm to the diet of broilers increased the digestibility of nutrients, such as protein and fat and with reference to high amount of energy in mealworm, a smaller amount of this diet, energy and nutrient needs are met and the amount of feed consumption is reduced [25]. The results of several experiments show no effect of supplementation of mealworm in the diet of broilers on carcass characteristics [26-28]. Positive effect of 15% replacement of mealworm with soybean meal on carcass percentage and relative breast.
weight can be attributed to more energy supply, better digestion of nutrients and higher weight average at this level.

**Conclusion**

The present research results indicated that substitution of soybean meal in the diet of broilers with mealworm at the level of 15% can improve growth performance and carcass characteristics. It is better to investigate the effect of replacing dried meal worm with soybean meal on blood parameters and the immune system of broilers in other researchers.

**Ethics Committee Approval**

All animal experiments were approved by the state committee on animal ethics, Karaj Branch, Islamic Azad University, Karaj, Iran (IACUC no:4687; 4/10/2018). The recommendations of the European Council Directive (2010/63/EU) of September 22, 2010, regarding the standards in the protection of animals used for experimental purposes were also followed.

**Peer-review**

Externally peer-reviewed

**Author Contributions**

Concept-M.R.; Design- M.R., N.N.; Supervision- M.R.; Resources-M.R., N.N.; Materials- M.R.; Data Collection and/or processing- N.N.; Analysis and/or Interpretation- M.R., N.N.; Literature Search-N.N.; Writing Manuscript – N.N., M.R.; Critical Review-M.R.; Other- N.N.

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**Conflict of interest**

The authors have no conflicts of interest to declare.

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