Productive performance, egg-related indices, blood profiles, and interferon-Ɣ gene expression of laying Japanese quails fed on Tenebrio molitor larva meal as a replacement for fish meal

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ABSTRACT
The present experiment was conducted to investigate effects of Tenebrio molitor (TM) as a replacement for fish meal on egg productive and qualitative traits, serum biochemical parameters, haematology and interferon-Ɣ gene expression in laying quails. A total of 250 laying Japanese quails (Coturnix japonica), at 74 days of age were randomly allotted to five dietary treatments in a completely randomised design during a five-week period. Experimental treatments consisted of a basal diet (CTL) or basal diet supplemented with 25% (7.5 g/kg; TM25), 50% (15 g/kg; TM50), 75% (22.5 g/kg; TM75) and 100% (30 g/kg; TM100) TM larvae meal in replace of fish meal. Total replacement of fish meal with TM larva meal significantly increased daily feed intake (DFI) of laying quails compared to CTL (p < .05) but MT25, MT50 and MT75 decreased DFI in comparison to CTL (p < .05). Feed conversion ratio of quails fed with insect larva meal was not different from those received CTL. Serum cholesterol concentration was lower in quails of M75 and M100 groups than those fed on the other dietary treatments (p < .05). Haematological traits and interferon-Ɣ gene expression of quails were not affected by dietary supplemental TM larva meal. It was concluded that dietary inclusion of TM larva meal can be used as replacement for fish meal in diet of laying quails up to 30 g/kg. Dietary supplementation of TM larva meal did not have any adverse effect on health status of laying quails.

HIGHLIGHTS
- Dietary inclusion of TM larva meal can be used as replacement for fish meal in diet of laying quails.
- Dietary supplementation of TM larva meal did not have any adverse effect on health status of laying quails.
- Serum cholesterol concentration decreased by dietary supplementation of TM larva meal in laying quails.

Introduction
As the growth of human population is anticipated, the need to increase in animal-based protein production is unavoidable. The report of FAO (2011) shows that demand for the production of animal protein is estimated to be with 60% increase in the current level by 2050. Poultry production represents one of the most economical and easiest way to obtain animal protein supply owing to the rapid production of egg and meat. Eggs are low-cost source of high-quality protein, lipids, vitamins and minerals (Miranda et al. 2015) with rapidly increasing production, expected to reach the 89 million ton by 2030 (Secci et al. 2018). In this respect, quail egg production has been increasing in recent years due to early sexual maturity, short generation interval, high laying rate and limited feed and space requirements per bird (González 1995; Silva et al. 2018). On the other hand, the higher need to poultry production increases the need to further feed, accounting for more than 65% of variable expenses of producing poultry products (Kiarie et al. 2013). Thereby, producers are trying to decrease feed costs to increase their benefits. Fish meal is an animal by-product and one of the conventional sources of protein used in poultry diets with considerable balance of amino acids and vitamin content. However, the cost-efficiency and market availability are the major constraints to use fish meal in poultry diets (FAO 2013).
In recent years, the use of insects as an animal feedstuff have become more prevalent. Insects contain high amounts of energy, amino acids, fatty acids and micronutrients (Makkar et al. 2014; Rumpold and Schlüter 2013). Three groups of insects including black soldier fly (Hermetia illucens), common housefly (Musca domestica), and yellow mealworm (Tenebrio molitor; TM) are species with the highest potential for large-scale production to be used in diet of poultry (Veldkamp and Bosch 2015). There are many studies which supplemented insect meal in replace of fish meal or soybean meal in diet of broiler chickens (Biasato et al. 2018), laying hens (Bovera et al. 2018; Marono et al. 2017), broiler quails (Shariat Zadeh et al. 2019) and laying quails (Dalle Zotte et al. 2019). Amao et al. (2010) reported that westwood larva meal could be replaced up to 75% fishmeal in diet of laying hens. Maurer et al. (2016) replaced soybean cake by Hermetia illucens meal in diet of layer hens and concluded that Hermetia illucens larvae meal can be supplemented as a valuable replacer for soybean products without any deleterious effect on egg production and feed intake. Black soldier fly meal was successfully substituted for soybean meal at 100% level in the diet of Lohmann Brown Classic laying hens (Secci et al. 2018). Moreover, Dalle Zotte et al. (2019) indicated that defatted black soldier fly larvae meal can be a possible alternative ingredient for soybean meal in diet of laying quails up to the 15% inclusion level. Furthermore, effect of TM larva meal on immune-related gene expression in quails has not been studied. The effect of dietary insect meal on some immune response of poultry has been already studied. Hussain et al. (2017) showed that supplementation of TM larva meal in diet of broiler chickens had no adverse effects on the antibody titre against Newcastle disease virus. Moreover, the ratio of albumin to globulin was decreased when broilers fed on diets containing insect meal. To the authors knowledge, there is lack of research on supplementation of TM larva meal in diet of laying quails and its subsequent impact on immune-related gene expression. Therefore, further research in this field is warranted. Furthermore, effect of TM larva meal on immune-related gene expression in quails has not been studied.

It was hypothesised that TM larva meal could be dietary supplemented as a replace of fishmeal without any adverse effects on egg production variables, egg qualitative indices, blood attributes and interferon-γ (IFN-γ) gene expression. Therefore, this study was conducted to evaluate dietary substitution of TM larva meal (0%, 25%, 50%, 75% and 100%) for fish meal on egg production and qualitative traits, serum biochemical attributes, haematology and IFN-γ gene expression of laying quails.

Materials and methods

All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee of the Faculty of Animal Science, Islamic Azad University of Shahrekord (approval ref. no. 2017-056). The experiment was conducted according to the regulations and guidelines established by this committee.

Analysis of feed samples and Tenebrio molitor larva meal content

Prior to trial commence, corn, soybean meal, fish meal and TM dried and ground larva meal were analysed for crude protein (Method 990.03; AOAC 2006) and ether extract (Method 920.39 A; AOAC 2006). Furthermore, amino acids contents (Methods 982.30E a, b, and c; AOAC 2006) were analysed for TM larva meal. Calcium (Ca), total P (tP) contents of TM larva meal, fish meal and feed samples were determined by inductively coupled plasma – optical emission spectrometry (Method 2011.14; AOAC 1990). Analysis of yellow mealworm content and fish meal are shown in Table 1.

Birds and management

A total of 250 laying Japanese quails (Coturnix japonica), at 74 days of age were weighed (initial BW: 240 ± 0.05) and randomly distributed in 25 cages (40 x 40 x 30) in a power-ventilated house during a five-week period. Five replicate cages of 10 laying quails each were used with five different diet treatments. Each cage consisted of twelve nest boxes (20 cm by 20 cm) and a food-basin (20 cm by 20 cm) placed in the middle of the cage. Each cage contained five birds. The feeding regimen was as follows: 4 meals of 2% (w/w) of body weight per day, at 06.00, 09.00, 12.00 and 14.00 h. The following feed was offered: 0% fishmeal (control), 25% fishmeal, 50% fishmeal, 75% fishmeal and 100% fishmeal. Diet composition is shown in Table 1. The quails were given access to fresh water and feed ad libitum. The eggs were collected daily and the number of eggs laid and the egg losses were recorded over the five-week period. To determine egg production, all eggs were weighed and measured. The daily egg production was recorded as the number of eggs collected divided by the number of birds in the cage. The egg production was calculated as the sum of the daily egg production, divided by the total number of days in the laying period.

Table 1. Analysis of the mealworm larvae (Tenebrio molitor) and fish meal, g/kg as fed.

|                | Mealworm larvae | Fish meal |
|----------------|----------------|-----------|
| Total protein  | 464.40         | 639.00    |
| Ash            | –              | 13.90     |
| Ether extract  | –              | 10        |
| Arginine       | 22.28          | –         |
| Histidine      | 13.79          | –         |
| Isoleucine     | 18.29          | –         |
| Leucine        | 31.28          | –         |
| Lysine         | 25.04          | –         |
| Methionine     | 5.22           | –         |
| Cysteine       | 6.67           | –         |
| Phenylalanine  | 15.44          | –         |
| Threonine      | 17.03          | –         |
| Valine         | 25.74          | –         |
| Calcium        | 0.43           | 20.50     |
| Total phosphorus| 7.06           | 34.50     |
Japanese quails each were randomly allotted to five dietary treatments in a completely randomised design. Experimental treatments consisted of a basal diet (CTL) or basal diet supplemented with 25% (7.5 g/kg), 50% (15 g/kg), 75% (22.5 g/kg) and 100% (30 g/kg) TM larvae meal. The basal diet included 30 g/kg fish meal that was replaced by the same amount of TM larva meal in the corresponding diets. All experimental diets were formulated to be iso-energetic and iso-nitrogenous. Furthermore, diets were formulated to meet or exceed the nutrient requirements of laying Japanese quails as recommended by NRC (1994; Table 2). Dietary treatments were fed in mash form and offered ad libitum throughout the study. All birds had free access to water during the experiment. The lighting programme consisted of 23-hour light and one-hour darkness. Temperature was 23 ± 2 °C.

**Egg production variables and egg qualitative indices**

Provided feed and refusals were recorded for each treatment replicate to calculate daily feed intake (DFI) for the whole period of the experiment. All laid eggs were collected daily and weighed individually. Egg weight, daily egg mass production (g egg/hen/day) and feed conversion ratio (FCR, g feed/g egg) were calculated for the entire production period. In total, 120 eggs (10 eggs/cage) were used for egg quality examination. After weighing the individual eggs, each egg was broken onto a flat surface, and the albumen height, as well as yolk weight, height, and diameter, were recorded. The albumen was removed from shells and shells plus membranes were weighed after 24 hours of air drying. All noted parameters were determined according to Kondiah et al. (1981).

**Serum biochemical parameters and haematological traits**

On day 120 of experiment, blood samples were taken from two birds of each cage and collected in non-heparinised tubes by brachial vein puncture. Serum was separated via 2000 g centrifuge of blood samples for 15 minutes (SIGMA 4-15 Lab Centrifuge, Germany). Individual serum samples were analysed for total protein, albumin, uric acid, total cholesterol, triacylglycerol, high-density lipoprotein (HDL), calcium and phosphorus with a spectrophotometer using the kit package (Pars Azmoon CO; Tehran, Iran). Globulin concentration in serum was computed by subtracting albumin concentration from proteins. Subsequently, albumin to globulin ratio (A/G) was determined. Furthermore, serum was analysed for haematocrit, haemoglobin, and blood cell count (white blood cells, WBC and red blood cells, RBC) using an automatic haematological traits

**Table 2. Dietary composition and nutrients.**

| Ingredients (g/kg) | CTL | M25 | M50 | M75 | M100 |
|-------------------|-----|-----|-----|-----|------|
| Corn (80 g/kg crude protein) | 544.6 | 538.8 | 534.9 | 530.0 | 525.3 |
| SBM (440 g/kg crude protein) | 315.0 | 320.0 | 325.0 | 330.0 | 335.0 |
| Fish meal (639 g/kg crude protein) | 30 | 22.5 | 15.0 | 7.5 | 0.0 |
| TM meal (464 g/kg crude protein) | 0 | 7.5 | 15.0 | 22.5 | 30.0 |
| Soybean oil | 35.0 | 33.5 | 32.0 | 30.5 | 29.0 |
| Dicalcium phosphate | 9.9 | 11.9 | 11.8 | 12.7 | 13.6 |
| Calcium carbonate | 54.3 | 54.6 | 54.9 | 55.3 | 55.6 |
| DL-Methionine | 0.6 | 0.6 | 0.7 | 0.8 | 0.8 |
| Choline | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Vitamin and mineral premixa | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Sodium chloride | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| NaHCO3 | 1.6 | 1.6 | 1.7 | 1.7 | 1.7 |

Calculated nutrient level (as fed basis)

| Items | CTL | M25 | M50 | M75 | M100 |
|-------|-----|-----|-----|-----|------|
| ME (Kcal/kg) | 2900 | 2900 | 2900 | 2900 | 2900 |
| Crude protein (g/kg) | 200 | 200 | 200 | 200 | 200 |
| Lysine (g/kg) | 10.2 | 10.2 | 10.2 | 10.2 | 10.2 |
| Met + Cys (g/kg) | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |
| Threonine (g/kg) | 7.6 | 7.6 | 7.6 | 7.6 | 7.6 |
| Arginine (g/kg) | 12.7 | 12.7 | 12.7 | 12.6 | 12.6 |
| Valine | 9.7 | 9.7 | 9.7 | 9.7 | 9.7 |
| Isoleucine | 8.6 | 8.6 | 8.6 | 8.6 | 8.6 |
| Tryptophan | 2.3 | 2.3 | 2.3 | 2.3 | 2.3 |
| Sodium | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Chloride | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| Calcium (g/kg) | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Available phosphorous (g/kg) | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |

Calculated nutrient level (as fed basis)

**Quantitative real-time PCR**

At the end of the experiment, all quails were slaughtered after 6 hours of feed withdrawal, samples from the liver tissues were collected and quickly snap-frozen in liquid nitrogen that was further used to...
measure IFN-γ mRNA levels. Total RNAs were extracted from the homogenised tissues using TRIZOL reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). RNA concentration was quantified by spectrophotometer nano-drop (MD-1000) in wavelength of 250 nm. Complementary (c) DNA was synthesised from 1 μg of RNA samples with an MiScript II RT kit (QIAGENE, Germany), according to the manufacturer’s recommended protocol. All primers were synthesised and purified by Sigma Company. The β-actin was used as reference gene to normalise the expression of target gene. The primer pairs for the amplification of IFN-γ and β-actin cDNA fragments are listed in Table 3. Quantitative real-time PCR (qRT-PCR) was performed to determine the levels of inducible IFN-γ mRNA. Two microliters of 10-fold dilution reverse transcription products was used for PCR in a final volume of 25 μL containing 0.4–0.8 μM primers and 12.5 μL of QuantiTect SYBR Green master mix (Life Technologies, Cat # 4367659). Cycling parameters were as follows: 10 min at 95°C, then 40 cycles of 95°C for 30 s, annealing temperature for 30 s, and 72°C for 30 s, and extension for 2 min at 72°C. To confirm amplification specificity, the PCR products from each primer were subjected to a melting curve analysis and subsequent agarose gel electrophoresis. The final IFN-γ concentrations were calculated as arbitrary unit of band density relative to total protein concentration of each sample.

Statistical analysis

Data for recorded traits were subjected to analysis of variance procedures appropriate for a completely randomised design using the General Linear Model procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The cage was considered as the experimental unit for different parameters. For all statistical analyses, significance was declared at p ≤ .05, unless otherwise stated. The Fisher’s protected least significant difference test was used for multiple treatment comparisons. Means were presented with their standard error of means.

Results

Egg production variables and egg qualitative indices

Effect of dietary treatments on egg production variables of laying quails are summarised in Table 4.

Table 3. Primers used for quantitative real-time PCR.

| Target gene | Gene bank accession | Forward sequence (5’ to 3’) | Reverse sequence (5’ to 3’) |
|-------------|---------------------|-----------------------------|-----------------------------|
| INF-γ       | NM_205149           | CTGGCAVVTAGCACAATGAA        | TGCTTGCTGATCCATCT           |
| β-actin     | NM_205518           | AACAACCTTAATGATGGCAGC       | GCATCTCCTCAACACTGGCT        |

Substitution of 100% TM larva meal significantly increased DFI of laying quails compared to the other dietary treatments (p<.05). Laying quails received CTL had greater egg mass than those fed on M25 and M75 during the 1 to 5 week of production (p<.05). The lowest FCR observed in quails fed on M50 and M75 which was lower than those fed with M25 and M100 (p<.05).

The use of TM larva meal as a replacement for fish meal at the levels of 25% (M25) significantly decreased yolk weight compared to the other dietary treatments at the first week of production period (p<.05; Table 5) while it was not affected during the remaining weeks of the experiment. Height of the yolk in quails fed on M100 was significantly higher than those received M50 and M75 during the second week of egg production (p<.05; Table 5). On the other hand, quails received the CTL diet had significantly higher yolk height than those given M25 across 14 to 21 days of egg production (p<.05, Table 5). Furthermore, the greatest shell weight observed in broilers received basal diet which was higher than the shell weight reported for the group of quails fed M75 during the third week of production (p<.05; Table 5).

Table 4. Egg production variables of laying quails.

| Parameters          | CTL | M25 | M50 | M75 | M100 | SEM | p-value |
|---------------------|-----|-----|-----|-----|------|-----|---------|
| Feed intake (g/d)   |     |     |     |     |      |     |         |
| 1 to 5 week         | 27.19 b | 27.02 c | 25.27 d | 24.63 e | 27.96 a | 0.032 | <.001   |
| Egg mass            |     |     |     |     |      |     |         |
| 1 to 5 week         | 11.62 b | 10.90 c | 11.13 d | 11.04 e | 11.14 b | 0.341 | <.001   |
| FCR (g/g)           |     |     |     |     |      |     |         |
| 1 to 5 week         | 2.34 b | 2.48 a | 2.27 c | 2.23 d | 2.51 e | 0.059 | .001    |
| Egg weight (g)      |     |     |     |     |      |     |         |
| 1 to 5 week         | 11.91 | 11.47 | 11.65 | 11.66 | 11.74 | 0.245 | .38     |

a,b,c,dMeans in the same row with different superscript differ significantly (p<.05).

Egg biochemical parameters and haematological traits

Table 6 shows the effect of experimental treatments on serum biochemical parameters in broiler chickens. Concentration of serum cholesterol was higher in

Table 5. Egg production variables of laying quails.

| Parameters          | CTL | M25 | M50 | M75 | M100 | SEM | p-value |
|---------------------|-----|-----|-----|-----|------|-----|---------|
| Feed intake (g/d)   |     |     |     |     |      |     |         |
| 1 to 5 week         | 27.19 b | 27.02 c | 25.27 d | 24.63 e | 27.96 a | 0.032 | <.001   |
| Egg mass            |     |     |     |     |      |     |         |
| 1 to 5 week         | 11.62 b | 10.90 c | 11.13 d | 11.04 e | 11.14 b | 0.341 | <.001   |
| FCR (g/g)           |     |     |     |     |      |     |         |
| 1 to 5 week         | 2.34 b | 2.48 a | 2.27 c | 2.23 d | 2.51 e | 0.059 | .001    |
| Egg weight (g)      |     |     |     |     |      |     |         |
| 1 to 5 week         | 11.91 | 11.47 | 11.65 | 11.66 | 11.74 | 0.245 | .38     |

a,b,c,dMeans in the same row with different superscript differ significantly (p<.05).
quails fed 75 and 100% MT in replace of fish meal than the concentration observed for the birds in the other groups (p<.05). Other serum biochemical parameters remained unaffected (p<.05). As shown in Table 7, haematological traits were not affected in laying quails fed on dietary treatments.

### Discussion

The present study evaluated effects of dietary TM larva meal inclusion on egg productive and qualitative traits, serum biochemical parameters, haematology

### Table 5. Egg qualitative indices of laying quails.

| Parameters          | Yolk weight (g) | Yolk diameter | Yolk height | Albumen weight (g) | Shell weight (g) |
|---------------------|-----------------|---------------|-------------|-------------------|-----------------|
|                     | Week 1           | Week 2        | Week 3      | Week 4            | Week 5          |
| CTL                 | 3.15±0.26b       | 3.24±0.31a    | 3.16±0.33a  | 3.13±0.33a        | 3.13±0.33a      |
| M25                 | 3.63±0.33       | 3.74±0.32a    | 3.86±0.38a  | 3.86±0.32a        | 3.86±0.32a      |
| M50                 | 3.94±0.38a      | 3.74±0.32a    | 3.96±0.39a  | 3.96±0.32a        | 3.96±0.32a      |
| M75                 | 4.04±0.38a      | 3.74±0.32a    | 3.96±0.39a  | 3.96±0.32a        | 3.96±0.32a      |
| M100                | 3.97±0.36a      | 3.74±0.32a    | 3.97±0.39a  | 3.97±0.32a        | 3.97±0.32a      |
| SEM                 | 0.008±0.112     | 0.027±0.090   | 0.007±0.170 | 0.008±0.060       | 0.012±0.190     |
| p-Value             | <.001           | <.05          | <.05        | <.05              | <.05            |

### Table 6. Serum biochemical parameters of laying quails.

| Parameters                   | CTL     | M25     | M50     | M75     | M100    | SEM     | p-Value |
|------------------------------|---------|---------|---------|---------|---------|---------|---------|
| Total protein (g/dL)         | 5.56    | 4.19    | 4.49    | 5.47    | 4.05    | 3.795   | .629    |
| Albumin (g/dL)               | 2.33    | 1.88    | 1.88    | 2.24    | 1.82    | 0.804   | .520    |
| Globulin (g/dL)              | 3.23    | 2.31    | 2.61    | 3.23    | 2.23    | 0.850   | .257    |
| A/G                          | 0.99    | 0.94    | 0.99    | 0.87    | 0.83    | 0.085   | .322    |
| Uric acid (mg/dL)            | 3.71    | 4.62    | 4.150   | 4.90    | 5.34    | 0.545   | .557    |
| Cholesterol (mg/dL)          | 249.82± | 308.71± | 281.59± | 182.58± | 176.20± | 34.547  | .030    |
| Triglycerides (mg/dL)        | 233.48  | 271.54  | 261.32  | 202.11  | 240.35  | 32.009  | .220    |
| HDL (mg/dL)                  | 106.71  | 111.69  | 91.36   | 104.84  | 100.70  | 8.037   | .250    |
| Calcium (mg/dL)              | 4.9±ab  | 4.53±ab | 3.58±ab | 5.79±a  | 4.68±ab | 0.524   | .033    |
| Phosphorous (mg/dL)          | 7.2±a   | 6.9±b   | 6.67±b  | 6.17±b  | 6.9±b   | 0.340   | .180    |

### Table 7. Haematological traits of laying quails.

| Parameters                   | RBC (mmol × 10³) | Haemoglobin (g/dl) | WBC (mmol × 10³) | Monocyte (%) | Lymphocyte (%) |
|------------------------------|------------------|--------------------|------------------|--------------|----------------|
| CTL                          | 5.06±0.46        | 4.46±0.46         | 4.00±0.40        | 0.80±0.40    | 70.80±6.40     |
| M25                          | 5.06±0.46        | 4.46±0.46         | 4.00±0.40        | 0.80±0.40    | 70.80±6.40     |
| M50                          | 5.06±0.46        | 4.46±0.46         | 4.00±0.40        | 0.80±0.40    | 70.80±6.40     |
| M75                          | 5.06±0.46        | 4.46±0.46         | 4.00±0.40        | 0.80±0.40    | 70.80±6.40     |
| M100                         | 5.06±0.46        | 4.46±0.46         | 4.00±0.40        | 0.80±0.40    | 70.80±6.40     |
| SEM                          | 0.045±0.05       | 0.045±0.05        | 0.045±0.05       | 0.045±0.05   | 0.045±0.05     |
| p-Value                      | <.05             | <.05              | <.05             | <.05         | <.05           |

### Table 8. Effect of experimental treatments on mRNA expression of INF-γ gene.

| Item                      | CTL     | M25     | M50     | M75     | M100    |
|---------------------------|---------|---------|---------|---------|---------|
| mRNA expression           | 1.11±0.57 | 0.80±0.34 | 1.03±0.81 | 0.81±0.21 | .187±0.21 |
| p-Value                   | <.05    | <.05    | <.05    | <.05    | <.05    |

RT-PCR analysis showed that relative amount of IFN-γ expression was not affected by experimental treatments (Table 8).

### Table 8. Effect of experimental treatments on mRNA expression of INF-γ gene.

- **CTL**: a basal diet; **M25**: a basal diet supplemented with 25% (7.5 g/kg) *Tenebrio molitor* meal replace of fish meal; **M50**: a basal diet supplemented with 50% (15 g/kg) *Tenebrio molitor* meal replace of fish meal; **M75**: a basal diet supplemented with 75% (22.5 g/kg) *Tenebrio molitor* meal replace of fish meal; **M100**: a basal diet supplemented with 100% (30 g/kg) *Tenebrio molitor* meal replace of fish meal.
and IFN-γ gene expression of laying quails. In the present experiment, feeding quails with M100 increased DFI during the entire production period. This is consistent with the results found by Biasato et al. (2018) who indicated that DFI of male broiler chickens increased when incremental levels (0 g/kg, 50 g/kg, 100 g/kg and 150 g/kg) of TM larva meal supplemented to their diets. Conversely, there are many studies with adverse effects of insect meals on DFI of poultry. For example, DFI of laying hens decreased in response to dietary total replacement of soybean meal by *Hermetia illucens* (Marono et al. 2017). Moreover, feeding diets supplemented with either *Hermetia illucens* larva maggot meal or TM larva meal in replace of fish meal decreased DFI of laying (Widjastuti et al. 2014) and broiler quails (Shariat Zadeh et al. 2019), respectively. On the other hand, Maurer et al. (2016) and Bovera et al. (2018) did not find any effect of dietary supplemental *Hermetia illucens* larva meal on DFI of laying hens. Therefore, effect of dietary supplemental insect meal on DFI of poultry is equivocal. Because age, strain, nutrition and environmental condition is the same between birds of experimental treatments, DFI could be affected by palatability of diets. In this respect, Cullere et al. (2016) declared that broiler quails tended to prefer diets supplemented with *Hermetia illucens* meal in a feed-choice test. This might have originated from the innate tendency of chickens to insects as naturally consume it when reared in free-range systems (Zuidhof et al. 2003). In the current experiment, although quails increased their feed intake after feeding M100, but their intake decreased when fed on M25, M50 and M75 even worse than the time received control diet. It may show that substitution of fish meal by MT meal at levels below M100 adversely affected feed flavour and decreased the birds tendency to use diets. In the other words, quails preferred to consume diets containing either fish meal or insect meal per se, not both of them. Despite the increased DFI, egg mass and FCR of quails were not affected by M100. It may be attributed to reduction in protein digestibility of dietary TM larva meal in laying quails although crude protein digestibility is not measured in this experiment. Digestibility of crude protein in diets containing insect meal is mainly affected by chitin content with protein-binding activity which decreases protein availability for laying quails (Longvah et al. 2011). Bovera et al. (2018) observed the lower dietary protein digestibility in laying hens fed on diets containing *Hermetia illucens* larva meal. Results on the effect of experimental diets on FCR shows that supplementation of TM larva meal in diet of laying quails had no adverse effects on production performance of them, so fish meal can be successfully replaced by TM larva meal. The lack of insect meal effect on FCR has been observed in broiler chickens fed diets with increasing supplemental levels of defatted *Hermetia illucens* meal (Elwert et al. 2010), in laying hens fed with partially defatted *Hermetia illucens* larva meal as partial or complete replacement of soybean meal and oil (Cullere et al. 2016). Findings of our study disagrees with the results of Marono et al. (2017) who observed the better FCR in laying hens received diets containing *Hermetia illucens* larvae meal as total replace of soybean meal. Variations in results obtained for the effect of insect meal on growth performance of poultry can be ascribed to the nutritive value of the consumed insect meal, depending on the life stage, the substrate used for rearing (Sánchez-Muros et al. 2014) and also the birds rearing period. Because DFI of quails was variable in response to feeding dietary treatments, egg qualitative indices including yolk and albumen weights as well as yolk height affected in some weeks of production period, subsequently. It is thus rather difficult to explain why some of these traits were affected in a specific production week. Similar results observed in the study of Amao et al. (2010), indicating variable results of egg qualitative indices due to supplementing *Cirina forda* larva meal in diet of laying hens.

Uric acid is the final and major poultry nitrogenous waste product (Harr 2002). In the present experiment, uric acid was not affected by dietary treatments, suggesting that dietary treatments had no effect on protein metabolism. Analogous to our results, Marono et al. (2017) and Bovera et al. (2018) did not find any effect of dietary *Hermetia illucens* larva meal supplementation on serum uric acid concentration of laying hens. The serum cholesterol concentration decreased in quails that received M75 and M100 diets but was not affected by M25 and M50. Experimental evidence has shown that chitin content of insect larva meal is responsible for the reduction in serum cholesterol concentration (Hossain and Blair 2007) through the effect of its positive charge which is able to attract negatively charged bile acids and free fatty acids (Prajapati and Patel 2010). In agreement with our findings, Bovera et al. (2018) reported that laying hens fed dietary *Hermetia illucens* in substitution of soybean meal at two different levels (25 and 50%) had decreased serum cholesterol concentration. Moreover, there was
a reduction in serum cholesterol concentration of laying hens received Hermetia illucens larvae meal as total replacement of soybean meal from 24 to 45 weeks of age (Marono et al. 2017). In the present study, the values of the haematological traits fell within the normal physiological range and lack of significant difference between the birds received dietary treatments implies that ability of laying quails to respond to infection was not compromised with the inclusion of dietary TM larva meal as a substitution for fish meal.

As found herein, IFN-γ levels were not affected by substitution of TM larva meal for fish meal. Interferons are glycoproteins produced by white blood cells and viral-infected somatic cells in response to viral infections, immune activation, inflammatory stimulation and chemical stimulants. IFN-γ is a pleiotropic molecule that has some involvement in most stages of the inflammatory and immune responses. Our results may suggest that TM larva meal has no compromising impact on immunity and does not adversely affect health status of laying quails. Scanty information is available on the effect of nutrition on IFN-γ gene expression in poultry and further investigations are warranted.

Conclusions

In conclusion, feeding diets supplemented with TM larva meal as a replacement for fish meal at 100% level increased DFI of laying quails while this effect was not seen when quails received other dietary treatments. However, the increased DFI did not lead to improvement in the egg mass, FCR and egg production variables in laying quails. This could be attributed to lower protein digestibility of TM larva meal due to its chitin content. Serum concentration of cholesterol decreased by dietary supplementation of MT75 and MT100. Haematological traits and expression of IFN-γ gene remained unaffected, suggesting that MT larva meal substitution for fish meal in diet of laying quails had no compromising impact on health status of them.

Ethical Approval

All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee of the Faculty of Animal Science, Islamic Azad University of Shahrekord (approval ref. no. 2017-056).

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Geolocation information

The research was performed in Iran, Islamic Republic of, Shahrekord (Latitude: 32.32441, Longitude: 50.86320)

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