Feed supplementation with vermi-humus and earthworm (*Eisenia fetida*) powder on broiler productivity

Yousef Chashmidari, Ladan Esmaielzadeh, Mohammad-Amir Karimi-Torshizi, Alireza Seidavi, Cristiane Soares da Silva Araújo and Lucio Francelino Araujo

**ABSTRACT**

The objective of this study was to evaluate the use of earthworm meal (EW) and vermi-humus (VH) in broiler diets. A total of 225 day-old male broilers were divided into 5 treatment groups with 3 repetitions of 3 birds each. The treatment groups were: T1 (control; no EW or VH), T2 (1.0% EW and 0% VH), T3 (1.0% EW and 1.0% VH), T4 (1.0% EW and 1.5% VH), and T5 (1.0% EW and 2.0% VH). The birds received EW and VH for 14 d, and after this period, they received the common diet. The birds were reared for 42 d, and at 42 d, one representative broiler chicken per pen, close to the average body weight, was selected for blood sampling using a sterile needle and heparinised vacuum tube. Performance data, blood results, immunity, ileum morphology and microbiota, the relative weight of carcass components and gastrointestinal organs, malondialdehyde (MDA) content, and breast and thigh meat quality were evaluated.

EW and VH had a positive effect on the immune response of broilers, as well as producing a reduction in the aerobic bacteria in the birds’ intestines.

**HIGHLIGHTS**

- Protein is a limiting factor for broiler performance, carcass yield, and meat quality;
- Poultry industry is searching for new protein sources which can be used on broiler diets;
- Insects meal are good protein sources in broiler nutrition and can be used on diets without compromising its performance.

**Introduction**

An adequate protein supply is one of the major goals in broiler nutrition around the world. Protein sources can be of animal origin, such as meat and fishmeal, or of vegetable origin, such as soybean meal. However, animal sources have a more balanced amino acid profile, mainly lysine, which is the second limiting amino acid in diets based on corn and soybean meal.

Due to the high market price of protein sources, and particularly the seasonal variations in price, there is increasing interest in alternative protein sources for broiler chickens. An important novel source is EW (Kose and Ozturk 2017). According to Veldkamp et al. (2012) and Khan et al. (2016) EW may provide a viable protein source for poultry feed since it can be produced on a commercial scale and have good nutritional composition, up to 73% crude protein (Rumpold and Schlüter 2013), and higher levels of essential amino acids (Parolini et al. 2020).

According to Rezaeipour et al. (2014) VH is a source of humic acid with high biological value as a food additive. In addition, inhibits bacterial and fungal growth, improving intestinal health and the immune system of animals (Rath et al. 2006). The supplementation of diets with 2% of EW increased final body weight, feed intake, breast percentage and HDL level and decreased blood LDL levels in broiler chickens (Gholami et al. 2016).

The use of EW and VH together in the diet has great potential for improving the feeding efficiency of birds due to improved profiles of intestinal microbiota and its high protein level (Bahadori et al. 2017). Further, it has been reported the absence of anti-

**CONTACT**

Alireza Seidavi alirezaseidavi@iaurasht.ac.ir Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran

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nutrient factor in these ingredients (Bollido 2020). However, there is little reporting on how EW and VH influence microbiota and meat oxidative stability. In addition, the study of the economic viability of EW and VH in diets for broilers is lacking. Furthermore, this research is important because it seeks to identify possible alternative protein sources.

Based on the above description, the objective of the study was to see the effects of VH and EW (Eisenia fetida) on performance, carcass characteristics, immunity, blood constitutes, small intestinal microbiota and histomorphology, meat quality and meat oxidative stability of broilers.

Materials and methods

Animals and husbandry

Two hundred and twenty-five day-old male broilers (42 g) were purchased from a commercial hatchery. Fifteen broilers were placed in each 1.5 × 1.0 m cage, providing a floor area/bird of 0.1 m². Cages were centrally positioned in a thermostatically-controlled curtain side-walled poultry barn. The cage floors were covered with wood shavings as litter, and the birds remained in the cages for the duration of the study, which ended when they were 42 d old. The ambient temperature within the poultry barn was maintained with supplemental heat from thermostatically-controlled gasoline rocket heaters, and misting was used to maintain relative humidity in the barn at 55–65%. Ambient temperature was maintained at 31–33 °C at the time of placement of birds in the cages and decreased progressively to 18–21 °C by 6 weeks of age. Controlled lighting and tunnel ventilation were included in the barn. Lighting was provided by 20-watt lamps in ceiling fixtures. Constant light was provided on day 1, after which lighting was set at 23 h per day until the end of the study. Air circulation within the poultry barn was facilitated by 2 wall-mounted fans at one end of the barn to establish tunnel ventilation.

Feed and treatments

A three-phase feeding program was used, consisting of starter (1st–14th d of age), grower (15th–28th d of age), and finisher (29th–42nd d of age) diets. The ingredient and nutrient composition of the diets (Table 1) met or exceeded broiler recommendations (Aviagen 2009). The day-old chicks were allocated to 15 groups of 15 birds (5 treatments and 3 replicates of each Table 1. Feed ingredients and nutrient analysis of diets used during the starter (1st–14th d of age), grower (15th–28th d of age), and finisher (29th–42nd d of age) periods.

| Ingredient, % | 0EW0VH | 1EW0VH | 1EW1VH | 1EW1.5VH | 1EW2VH | Grower | Finisher |
|---------------|--------|--------|--------|----------|--------|--------|---------|
| Corn          | 51.32  | 50.02  | 51.28  | 52.54    | 52.75  | 58.59  | 60.97   |
| Corn gluten meal | 7.00   | 7.00   | 7.00   | 7.00     | 7.00   | 0.00   | 0.00    |
| Soybean oil   | 1.33   | 1.70   | 1.33   | 0.95     | 0.73   | 2.43   | 3.43    |
| Soybean meal  | 35.29  | 35.48  | 33.65  | 31.83    | 30.95  | 34.74  | 31.62   |
| DL-methionine | 0.25   | 0.25   | 0.5    | 0.24     | 0.23   | 0.27   | 0.23    |
| L-lysine-hydrochloride | 0.39 | 0.39 | 0.39 | 0.38 | 0.35 | 0.17 | 0.11 |
| L-threonine   | 0.07   | 0.07   | 0.07   | 0.06     | 0.04   | 0.06   | 0.04    |
| Choline       | 0.10   | 0.10   | 0.10   | 0.10     | 0.10   | 0.10   | 0.10    |
| Dicalcium phosphate | 2.00 | 2.00  | 2.00  | 1.86  | 1.77  | 1.62  |
| CaCO₃         | 1.29   | 1.07   | 1.07   | 1.06     | 1.06   | 1.03   | 1.03    |
| Sodium bicarbonate (NaHCO₃) | 0.35 | 0.35 | 0.34 | 0.33 | 0.31 | 0.30 | 0.00 |
| NaCl          | 0.10   | 0.10   | 0.10   | 0.10     | 0.11   | 0.34   | 0.35    |
| Vitamin and mineral premix* | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Earthworm     | 0.00   | 1.00   | 1.00   | 1.00     | 1.00   | 0.00   | 0.00    |
| Vermihumus    | 0.00   | 0.00   | 1.00   | 1.50     | 2.00   | 0.00   | 0.00    |
| Calculated composition | | | | | | |
| Metabolizable energy, kcal/kg | 2920 | 2920 | 2920 | 2920 | 3000 | 3100 |
| Crude protein, % | 24.29 | 24.34 | 24.34 | 24.34 | 24.34 | 20.44 |
| Lysine, % | 1.34 | 1.34 | 1.34 | 1.34 | 1.34 | 1.34 |
| Methionine, % | 0.61 | 0.61 | 0.61 | 0.61 | 0.61 | 0.61 |
| Methionine + Cysteine, % | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.83 |
| Threonine, % | 0.84 | 0.84 | 0.84 | 0.84 | 0.84 | 0.72 |
| Valine, % | 1.00 | 1.00 | 1.00 | 1.01 | 1.02 | 0.85 |
| Calcium, % | 1.03 | 1.03 | 1.03 | 1.03 | 1.03 | 0.88 |
| Available Phosphorus, % | 0.49 | 0.49 | 0.19 | 0.49 | 0.49 | 0.44 |
| Sodium, % | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 |
| DCAB, mEq/kg | 244.31 | 244.25 | 234.70 | 225.15 | 219.61 | 209.21 |

*Supplied per kilogram of feed: Vitamin A: 3,600,000 IU; vitamin D₃: 800,000 IU; vitamin E: 7.2 g; vitamin K₃: 0.8 g; B₁: 0.7 g; B₂: 2.64 g; B₃: 4 g; calcium pantothenate: 3.92 g; B₆: 1.176 g; B₉: 0.4 g; B₁₂: 6 mg; H₂: 40 mg. Choline chloride: 100 g; Mn: 40 g; Fe: 20 g; Zn: 33.88 g; Cu: 4 g; I: 0.4 g; Se: 0.08 g.

DCAB: Dietary Cation-Anion Balance.

Experiment was based on these 2 rows with values in bold.
treatment), so that mean body weights were similar across groups. Each cage of 15 chicks was assigned at random to a specific dietary treatment group.

**Treatments**

EW and VH were added to starter diets for the first 14 d and effects on performance, carcass characteristics, immunity, blood constitutes, small intestinal microbiota and morphology, meat quality and oxidative stability of chilled breast meat were evaluated. The chemical analyses of the EW and VH from Amize Tabiat Co (Iran) are shown in Table 2. Treatments were as follow:

- Treatment 1: control (without VH or EW)
- Treatment 2: 1.0% EW and 0% VH in starter diet
- Treatment 3: 1.0% EW and 1.0% VH in starter diet
- Treatment 4: 1.0% EW and 1.5% VH in starter diet
- Treatment 5: 1.0% EW and 2.0% VH in starter diet

**Measured variables**

Bodyweight and feed intake were measured. The feed conversion ratio was calculated by dividing feed intake by body weight gain for each replicate (Sigolo et al. 2019). At 42 d, one representative broiler per replicate was selected and euthanized. Feet were separated from the carcass at the tibiotarsal joint. Neck, wingtips, gut and liver were removed, and the empty or edible carcass was weighed and recorded. The total weight of all dissected organs was also calculated. Ratios were calculated according to the following formula: [(weight of component(s)/eviscerated carcass weight) × 100] (Poorghasemi et al. 2017). Carcass components, gastrointestinal organs and heart were measured according to the methods described by Seidavi et al. (2014) on 42nd day of age.

The antibody title against Newcastle disease (vaccine administrated in drinking water at 18 d of age), influenza (vaccine injected at 7 d of age) and sheep red blood cells (SRBC) (injected on 21st and 35th day of age) were measured as described in Pourhossein et al. (2015). Blood samples were collected for antibody titres against Newcastle disease and influenza on 42nd day and for antibody titres against SRBC on 28th and 42nd day of age.

On day 28, 12 birds per treatment, were sensitised by the single percutaneous application of 1-chloro-2,4-dinitrobenzene (DNCB-Merck). A total of 250 mL of DNCB (10 mg/mL of acetone and olive oil 4:1), was applied on a featherless area on the right side, whilst a similar area on the left side received the solvent without DNCB as a control. Changes in mean skin thickness before and 24 h post-challenge were assessed using digital callipers (Mitutoyo, Japan) as described by Karimi Torshizi et al. (2010).

For PHA-M induced lymphoproliferation, a phytohemagglutinin-M (Gibco, USA), T-cell mitogen (100 mg dissolved in 100 mL of sterile PBS) was injected into the right toe web of 9 birds per experimental group at 40 d. The increase in toe web thickness was measured 24 h after injection as described by Karimi Torshizi et al. (2010).

The ileum microbiota was measured based on methods of Dibaji et al. (2014). Meat quality and oxidative stability of chilled breast and thigh of broilers were measured based on Rostami et al. (2017). All blood constitutes measurements were based on Jahanpour et al. (2013). Intestinal histomorphology traits were measured based on conventional protocols. Ileal segments (approximately 2 cm) were taken from midway between Meckel’s diverticulum and the ileocecal junction. These segments were immediately flushed twice with phosphate buffer saline (PBS) to remove luminal digesta. Tissue samples were fixed in 10 mL of fresh formalin buffer/L, dehydrated, cleared, and embedded in paraffin. Sections were cut to a thickness of 6 μm, placed on glass slides, stained with hematoxylin-eosin (Kiernan 2008), and examined by light microscopy. Villi having a lamina propria were randomly selected on each slide. Villus height was defined as the distance from the tip to the base,

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**Table 2. Chemical composition and amino acid profile of earthworm and vermi-humus.**

| Nutrient analysis | Earthworm | Vermi-humus |
|-------------------|-----------|-------------|
| Dry matter, %     | 91.00     | –           |
| Crude protein, %  | 65.68     | 7.27        |
| Metabolizable energy, kcal/kg | 3258 | – |
| Crude fibre, %    | 7.03      | –           |
| Calcium, %        | 0.45      | 8.97        |
| Phosphorus, %     | 1.22      | 0.70        |
| Humic acid, %     | –         | 1.86        |
| Fulvic acid, %    | –         | <0.10       |
| Methionine, %     | 1.20      | –           |
| Cysteine, %       | 0.95      | –           |
| Methionine + Cysteine, % | 2.15 | – |
| Lysine, %         | 4.44      | –           |
| Threonine, %      | 2.99      | –           |
| Arginine, %       | 4.41      | –           |
| Isoleucine, %     | 2.95      | –           |
| Leucine, %        | 5.02      | –           |
| Valine, %         | 3.22      | –           |
| Hystidine, %      | 1.74      | –           |
| Phenilalanine, %  | 2.72      | –           |
| Glycine, %        | 3.46      | –           |
| Cerie, %          | 2.94      | –           |
| Proline, %        | 2.41      | –           |
| Alanine, %        | 3.44      | –           |
| Asparagine, %     | 6.54      | –           |
| Glutamine, %      | 8.76      | –           |
excluding the intestinal crypt, and crypt depth was defined as the distance from the villus base to the muscularis layer, not including the intestinal muscularis. The villus height/crypt depth ratio was calculated.

**Statistical analysis**

Data were analysed using SAS by analysis of variance using a completely randomised design (CRD) using a one-way ANOVA procedure. The Tukey test was used for pairwise comparison between treatments if the initial test result was significant at \( p < 0.05 \). Statements of significance were based on \( p < 0.05 \).

**Results and discussion**

**Performance**

During the initial period (0 to 14 d), birds that were fed the control diet (0EW0VH) and the 1EW2VH diet showed higher feed consumption (\( p = 0.005 \)) and had worse feed conversion (\( p = 0.034 \)) as shown in Table 3. However, these differences were not observed during the growth phase (15 to 28 d) nor the final phase (29 to 42 d), reflecting the absence of statistical differences, throughout the experimental period (1 to 42 d). In the initial period (0 to 14 d) the inclusion of 1% EW and its combination with 1% VH, resulted in a decrease in feed consumption and lower feed conversion. However, this decrease was not seen in the other evaluated periods. According to Prayogi (2011) and Bahadori et al. (2017) decrease in feed consumption may occur with the use of EW because of the lack of some essential nutrients. In addition, it seems there is decreased palatability of the diet because of the presence coleomic fluid (Ngoc et al. 2016) and higher levels of arginine and cysteine which can reduce the appetite of the broiler chickens (Prayogi 2011). However, in this study, feed consumption was not decreased even with the highest levels of EW and VH in the diet when 1–42 d period was considered.

No differences were observed for body weight gain (BWG) during any experimental phases, nor body weight of birds at 42 d (\( p = 0.135 \)). Likewise, Bahadori et al. (2017) observed that diets with different levels of EW and VH had no effect on weight gain. On the other hand, Nalunga et al. 2021 found a quadratic and cubic effect on BWG when EW (1%, 3%, 5% and 7% replacing fish meal) supplementation was increased. Perhaps, the difference between their results and the present study could be due to the different kinds of EW and the absence of VH. In their study, the broiler chickens were fed with EW from _Eudrilus eugeniae_, unlike in this study where EW from _Eisenia foetida_ was used.

Although there were numerical differences between treatments for the European Production Index, they were not statistically significant. With regard to the cost to produce 1 kg of live weight of broiler chicken, 0EW0VH was the cheapest and 1EW2VH the most expensive diet (\( p = 0.001 \)). The results were consonant to the study of Bollido (2020) that the effects of supplementation of vermi meal in commercial diets at 2%, 3%, and 5% had negative results in profit and return of investment. However, these results should not be seen separately, since the productive efficiency of a flock must not only take into account its performance.

**Table 3.** Effect of different amounts of earthworm meal (EW) and vermi-humus (VH) on broiler chicken performance.

| Treatments | 0EW0VH | 1EW0VH | 1EW1VH | 1EW1.5VH | 1EW2VH | SEM | \( p \) |
|------------|--------|--------|--------|---------|--------|-----|------|
| Feed intake, g/d |        |        |        |         |        |     |      |
| 0–14 d     | 33.570a| 29.810bc| 28.380c| 32.490ab| 33.680a| 0.033| .005 |
| 15–28 d    | 83.410 | 89.230 | 82.400 | 91.540  | 96.610 | 2.020| .137 |
| 29–42 d    | 160.360| 146.890| 158.870| 159.740 | 164.360| 1.227| .416 |
| 1–42 d     | 92.450 | 94.640 | 89.880 | 94.590  | 98.220 | 1.045| .128 |
| Body weight gain, g/d |        |        |        |         |        |     |      |
| 0–14 d     | 23.540 | 21.940 | 22.350 | 22.890  | 21.520 | 0.329| .336 |
| 15–28 d    | 51.820 | 53.900 | 53.420 | 54.320  | 56.130 | 0.635| .301 |
| 29–42 d    | 83.120 | 78.130 | 83.310 | 78.900  | 76.780 | 1.172| .274 |
| 1–42 d     | 54.220 | 51.320 | 52.940 | 52.040  | 51.470 | 0.410| .137 |
| Feed conversion, g/g |        |        |        |         |        |     |      |
| 0–14 d     | 1.420ab| 1.360b | 1.270a | 1.420ab | 1.580a | 0.033| .034 |
| 15–28 d    | 1.610  | 1.650  | 1.540  | 1.680   | 1.710  | 0.025| .280 |
| 29–42 d    | 1.930  | 2.110  | 1.910  | 2.030   | 2.150  | 0.036| .118 |
| 1–42 d     | 1.790  | 1.840  | 1.700  | 1.820   | 1.910  | 0.026| .136 |
| Body weight, 42 d-g |        |        |        |         |        |     |      |
| EPI*       | 268.800| 248.000| 276.000| 253.800 | 242.400| 4.940| .125 |
| Feed cost/kg BW US$/kg | 0.630a | 0.890bc| 0.820a | 0.890bc | 0.950c | 103.710| .001 |

*Means within each column of treatments with no common superscript differ significantly at \( p < 0.05 \).

*EPI: European production index; SEM: Standard Error of Means.
**Blood constituents**

The results of blood analysis of broilers-fed diets supplemented with EW and VH are shown in Table 4. Haemoglobin levels were higher in treatment groups 0EW0VH and 1EW2VH ($p = .001$). There were no statistical differences in total protein, haematocrit, albumin, globulin, glucose, total cholesterol, triglyceride, uric acid, calcium, nor phosphorus between the groups.

EW and VH supplementation decreased haemoglobin levels when compared to the control diet. At levels of 0, 1 and 2.5% humic acid in the broiler diet, Rath et al. (2006) observed a decrease in the levels of protein, albumin, glucose and calcium, blood phosphorus with the use of 2.5% dietary humic acid. According to these authors, the reduction in mineral levels may be due to the binding effect of humic acid, due to a large number of side chains of carboxylic acid. We did not observe this effect in the present study. In addition, Bahadori et al. (2017) observed an increase in protein and albumin levels, as well as a reduction in cholesterol level, and a quadratic effect on haemoglobin level with different inclusion of EW and VH in the diet. According to these authors, the effects of EW on blood protein and albumin may be due to the action of proteolytic enzymes and their precursors. Zang et al. observed increased globulin concentrations in broilers supplemented with 5% EW in the diet, suggesting that EW may improve the bird's antioxidant system. Prakash et al. (2007) state that EW has antioxidant potential and hepatoprotective properties. Also, Sun et al. (2020) found that layer hens fed EW had increased protein, globulin, and albumin levels, but decreased triglycerides, cholesterol, and glucose suggesting that EW could be a potential dietary substitute for these birds.

| Treatments       | 0EW0VH | 1EW0VH | 1EW1VH | 1EW1.5VH | 1EW2VH | SEM % |
|------------------|--------|--------|--------|---------|--------|-------|
| Total protein, g/dL | 5.130  | 4.740  | 4.800  | 4.810   | 4.550  | 0.079 .247 |
| Haemoglobin, g/dL | 12.580a| 11.120d| 12.030bc| 11.640c| 12.500ab| 0.130 .001 |
| Haematocrit, g/dL | 34.800 | 33.400 | 34.200 | 33.200  | 33.000 | 0.470 .756 |
| Albumin, g/dL    | 3.010  | 2.800  | 2.860  | 2.850   | 2.720  | 0.036 .117 |
| Globulin, mg/dL  | 2.070  | 1.940  | 1.940  | 1.960   | 1.950  | 0.034 .744 |
| Glucose, mg/dL   | 131.800| 127.930| 128.090| 129.770 | 130.480| 1.910 .971 |
| Total cholesterol, mg/dL | 154.620| 151.670| 149.830| 148.390| 138.160| 3.080 .541 |
| Triglyceride, mg/dL | 156.010| 153.200| 156.980| 148.390| 154.230| 1.900 .354 |
| Uric acid, mg/dL | 5.920  | 5.230  | 5.340  | 5.290   | 5.320  | 0.113 .354 |
| Calcium, mg/dL   | 8.380  | 8.170  | 8.360  | 8.310   | 7.540  | 0.153 .412 |
| Phosphorus, mg/dL| 5.630  | 5.290  | 5.350  | 5.180   | 5.880  | 0.159 .703 |

Means within each column of treatments with no common superscript differ significantly at $p < .05$.

**Immunity**

Antibodies to Newcastle disease and Influenza did not differ statistically between treatments (Table 5). However, the titres for Haemagglutinin, ($p = .041$), cell-mediated immunity by contact with PHA-M ($p = .001$) and DNCB ($p = .001$) were lower in birds fed the control diet.

The titres of anti-Newcastle and anti-Influenza antibodies were not influenced by the addition of EW and VH in the diet. However, the other immunological parameters improved with their use, corroborating the findings of Bahadori et al. (2017). In addition, Hesami et al. (2021) found a significant increase in the cell-mediated immune response to DNCB when breeder quail were fed a combination with 2.5% EW and 0,8% VH.

According to Popovic et al., the components of the humoral response such as lecithins, antimicrobial
peptides, and proteases are affected by EW. Humic acid binds to the epithelium and forms a protective epithelial layer against toxins and intestinal infections, in addition to its anti-inflammatory, immune stimulatory and antiviral action (Tohid et al. 2010).

**Ileum histomorphology and microbiota**

There were no statistical differences (Table 6) between treatments for villus diameter ($p = .153$). The greatest depth of crypt and the lowest ratio of villus height and crypt depth were observed for birds fed the 1EW0VH diet ($p = .001$ and $p = .015$, respectively). The greatest villus height was found in birds receiving the 1EW2VH diet ($p = .019$) and the lowest villus area for birds receiving the 1EW1VH ($p = .006$). Birds fed the 1EW0VH diet had a higher incidence of *Escherichia coli* and Lactic acid bacteria ($p = .003$ and $p = .001$, respectively). Birds receiving control diets had larger numbers of aerobic bacteria ($p = .001$) and the birds in treatment group 1EW2VH, had more aerobic spore bacteria ($p = .001$).

Morphologic assessment of the ileum gives an indication of the animals’ intestinal health. The addition of 1% EW in the diet resulted in greater villus height. However, there was no increase in villus diameter. Bahadori et al. (2017) found no statistical differences in intestinal morphology of birds supplemented with EW and VH in the diet.

Birds fed EW and VH diets had different intestinal microbiota, with decreased aerobic bacteria, and the use of 2% of VH increased the amount of *E. coli*; however, improved lactic acid bacteria, when compared to birds fed a control diet. It is well known that microbiota is essential to maintain gastrointestinal tract homeostasis and interact with the immune system present in there. Further, gut microorganisms are able to help in nutrient digestion and inhibit the presence of harmful bacteria (Borda-Molina et al. 2018).

**Relative weight of carcass components and gastrointestinal organs**

Birds receiving the 1EW1VH diet ($p = .041$) had an increase in proportional gizzard weight. However, no statistical differences were observed between treatments for eviscerated carcass weight, breast, thighs, wings, abdominal fat, pancreas, heart, and liver percentage (Table 7).

The use of different levels of EW and VH did not affect the carcass yield and its components, the only changes being an increase in the relative weight of gizzard in birds supplemented with 1% EW and 1% VH in the diet. Likewise, Bahadori et al. (2017) found no statistical differences for carcass characteristics of broilers fed different levels of EW and VH in the diet. However, our results partially disagree with Gunya et al. (2019) who provided up to 10% EW in the broiler diet and observed a decrease in wing yield with the

Table 6. Effect of different amounts of earthworm meal (EW) and vermi-humus (VH) on ileum morphology and microbiota, CFU/g.

| Treatments | 0EW0VH | 1EW0VH | 1EW1VH | 1EW1.5VH | 1EW2VH | SEM | Percentage |
|------------|--------|--------|--------|----------|--------|-----|------------|
| Morphology |        |        |        |          |        |     |            |
| Villus height, μm | 733.910ab | 769.990ab | 640.960b | 662.860ab | 817.270a | 21.380 | .019 |
| Villus diameter, μm | 94.940 | 93.420 | 83.220 | 72.720 | 88.880 | 1.146 | .153 |
| Crypt depth, μm | 38.120b | 50.640a | 35.740d | 34.040b | 37.190b | 1.460 | .001 |
| Villus area, μm² | 36.664a | 35.865a | 26.690b | 23.732b | 36.444a | 1606.830 | .006 |
| Villus height/villus depth | 20.490a | 15.240b | 18.380ab | 19.530a | 19.050a | 0.701 | .015 |
| Microbiota |        |        |        |          |        |     |            |
| *Escherichia coli* | 3.740c | 4.870a | 3.690c | 4.240bc | 4.330b | 0.131 | .003 |
| Lactic acid bacteria | 6.260b | 6.630b | 6.410bc | 5.970d | 6.480ab | 0.063 | .001 |
| Aerobic bacteria | 6.460a | 5.650b | 5.640d | 5.740b | 5.890b | 0.890 | .001 |
| Aerobic spore bacteria | 3.990b | 4.050b | 3.920c | 3.840c | 4.210a | 0.036 | .001 |

SEM: Standard Error of Means.

Table 7. Effect of different amounts of earthworm meal (EW) and vermi-humus (VH) on relative weight of carcass components and gastrointestinal organs, %.

| Treatments | 0EW0VH | 1EW0VH | 1EW1VH | 1EW1.5VH | 1EW2VH | SEM | Percentage |
|------------|--------|--------|--------|----------|--------|-----|------------|
| Eviscerated carcass | 61.310 | 62.760 | 61.990 | 60.330 | 62.240 | 0.5120 | .645 |
| Breast | 26.440 | 29.260 | 23.060 | 28.390 | 28.270 | 1.050 | .379 |
| Thighs | 25.310 | 24.860 | 24.950 | 24.430 | 23.400 | 0.225 | .058 |
| Wings | 3.440 | 3.570 | 3.410 | 3.440 | 3.390 | 0.035 | .540 |
| Abdominal fat | 2.240 | 1.770 | 1.790 | 1.540 | 1.990 | 0.132 | .568 |
| Pancreas | 0.200 | 0.200 | 0.200 | 0.210 | 0.180 | 0.070 | .716 |
| Gizzard | 2.440b | 2.580ab | 2.970c | 2.690bc | 2.350b | 0.071 | .041 |
| Heart | 0.380 | 0.480 | 0.460 | 0.410 | 0.460 | 0.013 | .152 |
| Liver | 1.810 | 2.120 | 2.420 | 2.390 | 2.220 | 0.069 | .025 |

Means within each column of treatments with no common superscript differ significantly at $p < .05$.

SEM: Standard Error of Means.
use of 10% EW and an increase in the yield of drumsticks with the use of 3% EW. However, the use of 10% EW in the diet increased the relative weight of gizzard. Gunya et al. (2019) speculated that the increase in the percentage of gizzard weight is due to hyperplasia of the gizzard as a result of increased mechanical activity dealing with the high dietary protein levels.

**Malondialdehyde (MDA) content**

The highest values for MDA (Table 8) were observed in fresh breast ($p = .001$), fresh thigh ($p = .001$), and stored thigh ($p = .003$) of birds in the 1EW2VH treatment group. However, the highest level of malondialdehyde in the stored breast was seen in birds fed the 1EW0VH diet ($p = .001$).

Good oxidative stability is essential to avert or delay possible progress rancidification in meat (Hudák et al. 2021). MDA levels are indicative of muscle lipid oxidation, particularly in meat and fish products, which tends to increase with increasing meat storage time. Our findings are in concordance with those of Bahadori et al. (2017) who observed an increase in MDA levels with the supplementation of EW and VH in the diet. In the present study, MDA levels were lower than 1 mg/kg. These results are important because MDA levels above 1 mg/kg can be associated with the beginning of organoleptic perceptibility of lipid oxidation and could be considered ‘rancid’ (Uzun Özcan et al. 2018).

**Breast and thigh meat quality**

No statistical differences were observed between treatments for odour, colour, texture, succulence, taste, and overall acceptance, for either breast or thigh meat (Table 9). Bahadori et al. (2017) observed a reduction in water retention in the thigh musculature, which was not seen in breast musculature and may be due to different characteristics of the musculature in the two sites. Gunya et al. (2019) observed an increase in water loss from the breast in broilers receiving 10% EW in the diet, which may be a result of the muscle’s ability to retain water (Abu et al. 2015).

**Conclusions**

Although there was no difference in the performance of birds, the use of EW and until 2% of VH has great
potential to improve the immune response of broilers and reduce aerobic intestinal bacteria. These characteristics may be of high value in intensive poultry production systems where birds experience significant health challenges.

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Ethical approval

The experimental procedures were approved by the Ethics Committee of Sanandaj Branch, Islamic Azad University.

Disclosure statement

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

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ORCID

Mohammad-Amir Karimi-Torshizi http://orcid.org/0000-0002-8141-4904
Alireza Seidavi http://orcid.org/0000-0002-1903-2753
Lucio Francelino Araujo http://orcid.org/0000-0003-1648-5949

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