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Use of earthworm meal with vermi-humus in diet for laying quail

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Abstract – The objective of this work was to evaluate the effect of dietary earthworm (*Eisenia fetida*) meal (EW), associated with vermi-humus (VH), on the performance, egg characteristics, immunity, and blood constituents of laying quails. A total of 336 female quails (163.94±1.5 g), with 30 days of age, was distributed in 7 treatments and 4 replicates of 12 birds during 42 days. The following treatments were evaluated: control diet without the inclusion of VH and EW; diet with the inclusion of only 0.8% VH; and diets with 0.8% VH supplemented with different EW levels (0.5, 1.0, 1.5, 2.0, and 2.5%). A greater body weight, weight gain, and feed intake were obtained with 1.5% EW; a higher egg productivity, with 0.5–1.0% EW; and a higher egg mass, with 0.5% EW. The inclusion of 2.5% EW reduced eggshell weight and thickness. Levels of 1.0–2.0% EW decreased malondialdehyde in the eggs, whereas 2.0% EW reduced cholesterol content. Higher blood cell volume and antibody titer were obtained with 1.0% EW, whereas higher total protein, globulin, and calcium were obtained with 0.5% EW. Levels of 1.0–1.5% EW + 0.8% VH improve egg production and characteristics, as well as the humoral response of quails, whose performance is not affected.

Index terms: alternative feed, egg quality, humoral response, livestock, nutrition, poultry farming.

Uso de farinha de minhoca com vermi-húmus em dieta para codornas de postura

Resumo – O objetivo deste trabalho foi avaliar o efeito de farinha de minhoca (EW) (*Eisenia fetida*) dietética, associada ao vermi-húmus (VH), sobre o desempenho, as características dos ovos, a imunidade e os constituintes do sangue de codornas poedeiras. Um total de 336 codornas fêmeas (163,94±1,5 g), com 30 dias de idade, foi distribuído em 7 tratamentos e 4 repetições de 12 aves, por 42 dias. Foram avaliados os seguintes tratamentos: dieta controle sem a inclusão de VH e EW; dieta com inclusão somente de 0,8% VH; e dieta com 0,8% VH suplementada com diferentes níveis de EW (0,5, 1,0, 1,5, 2,0 e 2,5%). Foram obtidos maiores peso vivo, ganho de peso e consumo de ração com 1,5% de EW; maior produção de ovos com 0,5–1,0% de EW; e maior massa de ovos com 0,5% de EW. A inclusão de 2,5% EW reduziu o peso e a espessura da casca do ovo. Níveis de 1,0–2,0% de EW diminuíram o malonaldeído nos ovos, enquanto o de 2,0% de EW reduziu o conteúdo de colesterol. Foram obtidos maiores volume de células sanguíneas e título de anticorpos com 1,0% de EW, enquanto maiores proteína total, globulina e cálcio foram obtidos com 0,5% de EW. Níveis de 1,0–1,5% + 0,8% de VH melhoram a produção e as características dos ovos, bem como a resposta humoral das codornas, cujo desempenho não é afetado.

Termos para indexação: alimento alternativo, qualidade do ovo, resposta humoral, animais de criação, nutrição, avicultura.

Introduction

Currently, one of the greatest challenges in animal production is to adapt diets to ensure maximum productivity with the lowest feed cost. In this sense, the inclusion of alternative foods that can replace corn and soybean meal are important. A potential alternative feedstuff is earthworm meal (EW) (Bahadori et al., 2017), whose greatest advantage is its efficiency in reproducing annelids in organic waste (Edwards, 1985), as well as its ease of being processed and stored (Rodriguez-Campos et al., 2014). Earthworms can be produced using fresh manure from cattle, swine, goat, and chicken, besides leaves, sawdust, rice hull, rice bran (Barcelo, 1988), and several fruit and vegetable wastes used as feedstock (Conti et al., 2019). Therefore, stimulating the adoption of this type of compost in animal feed is directly related to sustainability.

In their review paper, Parolini et al. (2020) found that EW contains a higher crude protein (CP) content in dry matter (DM) than soybean meal and fishmeal, as well as a lysine content similar to that of fishmeal, but with a lower digestible energy content. According to Bahadori et al. (2017), EW contains 91% DM, 66% CP in DM, 3,248 kcal kg^{-1} metabolizable energy, 7.0% fat, a calcium:phosphorus ratio of 0.37, 2.15% methionine + cystine, and 4.4% lysine. Prayogi (2011) observed that increasing EW up to 10%, as a replacement for fishmeal in diets, improved the performance of male quails. For laying hens, Son (2009) reported that the use of up to 0.6% dietary EW improved egg laying and quality, particularly the ω-6/ω-3 fatty acid ratio in the egg yolk. Other authors also found positive effects when combining EW with vermi-humus (VH) (Bahadori et al., 2017; Hesami et al., 2020).

VH is a source of humic acid, which is formed from the decomposition of organic materials in the soil. This acid can be used as a food additive because it inhibits the growth of bacteria and fungi and also prevents the occurrence of high mycotoxin levels in the feed (Rezaeipour et al., 2014). Ozturk et al. (2012) concluded that VH can improve the performance, welfare, and immune system of broiler chicken, besides preventing intestinal diseases (Bahadori et al., 2017). Therefore, mixing EW, a feedstuff with a high crude protein level, and VH, which can improve the profile of intestinal microbiota, can be interesting to improve animal performance and immune status. In fact, studies have already been carried out using these ingredients in diets for broilers (Bahadori et al., 2017) and breeding quails (Hesami et al., 2020). However, there are no known reports on the effect of these ingredients on the blood constitutes and egg quality of laying quails, which is important since the use of alternative protein sources could impair bird laying performance due to nutritional imbalances (Marono et al., 2017).

The objective of this work was to evaluate the effect of dietary EW, associated with VH, on the performance, egg characteristics, immunity, and blood constituents of laying quails.

Materials and Methods

The experimental procedures were approved by the ethics committee of Islamic Azad University (protocol number 13940629). A total of 336 female Japanese quails (*Coturnix japonica* Temmink & Schlegel, 1849), weighing 163.94±1.5 g and aged 30 days, were equally assigned to 28 cages (0.2×0.2 m), positioned at the center of a thermostatically-controlled poultry barn with side-wall curtains, during 42 days. The cage floors were covered with wood shavings as bedding. Throughout the entire experimental period, ambient temperature was maintained at 22°C within the barn, using supplemental heat from thermostatically-controlled gasoline rocket heaters, and misting was used to maintain relative humidity at 70%. The lighting program in the barn was: 20 lux, using 20 W fluorescent lamps in ceiling fixtures; and a photoperiod of 16 hours light:8 hours dark until the end of the experiment. The barn was tunnel ventilated, with air circulation being facilitated by fans mounted on the wall at one end of the barn. Quails had free access to the diets and clean fresh water during the entire experimental period.

A single-phase feeding program was used throughout the experiment. The chemical composition of the tested feedstuffs was determined (Table 1). The assessed treatments were: control diet, with 0% EW and 0% VH; a diet containing 0.8% VH; and five diets containing 0.8% VH supplemented with different EW levels (0.5, 1.0, 1.5, 2.0, and 2.5%) (Table 2). VH and EW are commercial products that were obtained from a local supplier (Amizeh Tabiat Co, Tehran, Iran). All diets were formulated to meet or exceed the recommendations for quails (NRC, 1994).

The experimental design was completely randomized, with 7 treatments and 4 replicates of 12 birds each. The mean body weights of the quails were similar across the evaluated groups.

Body weight was measured on the first and last days of the experiment, whereas feed intake was determined throughout the entire experimental period. The eggs were manually collected, and their number and weight were recorded daily in each cage. Egg productivity (%), ratio between number of produced eggs and feed, weight of produced eggs, ratio between weight of produced eggs and feed, and egg mass were calculated for each replicate during the experiment.

Four eggs of each replicate were collected at the end of the experimental period and were individually opened; yolks were separated from the albumen and then weighed. Shell thickness (with shell membrane) was measured, using a micrometer, at three locations on the eggs – air cell, equator, and sharp end. Eggshell weight (plus adhering membranes) was determined after eggs were washed and dried. A tripod micrometer was used to measure albumen height, whereas yolk color was determined using an egg yolk color fan. Yolk malondialdehyde (MDA) and egg cholesterol were measured using the standard protocols of commercial laboratory kits (Pars Azmun Co., Tehran, Iran).

On day 58, one bird per replicate with a body weight close to the average of the pen was selected for blood collection. Blood samples (2.0 mL per bird) from the wing vein were collected into heparinized tubes. Two hours after collection, blood samples were centrifuged at 1,300 × *g*, for 15 min, at room temperature. Plasma was stored in microcentrifuge tubes at -20ºC until analysis, using specific kits for glucose, globulin, albumin, triglyceride, uric acid, calcium, and phosphorus (Pars Azmun Co., Tehran, Iran).

Quails were vaccinated against Newcastle and influenza. Both vaccines were administrated by eye drop at 49 days of age on 2 birds per replicate and 8 birds per treatment. The quails also received sheep red blood cell (SRBC) injections at 42 and 49 days of age. The humoral immune response of the birds was evaluated by collecting blood at 63 days of age, to determine antibody titers against Newcastle and influenza, and at 49 and 56 days of age, to determine the antibody titer against SRBCs. For the analyses, a hemagglutination assay was carried out in serum, based on Pourhossein et al. (2015).

Data were subjected to the analysis of variance (Anova) using the general linear model procedures of the SAS/STAT, version 9.2, software (SAS Institute Inc., Cary, NC, USA). The Duncan multiple range test was used for pairwise comparison between treatments if the result of Anova was significant ($p \leq 0.05$). Polynomial (linear) contrasts were used to examine rate responses due to increasing amounts of EW in the diet. When data were not normally distributed or when some of the required assumptions for the Anova were violated, data were transformed before the analysis, following the guidelines of Büchse et al. (2007). Results were significant if $p \leq 0.05$.

Table 1. Chemical composition of the used earthworm (*Eisenia fetida*) meal and vermi-humus.

Properties	Earthworm meal	Vermi-humus
Dry matter (%)	91.0	-
Crude protein (%)	65.68	7.27
Crude fat (%)	-	0.14
Metabolizable energy (kcal kg^{-1})	3,258	-
Crude fiber (%)	7.03	-
Ash (%)	-	64.86
Calcium (%)	0.45	8.97
Phosphorus (%)	1.22	0.70
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	-
Histidine	1.74	-
Phenylalanine (%)	2.72	-
Glycine (%)	3.46	-
Serine (%)	2.94	-
Proline (%)	2.41	-
Alanine (%)	3.44	-
Asparagine (%)	6.54	-
Glutamine (%)	8.76	-
Humic acid (%)	-	1.86
Fulvic acid (%)	-	<0.10

Results and Discussion

Compared with the control, 0.8% VH did not influence the performance of laying quails, which was also not affected by the inclusion of up to 1.0% EW + 0.8% VH in the diets. In addition, there was no influence of the use of EW on bird mortality and egg weight.

Quails fed 1.5% EW showed a greater body weight at 70 days of age, weight gain, and feed intake than those fed 2.0 and 2.5% EW (Table 3). However, no differences were observed regarding these variables when comparing the different levels of EW with the control treatment. With 0.5% EW, egg production and mass were similar but slightly greater than those of the control, when compared with the higher inclusion levels. Moreover, the use of 1.5 and 2.5% EW increased the feed conversion of the quails.

The obtained results are indicative that low levels – between 0.5 and 1.0% – of EW, associated with VH, should be used in laying quail diets in order to not decrease egg production. This result

Table 2. Ingredients and chemical composition of the experimental diets with different amounts of earthworm (*Eisenia fetida*) meal (EW) and vermi-humus (VH).

Ingredient (%)	Experimental diet						
	Control	0.0 EW+ 0.8 VH	0.5 EW+ 0.8 VH	1.0 EW+ 0.8 VH	1.5 EW+ 0.8 VH	2.0 EW+ 0.8 VH	2.5 EW+ 0.8 VH
Corn	50.48	49.74	49.07	48.64	48.12	47.62	47.91
Soybean oil	1.92	1.89	2.17	2.15	2.14	2.14	2.14
Soybean meal 44%	32.36	32.41	32.50	32.54	32.59	32.64	32.68
DL-methionine 99%	0.29	0.28	0.28	0.27	0.26	0.25	0.24
L-lysine-hydrochloride 99%	0.06	0.03	0.03	0.00	0.00	0.00	0.00
Meat meal 55%	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Sodium bicarbonate	0.14	0.12	0.12	0.09	0.10	0.09	0.09
Dicalcium phosphate	0.19	0.17	0.14	0.12	0.10	0.08	0.06
Calcium carbonate	6.89	6.88	6.71	6.70	6.70	6.69	6.69
Sodium chloride	0.17	0.18	0.18	0.19	0.19	0.19	0.19
Vitamin/mineral premix(1)	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Earthworm(2)	0.00	0.00	0.50	1.00	1.50	2.00	2.50
Vermi-humus	0.00	0.80	0.80	0.80	0.80	0.80	0.80
Nutrient calculated							
Metabolizable energy (kcal kg^{-1})	2,800	2,800	2,800	2,800	2,800	2,800	2,800
Crude protein (%)	22.20	22.51	22.53	22.84	23.15	23.46	23.77
Lysine (%)	1.23	1.20	1.24	1.18	1.18	1.18	1.18
Methionine (%)	0.61	0.61	0.61	0.60	0.59	0.58	0.57
Methionine + cystine (%)	0.98	0.97	0.96	0.96	0.96	0.93	0.92
Threonine (%)	0.84	0.84	0.84	0.84	0.84	0.72	0.66
Tryptophan (%)	0.23	0.23	0.22	0.21	0.21	0.22	0.20
Arginine (%)	1.38	1.38	1.37	1.36	1.38	1.27	0.18
Isoleucine (%)	0.91	0.91	0.91	0.91	0.92	0.77	0.72
Valine (%)	1.00	1.00	1.00	1.01	1.02	0.85	0.80
Leucine (%)	2.17	2.17	2.17	2.18	2.20	1.59	1.51
Calcium (%)	3.10	3.10	3.10	3.10	3.10	3.10	3.10
Available phosphorus (%)	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Potassium (%)	0.88	0.89	0.87	0.86	0.86	0.86	0.81
Chloride (%)	0.18	0.18	0.18	0.18	0.18	0.29	0.28
DCAB (mEq kg^{-1})(3)	244	244	234	225	219	209	198
Linoleic acid (%)	1.34	1.31	1.32	1.34	1.33	1.39	1.41
Ether extract (%)	3.43	3.75	3.42	3.08	2.87	4.63	5.69
Crude fiber (%)	3.62	3.61	3.51	3.40	3.35	3.55	3.37

(1)Provided per kilogram of feed: 3,600,000 IU vitamin A; 800,000 IU vitamin D3; 7.2 g vitamin E; 0.8 g vitamin K3; 0.7 g thiamine; 2.64 g riboflavin; 4.0 g niacin; 3.92 g calcium pantothenate; 1.176 g pyridoxine; 0.4 g folic acid; 6.0 mg vitamin B_{12}; 40 mg biotin; 100 mg choline chloride; 40 mg manganese; 20 mg iron; 33.88 mg zinc; 4.0 mg copper; 0.4 mg iodine; and 0.08 mg selenium. (2)Obtained from Amize Tabiat Co., a local supplier, located in Tehran, Iran. (3)Dietary cation-anion balance.

was similar to those found by Hesami et al. (2020), who recommended levels between 1.0 and 1.5% for Japanese breeder quails.

In the literature, the effects of EW on bird feed intake and weight gain vary. Prayogi (2011) and Struti et al. (2018) observed a higher weight gain when using 10% earthworm meal in quail diets. For broilers, Zang et al. (2018) found a greater weight gain and feed intake when using 3.0 and 5.0% earthworm powder. However, Bahadori et al. (2017) reported a decrease in the growth performance of broiler chickens with an increasing amount of EW up to 3.0% in dry matter. These results can be attributed to the different characteristics of the used EW, such as age of the annelids and ways of processing the feedstuff.

Regarding VH, there are reports that this food increases the growth of broilers (Ozturk et al., 2012; Jad'uttová et al., 2019). However, in the present study, the diet containing only VH did not influence quail performance, probably because the tested amount was too low to affect the studied variables. These results are similar to those found by Nagaraju et al. (2014) when testing 0.05, 0.075, and 0.1% humic acid.

In the present study, the feed intake of the experimental diets by the birds did not differ from that of the control, and the crude protein content of the diets varied between 22.2 and 23.8% (Table 2). Regarding EW, only low levels (1.0%) did not impair the productive performance of the birds. The inclusion of up to 2.5% EW reduced the amount of arginine in 87%, leucine in 30%, and threonine, valine, and isoleucine in 20% (Table 2). This suggests that, for the use of higher EW levels, it may be necessary to supplement the diet with other amino acids, in addition to lysine and methionine, following the ideal protein concept in the formulation of diets (Emmert & Baker, 1997). This is important since the inclusion of alternative foods in farm animal diets can cause a nutritional imbalance, especially of amino acids.

The inclusion of EW + VH reduced linearly albumen height, eggshell weight, and egg MDA concentration and cholesterol (Table 4). Moreover, compared with the control, the inclusion of 2.5% EW + 0.8% VH reduced eggshell thickness and weight. The inclusion of 1.0–2.0% EW + 0.8% VH reduced MDA concentration in eggs, while that of 2.0% EW reduced cholesterol content. However, there was no effect of the tested feedstuffs on the weight and color of the yolk, although the inclusion of high amounts of EW impaired the physical quality of the eggs. This result is probably related to the nutritional imbalance caused by the high inclusion of this meal. According to Kaur et al. (2008), the wide variation in the composition and amount of protein and/or energy present in feedstuffs can influence bird performance.

The lack of changes in yolk color due to the evaluated diets was expected since there was no detectable presence of carotenoids in EW or VH and the reduction in corn meal was not significant. Franco & Sakamoto (2012) concluded that yolk color can vary

Table 3. Effect of different dietary amounts of earthworm meal (*Eisenia fetida*) (EW) and vermi-humus (VH) on the performance of laying quails from 30 to 72 days of age(1).

Parameter	Control	0.0 EW+ 0.8 VH	0.5 EW+ 0.8 VH	1.0 EW+ 0.8 VH	1.5 EW+ 0.8 VH	2.0 EW+ 0.8 VH	2.5 EW+ 0.8 VH	SEM(2)	p-value	Linear(3)
Mortality (%)	0.44	0.22	1.16	0.45	0.22	0.44	0.00	0.51	0.10	0.19
Body weight at 30 days of age (g)	164.16	164.75	161.50	164.75	163.75	163.51	165.16	3.35	0.79	0.75
Body weight at 70 days of age (g)	212ab	211ab	211ab	208abc	216a	202c	206bc	3.83	<0.01	0.01
Weight gain (g per day)	48.48ab	46.50ab	49.50ab	43.25ab	52.50a	39.09b	41.75b	5.52	0.03	0.75
Feed intake (g per day)	25.50ab	24.75b	25.50ab	27.07a	27.25a	25.05b	24.50b	0.83	<0.01	0.73
Egg productivity (%)	42.86ab	38.25ab	48.16a	45.50a	29.37b	39.23ab	30.38b	6.20	<0.01	<0.01
Egg weight (g)	11.86	11.54	11.97	11.32	11.72	11.74	10.78	0.70	0.05	0.11
Egg mass (g)	5.08ab	4.40abc	5.79a	5.09ab	3.46bc	4.62abc	3.27c	0.76	<0.01	<0.01
Feed conversion ratio	3.09b	3.37b	3.01b	3.34b	4.81a	3.33b	4.38a	0.44	<0.01	<0.01

(1)Means followed by different letters, in the lines, differ significantly by Duncan's test, at 0.05% probability. n=4. (2)Standard error of the mean. (3)Probability of a linear response to increasing levels of earthworm meal in the diet. The treatments were: control, basal diet without the inclusion of EW or VH (0 EW+0 VH); and diets containing 0.8% VH (0.8 VH) supplemented with 0, 0.5, 1.0, 1.5, 2.0, and 2.5% EW (0 EW, 0.5 EW, 1.0 EW, 1.5 EW, 2.0 EW, and 2.5 EW, respectively).

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from pale yellow to dark orange depending on the amount of carotenoids present in the bird diet.

The concentration of lipids increased from 3.43% in the control diet to 5.69% with the highest level of EW (Table 2). However, triglyceride levels in quail blood did not differ (Table 5), despite the decrease in the cholesterol in the eggs of the birds fed diets with high EW levels (Table 4). Likewise, Bahadori et al. (2017) observed that broiler chickens fed higher amounts of EW and VH had a lower blood cholesterol. Other authors also found that the decrease in blood cholesterol in broiler chickens is one of the effects of humic substances such as VH (Ozturk et al., 2012; Jad'uttová et al., 2019). However, since egg cholesterol is important for embryo and post-hatching development (Sutton et al., 1984), the use of higher EW levels in breeding needs to be better evaluated.

In the present work, the reduction of cholesterol in the eggs was accompanied by a reduction in MDA concentration. However, the increase in the amount of

Table 4. Effect of different dietary amounts of earthworm (*Eisenia fetida*) meal (EW) and vermi-humus (VH) on the egg quality of laying quails from 30 to 72 days of age[(1)].

Parameter	Control	0.0 EW+ 0.8 VH	0.5 EW+ 0.8 VH	1.0 EW+ 0.8 VH	1.5 EW+ 0.8 VH	2.0 EW+ 0.8 VH	2.5 EW+ 0.8 VH	SEM[(2)]	p-value	Linear[(3)]
Shell thickness (mm×10^{-2})	0.220ab	0.220ab	0.221ab	0.229a	0.222ab	0.224ab	0.212b	0.010	0.03	0.37
Albumen height (mm)	4.58	4.49	4.78	4.61	4.53	3.96	4.23	0.703	0.11	0.03
Yolk weight (g)	4.39	4.38	4.39	4.57	4.52	4.50	4.28	0.58	0.91	0.94
Shell weight (g)	1.04a	1.07a	1.05a	1.01ab	1.05a	1.09a	0.91b	0.11	<0.01	0.04
Yolk color	6.41	6.08	6.08	5.41	5.33	5.16	6.08	1.39	0.22	0.10
Malondialdehyde (μg g^{-1})	1.14a	0.62ab	0.59ab	0.50b	0.46b	0.50b	0.60ab	0.21	0.02	0.01
Cholesterol (mg dL^{-1})	216.39a	168.36ab	214.75a	165.98ab	187.04ab	150.40b	182.62ab	28.11	0.02	0.03

[(1)]Means followed by different letters, in the lines, differ significantly by Duncan's test, at 0.05% probability. n=4. [(2)]Standard error of the mean. [(3)]Probability of a linear response to increasing levels of earthworm meal in the diet. The treatments were: control, a basal diet without the inclusion of EW or VH (0 EW+0 VH); and diets containing 0.8% VH (0.8 VH) supplemented with 0, 0.5, 1.0, 1.5, 2.0, and 2.5% EW (0 EW, 0.5 EW, 1.0 EW, 1.5 EW, 2.0 EW, and 2.5 EW, respectively).

Table 5. Effect of different dietary amounts of earthworm (*Eisenia fetida*) meal (EW) and vermi-humus (VH) on the blood constituents of laying quails from 30 to 72 days of age[(1)].

Constituent	Control	0.0 EW+ 0.8 VH	0.5 EW+ 0.8 VH	1.0 EW+ 0.8 VH	1.5 EW+ 0.8 VH	2.0 EW+ 0.8 VH	2.5 EW+ 0.8 VH	SEM[(2)]	p-value	Linear[(3)]
Mean blood cell volume	39.75ab	40.75ab	45.0a	37.5b	44.0a	39.5ab	42.25ab	2.62	<0.01	0.57
Total protein (mg dl^{-1})	5.12bc	4.96c	5.90a	5.34bc	5.07bc	5.43abc	5.51ab	0.23	<0.01	0.05
Glucose (mg dl^{-1})	165.2	164.5	169.8	145.9	149.6	142.7	153.1	15.5	0.13	0.02
Globulin (mg dl^{-1})	1.92bc	1.68c	2.50a	2.12ab	2.04bc	2.20ab	2.44a	0.18	<0.01	<0.01
Albumin (g dl^{-1})	3.19	3.28	3.39	3.21	3.03	3.23	3.07	0.20	0.23	0.12
Triglyceride (mg dl^{-1})	290.7	254.1	292.2	292.4	201.3	205.7	312.3	81.5	0.33	0.57
Uric acid (mg dl^{-1})	5.97	5.51	6.43	5.86	5.64	5.54	6.75	0.61	0.06	0.33
Calcium (mg dl^{-1})	8.77b	9.31ab	10.73a	10.16ab	9.54ab	9.29ab	10.09ab	0.74	0.02	0.17
Phosphorus (mg dl^{-1})	5.46ab	5.72ab	4.58ab	6.13a	4.77ab	4.19b	4.42ab	0.84	0.02	0.01
Humoral immunity (antibody titer) (×$\log_2$)										
Anti-Newcastle	0.25	1.50	0.75	0.75	0.50	1.00	0.75	0.95	0.66	0.92
Anti-influenza	0.00c	0.00c	1.25ab	1.63a	1.25b	0.95ab	1.57a	1.01	<0.01	<0.01
Anti-sheep red blood cell	0.75c	2.00b	0.75c	1.75bc	1.50bc	3.00a	3.00a	1.13	0.04	<0.01

[(1)]Means followed by different letters, in the lines, differ significantly by Duncan's test, at 0.05% probability. n=4. [(2)]Standard error of the mean. [(3)]Probability of a linear response to increasing levels of earthworm meal in the diet. The treatments were: control, a basal diet without the inclusion of EW or VH (0 EW+0 VH); and diets containing 0.8% VH (0.8 VH) supplemented with 0, 0.5, 1.0, 1.5, 2.0, and 2.5% EW (0 EW, 0.5 EW, 1.0 EW, 1.5 EW, 2.0 EW, and 2.5 EW, respectively).

lipids in the diet (Table 2), associated with a reduction in egg cholesterol (Table 4), suggests a lower lipid content in the eggs. In this case, further studies should be conducted with EW and VH to investigate the effects of these feedstuffs on the lipid metabolism of birds.

The inclusion of EW + VH increased linearly the concentrations of total protein, globulin, and phosphorus in quail blood. Both phosphorus and calcium are essential elements for the structure and metabolism of bones and eggshells (NRC, 1994). However, higher levels of EW decreased the performance of laying quails and reduced the thickness and weight of the eggshells. According to DeLuca (1979), a lower level of blood phosphorus may indicate an imbalance in the calcium:phosphorus ratio of the diet due to phosphorus deficiency, which activates vitamin D, increasing the levels of blood calcium. Although the calcium:phosphorus ratio was corrected in all experimental diets in the present study, the effect of EW + VH on calcium and phosphorus metabolism needs to be further clarified.

The inclusion of EW + VH also reduced linearly glucose concentration and increased the antibody titers against influenza and SRBCs; however, there was no effect on the antibody titer against Newcastle. The inclusion of 0.5% EW + 0.8% VH increased the concentrations of total protein, globulin, and calcium, as well as the antibody titer against influenza. The use of 0.8% VH increased the antibody titer against SRBCs, except when associated with 0.5% EW. However, there was no effect of EW + VH inclusion on the concentrations of albumin, triglyceride, and uric acid in quail blood.

Similarly, Bahadori et al. (2017) found that the response of the antibodies of avian influenza was affected by EW supplementation, suggesting that this feedstuff may influence the systemic or humoral immunity of birds. In fact, the humoral components of EW include antimicrobial peptides, lectins, phenoloxidases, proteases, and pore-forming proteins (Popović et al., 2005). According to Cooper et al. (2002), specific molecules in the immune system of earthworms may be used as a natural antibiotic. Humic acid has immune-stimulatory, anti-inflammatory, and antiviral properties (Klocking, 1994). These mechanisms are probably related to the ability of this food to modify the intestinal microbiota (Bahadori et al., 2017). In this scenario, the benefits of using EW + VH at low levels go beyond egg quality and may even improve the health status of animals.

Conclusion

Earthworm meal (EW) and vermi-humus reduce the weight gain, production, and egg mass of quails, but low levels of 0.5% EW do not impair egg production and mass.

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