

Research Article



Effect of Dietary Supplementation of Humic Acid on Blood Parameters, Antioxidant Activity, Serum and Bone Minerals in Laying Hens

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Abstract | The study assessed the influence of different levels of humic acid supplementation on blood parameters, antioxidant activity, serum minerals, and bone mineralization in layers. A total of 160 white commercial laying hens, aged 37 weeks, were distributed into four treatment groups with 5 replicates and 8 hens per replicate. The experimental duration was 8 weeks. The basal diet was supplemented with 0, 0.125, 0.250, and 0.375% humic acid. Blood samples were collected from the wing vein at the beginning and end of the trial. Blood parameters (CBC, LDL, HDL, AST, ALT, and cholesterol), antioxidant activity (TAC, SOD, and GPx), and serum minerals (calcium, phosphorus, magnesium, sodium, potassium, and chloride) were analyzed. Tibial ash was measured as an indicator of bone mineral content. Results showed that supplementation with 0.375% humic acid significantly increased antioxidant activity parameters (TAC, GPx, and SOD) and tibial ash minerals (calcium and phosphorus). Blood parameters (white blood cells, red blood cells, hemoglobin, cholesterol, and HDL) and serum minerals (calcium and phosphorus) were positively affected by 0.375% humic acid. However, other blood parameters and serum minerals were not significantly affected. In conclusion, 0.375% humic acid supplementation seems to positively influence the laying hen performance.

Keywords | Humic acid, Tibia ash, Blood parameters, Cholesterol, Total antioxidant capacity, Laying hens

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INTRODUCTION

Over the past decade, probiotics, prebiotics, herbal compounds, and organic acids have been utilized as alternatives to antibiotics in poultry diets, as they do not have any harmful effects on consumers (Yoruk et al., 2004; Griggs and Jacob, 2005). The use of non-antibiotic growth promoters in commercial layer production has become increasingly crucial due to concerns about

the development of antimicrobial resistant bacteria (Woolhouse et al., 2015). Residues of antibiotic growth promoters in poultry products pose a significant risk to human health. Consequently, organic acids have emerged as one of the alternatives to enhance poultry production and are available in various forms as feed additives. These organic acids aid in the proper digestion of the diet and improve the availability of nutrients, thereby facilitating faster growth (Vasiliev et al., 2018) and preventing several

infectious diseases (Alagawany et al., 2018).

Among these feed additives, humic substances have been incorporated into poultry diets. Humic acid, in particular, is widely used as an alternative to antibiotic growth promoters due to its positive impact on poultry performance and health (Arif et al., 2019). Humic substances are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains, formed through the physical, chemical, and microbiological transformation (humification) of biomolecules from soil and lignite (MacCarthy, 2001; Kollist-Siigur et al., 2001). Humic acid, fulvic acid, and humin are the three main components of humates based on their solubility, with humic acid being the most well-known group among them (Yildiz et al., 2013). Humic acid is derived from decomposed plant and animal matter through bacterial action and contains microminerals that enhance the health, performance, and bone strength of poultry birds (Eren et al., 2004). Humic acid has the ability to restrict the formation of harmful oxyradicals during tissue injury (Addington and Alexander, 1999) and reduce oxidative stress (Ipek et al., 2008). Adding humic acid to the diet of laying hens increases the count of red blood cells, white blood cells and hemoglobin concentration, as well as calcium, phosphorus, and iron levels in the serum (Cetin, 2006; Rath et al., 2006). Similarly, it has been reported that its supplementation also reduces blood cholesterol levels and low-density lipoprotein levels in laying hens (Arif et al., 2019). Additionally, it has a positive effect on serum calcium and phosphorus concentrations (Abdel-Mageed, 2012), as well as improves bone development in birds (Disetlhe et al., 2017).

Previous studies on humic acid supplementation have primarily focused on broilers or other poultry birds, with limited investigation specifically targeting laying hens in terms of blood parameters. Therefore, this study aims to bridge the gap by evaluating the effects of humic acid on not only the normal parameters but also the blood indicators in laying hens, contributing new insights to the existing body of knowledge.

MATERIALS AND METHODS

All the practices, procedures or protocols mentioned in this experiment were approved (No. DGS/21797-800/2021/043) by the Graduate Studies and Research Board, University of Agriculture, Faisalabad, Pakistan.

One week before the purchase of laying hens, the shed was cleaned thoroughly and white-washed. Formalin and warter with a ratio of 1:10 was sprayed using back pump sprayer. The drinking lines and feeding lines were cleaned with solution of KMnO₄ and dried in sunlight. Fumigation of the shed was done (by using KMnO₄ and formalin

7.5gm and 35 ml). The dimensions of cage used in this trial were 1 feet length, 1.5 feet width, 1.6 feet height. One cage was used to place two laying hens with space of 0.75 sq feet for each hen.

The temperature was maintained at 75 °F throughtout the complete duriation of research. The hens were housed and managed under similar contitions like feeding, lighting schedual, temperature and ventillation. Strict compliance was maintained with all biosecurity prevention measures.

A total of 160 white commercial layers (Crystal Nick) at 37 weeks of age with an average body weight of 1,560±60 g was procured from local market for this research. Hens were randomly distributed into four treatments in such a way that each treatment had 5 replicates with 8 hens each. The birds were reared for 8 weeks and fed iso-nitrogenous (18%) as well as iso-caloric (2,740 kcal/kg, Table 2) basal diet. The control diet was HA0 without humic acid supplementation. Other dietary treatments, including HA2, HA3 and HA4, were prepared by supplementing 0.125, 0.250 and 0.375% humic acid into the basal diet (Table 1), respectively. The diet was fed to the hen according to the requirements of laying hens that is 110 grams daily. Light was made available 16 hours per day throughout the trial and water was offered *ad libitum*.

Table 1: Ingredient composition of basal diet for laying birds.

Ingredient	%
Corn	62.65
Soybean meal	26.25
Dicalcium phosphate	1.25
Sodium chloride	0.30
¹ Vitamin premix (Vitalink [®])	0.20
² Mineral premix (Nutrimin [®])	0.20
DL-Methionine	0.25
Choline chloride	0.05
Limestone	8.85
Total	100.00

¹Included per kg of ration: vitamin A derived from acetate of vitamin A, 4400.0 International Units; vitamin E from α-tocopherol acetate, 11.0 International Units; cholecalciferol, 1000.0 International Units; vitamin B¹², 0.0110 milligrams; D-pantothenic acid, 10.0 milligrams; riboflavin, 4.40 milligrams; menadione sodium bisulfite complex, 2.330 milligrams; niacin, 22.0 milligrams. ²Included per kg in ration: iron, 75.0 milligrams from iron sulphate; zinc, 75.0 milligrams from zinc oxide; manganese, 75.0 milligrams from manganese sulphate; iodine, 0.350 milligrams from potassium iodide; copper, 5.0 milligrams from copper sulphate.

Table 2: Calculated nutrient ³composition (%) of basal diet for laying birds.

Nutrients	Basal diet
Metabolizable energy (Kcal/kg)	2,740
Crude protein (%)	18.00
Calcium (%)	3.73
Total Phosphorous (%)	0.61
Non-phytate Phosphorus (%)	0.35
Sodium (%)	0.15
Lysine (%)	0.83
Digestible lysine (%)	0.79
Methionine (%)	0.50
Digestible methionine (%)	0.45
Methionine + Cysteine (%)	0.70
Digestible methionine + cysteine (%)	0.50

³Calculated using feed composition table of NRC (1994).

Two blood samples from each replicate were collected on the first and last days of the experimental trial from the wing vein using ethylene di-amine tetra acetate (EDTA) tubes. Each sample was about 5ml. Blood parameters, including hemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), as well as serum biochemical analysis (AST, ALT, cholesterol, HDL, and LDL), were evaluated using standard procedures (Jain, 1993; Benjamin, 1978).

Serum samples were taken by centrifugation of blood at 3000 rpm for 10 minutes and preserved in eppendorf tubes at -20 °C for further processing. Antioxidant analysis of serum samples was assessed by total antioxidant capacity (Anjum et al., 2020), superoxide dismutase (Giannopolitis and Ries, 1977; Hassan et al., 2023) and glutathione peroxidase (Hassan et al., 2023) procedures. Total antioxidant ability was assessed by measuring their scavenging ability to 1, 1-diphenyl-2-picrylhydrazyl stable free radicals (Anjum et al., 2020). Serum extracts were assayed for SOD activity photochemically, using the assay system consisting of methionine, riboflavin, and NBT. The reaction mixture was composed of 1.3 AM riboflavin, 13 mm methionine, 63 AM NBT, 0.05M sodium carbonate (pH 10.2), and the appropriate volume of extract. Distilled H₂O was added to bring to the final volume of 3 ml. The mixtures were illuminated in glass test tubes selected for uniform thickness and color. Identical solutions that were not illuminated served as blanks. A circular fluorescent lamp (Sylvania, FC 12 T 10-CW-RS) was attached on the outside wall of the water bath and the entire assembly was fitted in a box lined with aluminum foil. The reaction was

initiated and terminated by turning the light on and off. The initial rate of the reaction was determined as increase of absorbance at 560 nm. In the presence of SOD, the reaction was inhibited and the amount of inhibition was used to quantitate the enzyme (Giannopolitis and Ries, 1977; Hassan et al., 2023). Peroxidase was determined by ultraviolet spectrophotometry be carried out in 0.01 M phosphate buffer, at 23-28°C and 7.0 pH (Hassan et al., 2023). Serum minerals were evaluated by using technique of flame photometer for Na and K, titration method for Ca, Mg and Cl and P by using spectrophotometer. These all procedures were done after wet digestion.

At the start and end of the trial, 2 birds per treatment were slaughtered. After evisceration, the tibia bone was removed and cleaned from tissues. For the calculation of bone mineral contents tibia bone was processed through wet ashing (Hassan et al., 2023). During wet digestion, b one samples were treated with nitric acid and then dry-ashed with the help of muffle furnace at 550 °C and the solublized ion HCl. After wet digestion the final volume was made up to 250 ml and mineral contents were determined by using spectrophotometer for P and titration method for Ca (Finley and Tietz, 1996).

STATISTICAL ANALYSIS

Collected data were analyzed by complete randomized design (CRD) and Tukey's test was used to compare the treatment means (Steel et al., 1997).

RESULTS AND DISCUSSION

The study revealed significant changes ($P < 0.05$) in cholesterol and HDL levels in response to the inclusion of humic acid in the layer's diet. However, there were no notable effects ($P > 0.05$) observed on AST, ALT, and LDL across the various treatment groups in Table 3.

Results of initial sampling showed non-significant ($P > 0.05$) results regarding all blood count analyses while the results of WBC, RBC and Hb were observed to be significant ($P < 0.05$) after final blood sampling in response to the addition of humic acid in the diet of layer (Table 4). While there was non-significant effect ($P > 0.05$) on HCT, MCH, MCV, MCHC and PLT among treatments.

The effect of humic acid supplementation on serum minerals in laying hens was presented in Table 5. Initially, no significant ($P > 0.05$) differences were observed in K, Na, and Cl levels among the treatments. However, after the final sampling, the addition of humic acid to the diet resulted in significant changes ($P < 0.05$) in Ca, P, and Mg levels. No significant effects ($P > 0.05$) on K, Na, and Cl were observed in response to the different treatments.

Table 3: Effect of humic acid on serum biochemical analysis.

Parameters		Treatments				SEM*	P value
		HA0	HA2	HA3	HA4		
AST (units/l)	I ¹	53.92	52.12	50.47	50.07	1.040	0.169
	F ²	53.88	52.32	50.52	49.81	0.837	0.082
ALT (units/l)	I	10.66	10.4	9.64	9.72	0.040	0.040
	F	10.61	10.52	9.62	9.52	0.101	0.283
Cholesterol (mg/dL)	I	162.5	165.5	153	148	6.699	0.347
	F	141.75 ^a	142.13 ^a	140.41 ^b	139.24 ^c	0.170	0.031
HDL (mg/dL)	I	45.5	50.5	46.45	46	2.111	0.423
	F	46.5 ^c	50.5 ^b	52.25 ^{ab}	53.5 ^a	0.451	0.002
LDL (mg/dL)	I	150.5	159	162	157	13.27	0.827
	F	151.5	161	155	143	6.581	0.661

Means in a row with different superscripts differ significantly ($P<0.05$). (AST) aspartate aminotransferase, (ALT) alanine transaminase, (HDL) high density lipoprotein, (LDL) low density lipoprotein, Cholesterol. ¹Initial– Start of the trial, ²Final – End of the trial, *SEM Standard error of mean.

Table 4: Effect of humic acid on complete blood count analysis.

Parameters		Treatments				SEM*	P value
		HA0	HA2	HA3	HA4		
WBC	I ¹	40.37	41.51	43.2	44.94	2.755	0.689
	F ²	40.68 ^c	43.55 ^b	44.67 ^{ab}	46.1 ^a	0.3555	0.0018
RBC	I	2.71	2.505	2.64	2.64	0.1568	0.8254
	F	2.71 ^c	3.36 ^b	3.78 ^{ab}	3.92 ^a	0.0784	0.0014
Hb	I	10.15	11.3	10.55	11.6	1.2364	0.8308
	F	10.65 ^c	11.8 ^b	12.55 ^{ab}	13.6 ^a	0.2031	0.0022
HCT	I	36.85	34.3	34.45	36.05	2.3467	0.8376
	F	38.35	40.3	34.95	40.05	2.336	0.4419
MCV	I	136.15	136.7	130.7	136.35	2.767	0.4565
	F	130.15	138.2	136.7	137.85	2.3385	0.2307
MCH	I	41.4	51.25	49.55	46.65	2.0921	0.0978
	F	42.9	52.25	50.55	47.65	1.8137	0.0723
MCHC	I	29.35	35.7	34.1	35.65	3.5667	0.5973
	F	29.7	35.6	35.1	36.3	3.3098	0.5408
PLT	I	11,00	12,00	10.50	11,00	0.901	0.710
	F	10.5	12.5	11.5	11.5	0.866	0.519

Means in a row with different superscripts differ significantly ($P<0.05$). RBC (Red Blood Cell), WBC (White Blood Cell), Hb (Hemoglobin), HCT (Hematocrit), MCH (Mean corpuscular hemoglobin), MCHC (Mean corpuscular hemoglobin concentration), (MCV mean corpuscular volume), PLT (Platelets). ¹Initial– Start of the trial, ²Final – End of the trial, *SEM Standard error of mean.

In Table 6, the impact of humic acid supplementation on bone mineralization in laying hens was presented. Significant variations ($P<0.05$) were observed in tibial ash,

Ca, and P levels in response to the addition of humic acid to the layer's diet.

Table 5: Effect of humic acid on serum minerals.

Parameters		Treatments				SEM*	P value
		HA0	HA2	HA3	HA4		
Ca (mg/dl)	I ¹	6.06	6.86	7.11	9.125	1.0642	0.3428
	F ²	6.56 ^b	7.36 ^{ab}	7.11 ^b	11.625 ^a	0.7803	0.0302
P (mg/dl)	I	3.525	4.88	5.59	5.63	0.9481	0.454
	F	4.185 ^b	5.38 ^{ab}	6.09 ^{ab}	7.13 ^a	0.3887	0.0243
Mg (mg/dl)	I	1.635	3.25	2.46	2.485	0.5082	0.3065
	F	2.27	2.45	1.86	3.735	0.351	0.0701
K (mmol/l)	I	5.2	4.55	6.15	5	0.5557	0.3492
	F	4.46	5.43	4.8	5.82	1.4766	0.9102
Cl (mmol/l)	I	114.3	117.75	132.9	112.6	10.558	0.5683
	F	116.8	112.65	141.9	117.1	11.06	0.3524
Na (mmol/l)	I	149	145.5	143	151.5	3.0619	0.3424
	F	152.5	144.5	155	149.5	3.4187	0.2959

Means in a row with different superscripts differ significantly ($P<0.05$). (Ca) calcium, (P) phosphorus, (Mg) magnesium, (K) potassium, (Cl) chloride, (Na) sodium. ¹Initial– Start of the trial, ²Final – End of the trial, *SEM Standard error of mean.

Table 6: Effect of humic acid on bone characters.

Parameters		Treatments				SEM*	P value
		HA0	HA2	HA3	HA4		
Ash (%)	I ¹	31.8	33.8	37.55	37.7	1.312	0.079
	F ²	31.3 ^b	34.3 ^{ab}	38.55 ^a	36.7 ^{ab}	1.1814	0.0449
Calcium (%)	I	20.37	20.74	21.695	20.73	0.3994	0.2538
	F	19.48 ^c	20.175 ^b	21.42 ^{ab}	22.38 ^a	0.2713	0.0057
Phosphorus (%)	I	7.7	8.2	9.1	9.3	0.324	0.0681
	F	8.3 ^c	8.7 ^b	9.4 ^{ab}	9.8 ^a	0.180	0.0137

Means in a row with different superscripts differ significantly ($P<0.05$). ¹Initial– Start of the trial, ²Final– End of the trial, *SEM Standard error of mean.

Table 7: Effect of humic acid on antioxidant activity.

Parameters		Treatments				SEM*	P value
		HA0	HA2	HA3	HA4		
GPx	I ¹	33.39	29.83	28.18	28.45	2.599	0.534
	F ²	25.01 ^c	32.95 ^{bc}	36.271 ^b	43.743 ^a	0.5778	<0.001
SOD	I	8.808	16.118	11.483	26.017	11.232	0.7287
	F	9.033 ^b	26.811 ^a	14.88 ^b	28.728 ^a	1.1177	<0.001
TAC	I	14.279	46.302	35.605	36.047	7.2965	0.1338
	F	16.651 ^c	51.872 ^{ab}	37.674 ^b	52.686 ^a	2.1847	<0.001

Means in a row with different superscripts differ significantly ($P<0.001$). (GPx) glutathione peroxidase, (SOD) super Oxide Dismutase, (TAC) total antioxidant capacity. ¹Initial– Start of the trial, ²Final – End of the trial, *SEM Standard error of mean.

The addition of humic acid to the diet of laying hens, as shown in Table 7, resulted in significant ($P<0.05$) changes

in GPx, SOD, and TAC levels, indicating an impact on antioxidant activity.

BLOOD PARAMETERS

The current study demonstrated a significant effect on cholesterol and HDL levels, while no significant effect was observed on AST, ALT, and LDL in white commercial layers fed a diet supplemented with 0.375% humic acid. These findings align with previous research of [Disetlthe \(2017\)](#), reporting a non-significant effect of humic acid on AST and ALT levels among different groups. Similarly, [Avci et al. \(2007\)](#) found no impact of dietary humic acid on biochemical parameters such as LDL, AST, and ALT. However, [Arif et al. \(2016\)](#) clarified that blood cholesterol and LDL levels significantly decreased with the addition of humic acid (2.25 and 3 g/kg) in the layer diet. Furthermore, [Arif et al. \(2018\)](#) stated that supplementing humic acid in the layer's diet at 1.5 and 2.25 g/kg resulted in a decrease in serum cholesterol concentration. [Hayirli et al. \(2005\)](#) reported that the inclusion of humic acid increased serum HDL and decreased LDL concentrations in layer birds, possibly due to a decrease in microbial intracellular pH, which leads to reduced blood lipids and cholesterol. The addition of humic acid to the diet likely leads to the inhibition of certain microbial enzymes present in the gut of the laying hens. As a consequence, the inhibition of these enzymes can disrupt the normal metabolism of bacteria in the gut, leading to the release of acidic protons. This, in turn, causes a reduction in the intracellular pH within the bacterial cells. As a result of these changes in the gut environment, there can be alterations in the overall metabolic processes and interactions with the host, leading to the observed impact on biochemical parameters ([Abdo, 2004](#); [Young and Foegeding, 1993](#)). In contrast, [Hakan et al. \(2012\)](#) demonstrated that humic acid had a non-significant effect on blood cholesterol in laying hens. [Koksall and Kucukersan \(2012\)](#) found that humic acid just slightly increased blood cholesterol levels. The results of the present study revealed a significant increase in WBC, RBC, and Hb levels with the addition of humic acid to the layer's diet. However, there was no significant effect on HCT, MCH, MCV, MCHC, and PLT among all groups. [Hammod et al. \(2019\)](#) obtained similar results, reporting a significant increase in WBC and RBC levels when birds were fed diets supplemented with humic acid. [Maysa and Sheikh \(2008\)](#) concluded that the administration of different levels of humic acid had a positive impact on RBC, WBC, and Hb compared to other groups, which may attribute to the increase in hen age. Similarly, [Elnaggar et al. \(2018\)](#) observed an increase in RBC and WBC levels with the supplementation of humic acid. The enhancement of the bird's ability to utilize nutrients involved in the composition of RBC, WBC, and Hb may be attributed to the effects of humic acid ([Ipak et al., 2008](#)).

SERUM MINERALS

The results of the current research indicate that the addition of humic acid to the layer's diet significantly increased the levels of Ca and P. However, there was no significant effect on K, Na, and Cl among treatment groups. These findings are consistent with the previous study ([Kovacik et al., 2020](#)) showing a non-significant decrease in Na concentration in the group fed 1.0% humic acid as compared with the supplementation of 0.5 and 0.75% humic acid. Similarly, [Kalafova et al. \(2018\)](#) reported a non-significant increase in serum Na and K levels in the group treated with humic acid. In contrast, [Kovacik et al. \(2020\)](#) demonstrated that serum K concentration was significantly increased in the group supplemented with 0.75% humic acid. [Avci et al. \(2007\)](#) reported a significant increase in serum Ca and P concentrations in the group supplemented with humic acid (600 mg/kg) compared to the control group. Similarly, [Islam et al. \(2005\)](#) found that the addition of humic acid improved Ca utilization in the layer's diet. [Ozturk et al. \(2012\)](#) also observed an increase in serum Ca and P levels with the supplementation of humic acid. These results align with the findings of [Kalafova et al. \(2018\)](#), who reported a significant increase in serum Ca and P with the inclusion of humic acid in the diet. This increase could be attributed to the ability of humic acid to bind inorganic ions and transport minerals to cells ([Islam et al., 2005](#)). However, [Jaduttova et al. \(2019\)](#) demonstrated that the supplementation of humic acid at 0.8% decreased Ca and P levels. The increase in serum mineral concentration observed in laying hens after humic acid supplementation can be explained by the chelating properties of humic acid. Humic acid has the ability to form complexes with various minerals, including Ca and P. When humic acid binds to these minerals, it may reduce their availability for absorption and utilization in the gut. As a result, more minerals may remain in the bloodstream, leading to an increase in serum mineral concentrations. This mode of action was proposed by [Rath et al. \(2006\)](#). Furthermore, [Arif et al. \(2018\)](#) observed that the supplementation of humic acid had no effect on serum calcium and phosphorous levels in birds.

BONE MINERALIZATION

The results of the present study demonstrated that the inclusion of humic acid in the diet of layers significantly increased the levels of Ca and P. These findings are consistent with the recent research of [Jaduttova et al. \(2019\)](#), who reported a higher amount of calcium in the tibia bone with the supplemented 0.8% humic acid in layers' diet. Similarly, [Ozturk and Coskun \(2010\)](#) found that adding 450 ppm of humic acid to drinking water significantly improved mineral levels and tibia ash content. This suggests the potential benefits of humic acid supplementation for enhancing mineral absorption and bone health. According to [Talaty et al. \(2009\)](#), a lack

of calcium in the diet can lead to blood hypocalcemia, resulting in decreased bone strength and mineralization. Therefore, the supplementation of humic acid in the layer's diet significantly enhances the mineralization of the bone matrix (Stepchenko et al., 1991). However, in contrast to the current results, Yildiz et al. (2013) reported that humic acid supplementation had a non-significant effect on bone characteristics and calcium levels.

ANTIOXIDANT ACTIVITY

Kovacik et al. (2020) reported a significant effect on SOD and GPx activities in the experimental groups when humic acid was included in the diet. The SOD and GPx play a role in suppressing the formation of oxygen free radicals. Vaskova et al. (2011) found that the addition of humic acid to the layer's diet enhanced the antioxidant defense system, leading to a decline in superoxide anion, which acts as a generator of toxic particles in the body. Kamel et al. (2015) stated that humic acid had a positive effect on TAC and GPx activity while decreasing the synthesis of toxic free radicals. Cetin et al. (2006) reported a significant effect of humic acid supplementation on TAC values in the diet of laying hens. Similarly, Ipek et al. (2008) investigated the effects of humic acid supplementation on TAC in birds and observed a significant increase in TAC with the highest dosage of humic acid (600 mg/kg) compared to other groups.

CONCLUSIONS AND RECOMMENDATIONS

On the basis of the results of present study, it can be concluded that there was positive influence of 0.375% humic acid supplementation on blood parameters, antioxidant capacity, serum minerals and bone mineralization in caged layers.

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NOVELTY STATEMENT

Studies on humic acid in laying birds are few with main focus on production parameters but present study focused on biochemical, serological and hematological parameters

AUTHOR'S CONTRIBUTION

SH, SAB and FA planned and designed the experiments. SH conducted the feeding trial. SAB supervised the

experiments. SAB and AUH and MUY performed the statistical analysis. MA, MN and AM did proofread and editing. MS did correspondence.

CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

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