Indian J. Anim. Nutr. 2025. 42 (3): 445-454

doi: 10.56093/IJAN.v42i3.16

Indian Journal of Animal Nutrition

Effect of Humic Substance Supplementation on Performance and Gut Health in Broilers

Chamarthi Jyothi, Nirmala Muwel*, Sunil Nayak, Ankur Khare, Rahul Sharma, Ajay Rai, Anil Shinde and Nidhi Gupta

Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur-482001, Madhya Pradesh, India

* Correspondence:nmuwel005@gmail.com

ABSTRACT

The study was conducted to investigate the effect of dietary supplementation of humic substance (HS) on performance of broilers. 120 day old broiler chicks were randomly distributed into 6 dietary treatments with five replicates of four chicks each. Broiler diets were formulated as per commercial chick feed specification (2011). Antibiotic growth promoters (AGP) group diet was same as CON with addition of AGP whereas, in HS0.1, HS0.2, HS0.3 and HS0.4 replacing antibiotic with HS @ 1, 2, 3 and 4 g/kg feed, respectively. Individual body weight and feed intake were recorded and thus feed to gain ratio and European production efficiency index (EPEI) % was calculated. Birds were sacrificed to estimate carcass traits, intestinal histomorphometry and caecal microbial count. Immunological response and liver function tests (SGOT and SGPT) were estimated. Broiler performance (0-6 weeks) indicate that supplementation of HS0.4 significantly ($p<0.05$) improved body weight. Better feed to gain ratio and EPEI % was observed with HS0.2 compared to CON. Crude protein and crude fat utilization improved significantly ($p<0.05$) in broilers fed with HS0.2 and HS0.4. Supplementation of HS significantly ($p<0.05$) improved intestine villus height (VH), crypt depth (CP) and VH:CD ratio. Caecal *E. coli* count was lowered, humoral immune response, weight of bursa of fabricius, spleen, serum IgG and IL-10 levels were increased in HS supplemented groups compared to CON. HS inclusion had no effect on the carcass traits, nutrient composition of meat and liver function tests. Lower feed cost per kg weight gain and improved economic efficiency was observed in broilers fed on diets supplemented with HS0.2. It can be concluded that supplementation of HS0.2 in feed can be used as an economically efficient alternative to antibiotic growth promoters in broilers diet.

KEYWORDS: Broiler, Histomorphometry, Humic Substance, Nutrient Utilization

Performance

Article received: 11 June 2025; Article accepted: 26 September 2025

INTRODUCTION

The poultry industry is rapidly expanding, especially in developing countries with both the production and consumption of poultry meat rising steadily. As per the 20th livestock census, the poultry population stands at 851.81 million, reflecting a growth rate of 16.81%. However, despite this growth, the current annual per capita availability of meat in India is only 3.5 kg/year, significantly lower than the recommended 10.5 kg/year suggested by the Indian Council of Medical Research.

Antibiotic growth promoters (AGPs) are widely used in poultry diets to promote growth and control of diseases. However, European Union has prohibited the use of feed grade antibiotic growth promoters due to concerns about potential drug resistance in human pathogens (Anadon, 2006). Consequently, this has led to a need for safe alternative additives to enhance poultry production without relying on antibiotics. The adoption of humic substance (HS) as substitutes for antibiotics in poultry farming has garnered significant attention. Humic substance are natural organic compounds formed from the decomposed plants matter in soil, coal and other sources. These substances have a complex structure and contain humic, humin and fulvic acids (Bezuglova and Klimenko, 2022), and are known for natural growth enhancers, antioxidant, antifungal, detoxifying and antiseptic properties. Their colloidal nature allows the formation of a protective layer on the intestinal epithelium, reducing pathogen colonization and toxins penetration in intestine (Gonzelez et al., 2018). Additionally, they enhance nutrient utilization and growth performance by promoting villus length and maintaining gut microflora. Furthermore, there buffering capacity also regulate gut pH and inhibit the growth of pathogenic bacteria (Marcincak et al., 2023). Despite their potential benefits, limited reports are available on the supplementation in broiler performance. Therefore, this study aimed to evaluate the effect of dietary supplementation of humic substance on performance, gut health and immune response of broilers.

MATERIALS AND METHODS

The experiment was conducted at Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Jabalpur, India. Study was approved by Institutional Animal Ethical Committee (IAEC) vide D.No.39/IAEC/Vety/2023 dated 01.10.2023. Affiliated with CPCSEA, Ministry of Animal Husbandry and Dairying, India.

Experimental design

A total of 120 day-old broiler chicks were procured from a commercial hatchery in Jabalpur and vaccination were done as per stander protocol/ schedule. (Chicks were vaccinated against Ranikhet disease (F1 strain), infectious bursal disease, and given a booster dose of Ranikhet disease on days 7, 14, and 28, respectively). Experimental diets were formulated as per commercial chick feed specifications (2011). Water were offered *ad-libitum* throughout the experimental period. The experiment was conducted under a completely randomized design with six isonitrogenous and isocaloric dietary treatments, each with five replicates of four chicks: CON (control, VENCOBB 400 specification), AGP (CON + AGP, 0.03%), HS0.1 (CON + 1 g/kg HS), HS0.2 (CON + 2 g/kg HS), HS0.3 (CON + 3 g/kg HS), and HS0.4 (CON + 4 g/kg HS).

Growth performance

Individual live body weight (g) and feed intake of broilers were recorded on weekly basis throughout the experiment. Feed to gain ratio (F:G) was calculated by considering cumulative feed intake and weight gain for each groups. European production efficiency index (EPEI) was calculated as formula given by Hubbard (1999).

Economics of production

Economics of broiler production over feed cost was calculated as per cost of feed consumed and per kg weight gain for each dietary treatment. The price of the diet and HS was calculated according to the local market price.

Nutrient utilization

A 3- day metabolic trial was conducted during 6th week of the experiment and excreta of each replicate were collected in acidic medium quantitatively at every 24 hours. Dry matter (DM), crude protein (CP) and crude fat (CF) of leftover feed and faeces were determined accoding to AOAC methods (2012).

Carcass traits and nutrient composition of meat

Two broilers per replicate were humanely sacrificed on day 35 after a 12-h fasting period to study carcass traits. Dressing yield, eviscerated weight, and drawn weight were recorded. Processing losses (% of live weight; blood, head, feathers, shank, separable fat, and wing tips) were also recorded. Organs (liver, heart, giblet, gizzard, pancreas) were collected replicate-wise, and their weight expressed as % of dressed weight. Breast muscle samples (two per replicate) were analyzed for proximate composition (DM, CP, CF) following AOAC (2012).

Histomorphometrical analysis

Tissue samples (approx.2 cm) from the duodenum, jejunum, and ileum of sacrificed birds were collected and fixed in 10% neutral buffered formalin for 72 h. Samples were processed, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin (Drury and Wallington, 1980). Measurements included intestinal wall thickness, villus height, crypt depth, villus height-to-crypt depth (VH:CD) ratio, and goblet cells per field was measured from middle part of the villi.. Sections were examined using a Leica DM 1000 LED microscope with digital imaging software (LAS v4.10).

Caecal microbial analysis

Caeca from sacrificed birds were aseptically collected, homogenized, and stored at –20°C until analysis. Total *E. coli* and *C. perfringens* counts were determined using standard plate count methods (Markey et al., 2013). Serial dilutions of caecal contents were plated on MacConkey agar for *E.*

coli (aerobic, 37°C, 24 h) and on tryptose-sulfite-cycloserine (TSC) agar with egg yolk emulsion for *C. perfringens* (anaerobic, 37°C, 48 h) in gas-pack anaerobic jars. Colony counts were expressed as log10 cfu/g of caecal digesta.

Immunological response

Lymphoid organs (bursa, spleen, and thymus) were collected and weighed as a percentage of live weight. Blood samples from vaccinated birds were taken on days 30 and 42, and serum was separated and stored at –20°C. Humoral immunity against NDV was assessed by haemagglutination inhibition test (OIE, 2004), with titres expressed as log10 of the reciprocal endpoint dilution. Serum IgG and IL-10 concentrations were measured using chicken-specific ELISA kits (Chongqing Biospes Co., Ltd, China).

Estimation of liver function test

Serum glutamic oxalo-acetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels estimated by (Reitman and Frankel, 1957) spectrophotometrically using commercial diagnostic kits (Erba Diagnostics Ltd., India).

Statistical analysis

Data were analyzed by one-way ANOVA for a completely randomized design (CRD) as per Snedecor and Cochran (1994) using SPSS software (version 22.0). Significant differences among treatment means were compared using Duncan's Multiple Range Test (1955). Results are expressed as pooled standard error of mean (SEM), with significance set at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance, nutrient utilization and economics of production

The effects of supplementing different levels of HS on growth performance, nutrient utilization and economics of production of broilers are shown in Table 1. The current study showed definite trend for increase in live weight of broiler supplemented with humic substance. Maximum and significantly ($p<0.05$) higher live weight was observed in group HS0.4 followed by HS0.2 and HS0.3 compared to the CON. The improved body weight in HS supplemented groups may be due to their effect on improving the intestinal morphometry, which increases intestinal absorption surface available for the nutrients for necessary growth. There are number of studies reporting improvement in growth performance of broilers with HS supplementation in feed (Ghazalah et al., 2022 and Elnaggar and El-Kelawy, 2018) or in drinking water with different levels (Kati, 2018).

There were no significant differences ($p>0.05$) in feed intake among broilers supplemented with different levels of HS. Results of present study are in line with the Arif et al. (2016) and Saleh et al. (2022) who reported no effect of HS supplementation on feed consumption in broilers.

However, significantly ($p<0.05$) lower or better feed to gain ratio was observed in broilers fed with HS0.4 and HS0.2 was best. While the groups supplemented with HS0.1 and HS0.3 were having at par feed to gain ratio with the AGP supplemented group. The European production efficiency index was significantly better ($p<0.05$) in the HS @ 2 g/kg feed group (HS0.2) compared to the control group but with no significant difference ($p>0.05$) between different levels of HS. The improved feed to gain ratio with HS supplementation may be attributed to improved nutrient utilization and body weight gain with similar feed intake. Humic substance stabilizes the intestinal microbiota which enhances nutrient utilization and body weight gain (Lala et al., 2017). Our results were in accordance with Ghazalah et al. (2022) and Arif et al. (2016), who observed improved FCR in humic acid supplemented groups.

Overall, these findings suggest that HS supplementation, particularly at a concentration of 2 g/kg feed, positively influences live weight, feed-to-gain ratio and EPEI in broilers, demonstrating its potential as a beneficial natural growth promoter in poultry diets.

In the present study, significantly ($p<0.05$) higher crude protein and crude fat utilization was observed in broilers fed a basal diet supplemented with HS compared to the control, with maximum values in HS0.2 and HS0.4 groups. However, HS supplementation had no significant effect ($p>0.05$) on dry matter utilization. Improved villus length in HS-supplemented birds likely contributed to better digestibility of crude protein and crude fat/EE. These findings are in agreement with Elnaggar and El-Kelawy (2018) and Ghazalah et al. (2022), who also reported improved apparent digestibility of crude protein and ether extract with humic acid supplementation.

Broilers supplemented with HS0.2 had significantly ($p<0.05$) lower feed cost per kg weight gain and higher economic efficiency compare to the CON and AGP fed group. In present study the reduced cost per kg live weight gain and improved economic efficiency might be due to better feed to gain ratio, higher nutrient utilization and better improved weight gain in broilers fed basal diet with humic substance. These findings are accordance with Hammod et al. (2021) and Omidiwura et al. (2021) who reported that supplementation of humic acid in diet improved economics of broiler production.

Table 1. Growth performance and economics of production in broilers of different treatment groups

Parameter	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p- value
Growth performance								
Final body weight (g)	2219.37^{d}	2318.85^{cd}	2358.10^{bc}	2469.73^{ab}	2303.57^{cd}	2495.75^{a}	22.99	0.01
Overall feed intake (g)	3779.55	3788.48	3753.73	3730.01	3775.43	3834.37	15.30	0.52
Average F:G	1.74a	1.67^{ab}	1.63^{bc}	1.54^{d}	1.56^{cd}	1.57^{cd}	0.02	0.01
EPEI(%)	297.99^{d}	324.64^{cd}	339.70^{bc}	374.99^{a}	368.05^{ab}	373.44^{a}	6.59	0.01
Nutrient utilization (%)								
Dry matter	70.98	72.69	71.81	72.12	71.79	71.91	0.23	0.43
Crude protein	71.80^{b}	73.93^{a}	72.87^{ab}	74.07^{a}	73.69^{a}	73.81^{a}	0.25	0.05
Crude fat	80.77^{b}	83.07^{a}	82.62^{a}	83.19^{a}	83.18^{a}	83.20^{a}	0.27	0.04
Economics of production								
Feed cost (Rs)/kg body weight gain	72.73^{a}	70.19^{ab}	68.51^{bc}	65.62^{c}	67.36^{bc}	68.31^{bc}	0.59	0.01
Economic efficiency (%)	51.92^{c}	57.00^{bc}	61.09^{ab}	67.74^{a}	52.95^{c}	61.43^{ab}	1.39	0.02

EPEI- Europian production efficiency index
Means within the same row with different superscripts are significantly different ($p<0.05$).

Carcass traits

Effect of different levels of humic substance supplementation on carcass traits of broilers in terms of carcass yield, organs weight, processing losses and nutrient composition of broiler breast meat at 35 days is described in Table 2. Diets with different levels of HS did not significantly ($p>0.05$) affect the carcass yield (dressing yield, eviscerated weight and drawn weight), organs weight (% of dressed weight) and processing losses (% of live weight). Similarly, the proximate composition of breast meat (DM, CP, and EE) was not influenced ($p>0.05$) by dietary treatments.

These findings agreed with the results reported by Nagaraju et al. (2014) and Khalaquzzman (2022) for dressing percentage, Samudovska et al. (2022) and Pistova et al. (2016) for relative organ weight of liver and heart in broilers and Hascik et al. (2018) and Korsakov et al. (2019) for nutrient composition (DM, CP and EE) of broiler meat with humic acid supplementation.

Table 2. Carcass traits of broilers in different treatment groups

Parameter	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p- value
Carcass yields (% of live weight)								
Dressing yield	82.87	82.97	83.45	83.46	83.23	83.67	0.26	0.97
Eviscerated weight	79.65	79.50	79.79	79.70	79.48	79.67	0.26	1.00
Drawn weight	82.70	82.79	83.29	83.28	83.04	83.49	0.26	0.97
Organ weight (%)								
Heart	0.42	0.55	0.53	0.56	0.57	0.63	0.04	0.85
Gizzard	1.76	1.76	1.84	1.97	1.90	2.07	0.04	0.27
Liver	1.47	1.61	1.86	1.87	1.83	1.86	0.08	0.67
Giblet	3.69	3.92	4.22	4.39	4.31	4.56	0.11	0.22
Pancreas	0.21	0.22	0.21	0.22	0.22	0.23	0.01	0.55
Processing losses (% of live weight)								
Blood	3.75	3.57	3.54	3.68	3.27	3.46	0.09	0.79
Feather	6.26	6.18	5.77	5.85	5.91	5.22	0.29	0.97
Head	2.10	2.21	2.41	2.33	2.55	2.50	0.06	0.25
Separable fat	1.19	1.26	0.95	0.80	1.17	0.66	0.10	0.47
Appendages	5.01	5.07	4.83	4.68	5.06	5.15	0.07	0.36
Meat nutrient composition (on DM basis)								
Dry matter	23.51	24.33	24.00	24.39	25.12	25.03	0.34	0.79
Crude protein	20.93	21.26	20.95	21.13	21.26	21.39	0.11	0.80
Crude fat	5.51	5.55	5.50	5.54	5.69	5.73	0.07	0.88

Means within the same row with different superscripts are significantly different ($p<0.05$).

Histomorphometry

The results of histomorphometrical analysis of small intestine in broilers are given in Table 3 and Figure 1. Broilers fed on basal diets supplemented with different levels of HS did not significantly ($p>0.05$) influenced duodenum, jejunum and ileum wall thickness and goblet cells per field. Maximum and significantly higher ($p<0.05$) villus height was recorded in the broilers fed with HS0.4 in both duodenum and ileum compared to CON. Whereas, in jejunum significantly ($p<0.05$) higher villus height was observed with HS0.3 feed compared to the CON. Diet supplemented with humic substance @ 4 g/kg feed (HS0.4) showed significantly ($p<0.05$) lower crypt depth in duodenum, jejunum and ileum. Similarly, significantly ($p<0.05$) higher villus height to crypt depth ratio was observed in HS0.4 group compared to CON group. Birds fed with HS0.4 showed significantly ($p<0.05$) better intestinal histomorphometry compared to CON but statistically non significant ($p>0.05$) to different HS supplemented groups.

Humic acid lowers intestinal pH and bacterial colonization, reducing intestinal mucosa inflammation and enhancing villus height, thereby improving secretion, digestion and nutrient absorption (Taklimi et al., 2012). It may also promote crypt cell proliferation and tissue turnover (Panda et al., 2009). Several studies agreed with present study who reported improved villus height and crypt depth with HS supplementation (Taklimi et al., 2012, Omidiwura et al., 2021 and Lala et al., 2017).

Table 3. Histomorphometry of small intestine in different treatment groups

Treatment	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p-value
Villus height (µm)								
Duodenum	1420.43^{c}	1484.25^{b}	1496.40^{ab}	1513.96^{ab}	1508.14^{ab}	1516.18^{a}	8.58	0.01
Jejunum	1125.71^{c}	1218.42^{b}	1225.38^{ab}	1268.30^{ab}	1304.95^{a}	1277.69^{ab}	16.39	0.01
Ileum	732.27^{c}	808.30^{ab}	790.99^{b}	849.09^{a}	823.01^{b}	869.45^{a}	10.94	0.01
Crypt depth (µm)								
Duodenum	189.20^{a}	171.20^{b}	167.65^{bc}	159.32^{bc}	157.73^{bc}	153.42^{c}	3.29	0.02
Jejunum	170.92^{a}	155.72^{b}	160.81^{ab}	152.06^{b}	156.45^{ab}	147.41^{b}	2.37	0.05
Ileum	169.62^{a}	161.58^{ab}	155.31^{bc}	152.70^{bcd}	149.50^{cd}	144.03^{d}	2.37	0.01
Intestinal wall thickness (µm)								
Duodenum	345.01	339.75	346.13	341.07	338.01	342.88	1.71	0.79
Jejunum	377.43	376.06	374.25	377.06	363.73	371.75	2.22	0.53
Ileum	426.37	423.18	428.38	420.25	415.09	417.30	4.51	0.97
Goblet cells per field								
Duodenum	23.66	25.66	24.00	25.00	23.00	23.66	0.72	0.94
Jejunum	35.67	33.67	34.00	34.33	33.33	34.66	0.67	0.96
Ileum	84.67	83.33	84.00	85.00	84.33	83.67	0.64	0.99
Villus height/Crypt depth ratio								
Duodenum	7.52^{c}	8.68^{b}	8.94^{ab}	9.51^{ab}	9.58^{ab}	9.91^{a}	0.22	0.01
Jejunum	6.61^{c}	7.85^{ab}	7.62^{b}	8.34^{ab}	8.37^{ab}	8.67^{a}	0.19	0.02
Ileum	4.32^{d}	5.00^{c}	5.10^{c}	5.56^{b}	5.51^{b}	6.04^{a}	0.14	0.01

Means within the same row with different superscripts are significantly different ($p<0.05$).

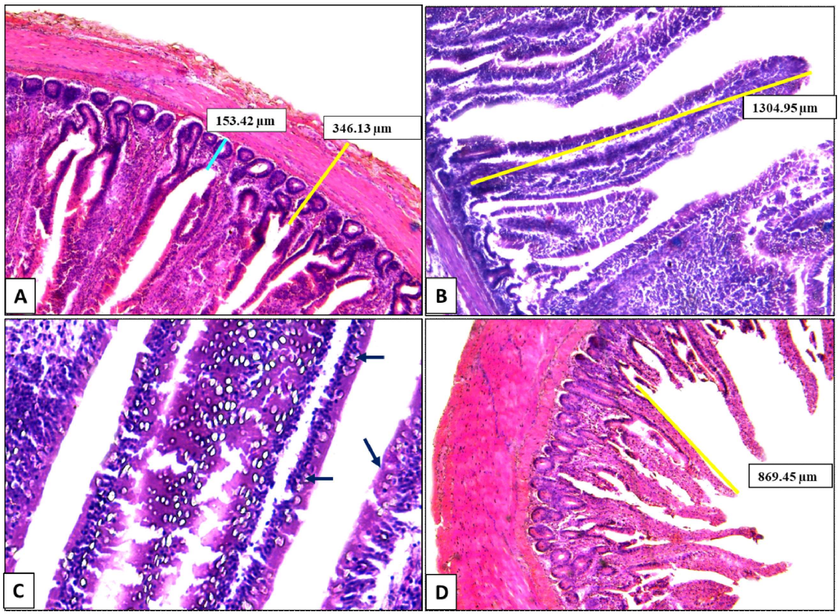

Fig 1. Photomicrographs (H&E) of small intestine showing: A. Total wall thickness of duodenum (yellow bar) and crypt depth (blue bar) X50. B. Villus height measurement of jejunum X50. C. Goblet cells in epithelium of jejunum (arrow) X200. D. Measurement of villus height in ileum X50

The effect of different levels of HS supplementation on caecal microbial count (cfu/g) of broilers are presented below in Table 4. Supplementation of HS0.4 had significantly lower ($p<0.05$) *E. coli* count compared to CON group but statistically ($p>0.05$) comparable to other supplemented groups. There was no significant ($p>0.05$) difference observed in *C. perfringens* count in caecal contents in different dietary treatments. However, numerically lower *C. perfringens* count was observed in HS supplemented groups compared to control group.

Humic substance reduces the gut pH ((Marcincak et al., 2023) which decreases the pathogenic bacteria colonization and toxins production. They may also disturb the microbial proteins and carbohydrates metabolism leads to the destruction of bacterial cells or viral particles (Huck et al., 1991). Studies have reported lower *E. coli* count in the intestinal contents of broilers in HS supplemented groups (Omidiwura et al., 2021, Mudronova et al., 2020 and Khalaquzzman, 2022), although Dominguez-Negrete et al. (2019) found that supplementation of HS had no significant ($p>0.05$) effect on *C. perfringens* count in caecal contents of broilers.

Table 4. Caecal microbial count (log_{10}cfu/g) of broilers in different treatment groups

Caecal microbiota	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p- value
E. coli	8.37^{a}	7.78^{ab}	7.44^{b}	7.18^{b}	6.97^{b}	6.98^{b}	0.14	0.01
C. perfringens	6.11	5.41	5.51	5.43	5.39	5.38	0.09	0.15

Means within the same row with different superscripts are significantly different ($p<0.05$).

Immunity

Humoral immune response, lymphoid organ weight, IgG and IL-10 and liver function test were estimated and presented in Table 5 and Table 6. Antibody titre against NDV on 30^{th} and 42^{nd} day in broilers indicated that humoral immune response was significantly improved ($p<0.05$) by increase of age with dietary supplementation of graded levels of HS compared to CON with significantly ($p<0.05$) higher antibody titre observed in broilers fed HS @ 4 g/kg diet (HS0.4).

Lymphoid organ weights (% of live weight), particularly the spleen and bursa, were significantly ($p<0.05$) higher in broilers supplemented with graded levels of HS in the diet.

Serum IgG and IL-10 levels were significantly improved in birds fed HS at 2, 3, and 4 g/kg feed, whereas SGOT/AST (aspartate aminotransferase) and SGPT/ALT (alanine aminotransferase) showed no significant effect ($p>0.05$).

The improved antibody titre against NDV might be due to their antiviral properties, phagocytic activity of leukocytes, ability to reduce colonization of pathogens in the gastrointestinal tract and improving immune functions. Results of the study agreed with the findings of Ahfeethah et al. (2023) and Mehdi and Hasan (2012) who demonstrated that supplementation of humic acid increases antibody titre against NDV. Increased spleen and bursa weight were observed with supplementation of HS (Korsakov et al., 2019; Elnaggar and El-Kelawy, 2018). Increase in serum IgG levels suggested that HS has capacity of elevating natural antibody production. Findings are agreement with Zhang et al. (2020) who noticed that supplementation with 0.1, 0.3 and 0.5% sodium humate significantly ($p<0.05$) increased serum IgG levels compared to the birds in the control group. Ma et al. (2021) observed that broiler diet supplemented with mixed organic acids has significant ($p<0.05$) effect on IL- 10 levels in the broiler serum. However, there is no increase in serum AST and ALT levels which indicates no negative effect on liver function. Results of our present study agreed with Saleh et al. (2022).

Table 5. Humoral immune response against Newcastle disease virus (log_{10}) of broilers in different treatment groups (30^{th} and 42^{nd} day)

Days	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	Period mean	SEM
30^{th}	1.51	1.66	1.70	1.70	1.80	1.86	1.70^{b}	0.11
42^{nd}	1.61	1.76	1.80	1.86	1.96	2.02	1.83^{a}	0.05
Treatment mean	1.55^{c}	1.71^{bc}	1.75^{abc}	1.78^{ab}	1.88^{ab}	1.95^{a}		
SEM	0.14	0.06	0.05	0.08	0.13	0.05		
p- value	Treatment		Period		Treatment*period			
	0.011		0.032		0.998			

Means within the same row with different superscripts are significantly different ($p<0.05$).

Table 6. Immunological parameters of broilers in different treatment groups

Parameter	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p- value
				Lymphoid organs (% of Live weight)				
Spleen	0.07^{b}	0.08^{b}	0.11^{a}	0.11^{a}	0.12^{a}	0.13^{a}	0.12	0.01
Thymus	0.37	0.44	0.50	0.52	0.55	0.58	0.55	0.25
Bursa of fabricius	0.11^{b}	0.15^{ab}	0.16^{a}	0.16^{a}	0.18^{a}	0.19^{a}	0.18	0.04
				IgG and IL-10				
IgG (µg/ml)	8.92^{c}	10.30^{bc}	10.08^{bc}	11.72^{ab}	12.15^{ab}	13.25^{ab}	0.43	0.03
IL-10 (ng/L)	15.46^{c}	18.01^{c}	19.05^{bc}	22.29^{ab}	23.39^{a}	25.10^{a}	0.83	0.01
				Liver function tests				
SGOT(IU/L)	244.14	215.78	229.42	236.88	256.64	206.72	6.83	0.31
SGPT(IU/L)	8.58	12.78	10.18	11.24	10.44	12.90	0.79	0.63

Means within the same row with different superscripts are significantly different ($p<0.05$).

CONCLUSION

The present study concluded that supplementation of humic substance @ 2 g/kg feed to the broilers diet improved feed to gain ratio and nutrient utilization (crude protein and crude fat) which resulted in better growth. Humic substance improved intestinal histomorphometry, immunological response and lowered caecal *E. coli* count which improves gut health. Substituting AGP with HS0.2 had lower feed cost Rs. per kg body weight gain and higher economic efficiency. Therefore, humic substance @ 2 g/kg feed can be used as an economically efficient alternative to antibiotic growth promoters in broilers diet to reduce the risk of antimicrobial resistance.

REFERENCES

Ahfeethah, F., Elazomi, A. and Kammon, A.2023. Effect of humic acid and probiotics on immunity of broiler chickens. Open Veterinary Journal. 13(7): 839-845.

Anadon, A. 2006. WS14 the EU ban of antibiotics as feed additives. 2006. Alternatives and consumer safety. Journal of Veterinary Pharmacology and Therapeutics. 29(1): 41-44.

AOAC.2012. Official Methods of Analysis, 19th Edn. Association of Analytical Chemists, Washington, D.C,USA.

Arif, M., Rehman, A., Saeed, M., Abd El-Hack, M.E., Arain, M.A., Haseebarshad, M., Zakria, H.M. and Abbasi, I.H. 2016. Impacts of dietary humic acid supplementation on growth performance, some blood metabolites and carcass traits of broiler chicks. Indian Journal of Animal Science. 86(9): 1073-1078.

Bezuglova, O. and Klimenko, A. 2022. Application of humic substances in agricultural industry. Agronomy. 12(3): 584-597.

Commercial chick feed specification. 2011. Feed specification for VENCOBB 400 broilers, Venkateshwara Hatcheries Private Limited, Pune, India.

Dominguez-Negrete, A., Gomez-Rosales, S., Angeles, M.D.L., Lopez-Hernandez, L.H., Reis-de Souza, T.C., Lopez-Garcia, Y., Zavala-Franco, A. and Tellez-Isaias, G.2019. Effect of the addition of humic substances as growth promoter in broiler chickens under two feeding regimens. Animals. 9(12): 1101-1116.

Drury, R.A.B. and Wallington, E.A. 1980. Carleton's Histological Technique, 5th Edn. Oxford University Press, pp 99-113.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.

ELnaggar, A.S. and El-Kelawy, M.I. 2018. Effect of humic acid supplementation on productive performance, blood constituents, immune response and carcass characteristics of sasso chicken. Egyptian Journal of Animal Production. 55(1): 75-84.

Ghazalah, A.A., El-Tahawy, W.S., Ghalwash, A.A. and Elnaggar, A.S. 2022. Productive and physiological response of broiler chicks to dietary humic acid. Egyptian Poultry Science Journal. 42(2): 157-170.

Gonzelez, M.J.A., Michel, M.A., Baxter, M.F.A., Tellez, G., Moore, P.A., Solis, C.B., Patlan, D.H., Guzman, R.M., Velasco, X.H., Latorre, J.D., Hargis, B.M., Rosales, S.G. and Isaias, G.T. 2018. Effect of humic acids on intestinal viscosity, leaky gut and ammonia excretion in a 24 hr feed restriction model to induce intestinal permeability in broiler chickens. Animal Science Journal. 89(7): 1002-1010.

Hammod, A.J., Zeny, Z.A.H., Mahdi, A.S. and Alfertosi, K.A. 2021. Probiotic and humic acid as feed additives and their effects on productive and economic traits of broiler. Indian Journal of Ecology. 48(13): 35-37.

Hascik, P., Arpasova, H., Pavelkova, A., Bobko, M., Cubon, J. and Bucko, O. 2018. Chemical composition of chicken meat after application of humic acid and probiotic *Lactobacillus fermentum*. Potravinarstvo Slovak Journal of Food Sciences. 12(1): 694–700.

Hubbard, S.A.S.1999. Broiler management guide, Hubbard SAS, Quintin, France.

Huck, J.A., Porte, N., and Bushell, M.E. 1991. Effect of humates on microbial activity. General Microbiology. 137: 2321-2329.

Kati, S.M. 2018. Effect of addition of different levels of humic acid to drinking water on productive and carcass characteristics of broiler chick. Journal of Kerbala for Agricultural Sciences. 5(3): 1-13.

Khalaquzzman, M. 2022. Effect of humic acid, probiotics and their combination on growth performance, immune response and carcass characteristics of broiler. MS thesis (Animal Nutrition), Sher-e-Bangla Agricultural University, Dhaka.

Korsakov, K., Simakova, I., Vasilyev, A., Lifanova, S. and Gulyaeva, L. 2019. The effect of humic acids on the natural resistance of the body of broiler chickens and the quality of their meat. Agronomy Research. 17(S2): 1356-1366.

Lala, A.O., Kwelum, N.O., So, A.O.O., Ajao, A.O. and Degbenjo, A.A.A. 2017. Response of broiler chickens to varying dosage of humic acid in drinking water. Journal of Animal Production Research. 29(1): 288-294.

Ma, J., Mahfuz, S., Wang, J. and Piao, X. 2021. Effect of dietary supplementation with mixed organic acids on immune function, antioxidative characteristics, digestive enzymes activity and intestinal health in broiler chickens. Frontiers in Nutrition. 8: 673316-673331.

Marcincak, S., Semjon, B., Marcincakova, D., Reitznerova, A., Mudronova, D., Vaskova, J. and Nagy, J. 2023. Humic substances as a feed supplement and the benefits of produced chicken meat. Life. 13(4): 927-940.

Markey, B., Leonard, F., Archambault, M., Cullinane, A., Maguire, D., Edwards, R. and Hewat, C. 2013. Clinical Veterinary Microbiology, 2nd Edn.Mosby Elsevier, London, pp 215-239.

Mehdi, A. and Hasan, G. 2012. Immune response of broiler chicks fed yeast derived mannan oligosaccharides and humate against Newcastle disease. World Applied Sciences Journal. 18(6): 779-785.

Mudronova, D., Karaffova, V., Pesulova, T., Koscova, J., Marussakova, I.C., Bartkovsky, M., Sevsikova., Z. and Marcincak, S. 2020. The effect of humic substances on gut microbiota and immune response of broilers. Food and Agricultural Immunology. 31(1): 137-149.

Nagaraju, R., Reddy, B.S., Gloridoss, R., Suresh, B.N. and Ramesh, C. 2014. Effect of dietary supplementation of humic acids on performance of broilers. Indian Journal of Animal Science. 84(4): 447-452.

Office International des Epizooties. 2004. Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). Office International des Epizooties, Paris, pp 258-269.

Omidiwura, B.R., Agboola, A.F., Emeruwa, O.M. and Awofodu, O.O. 2021. Effect of humic acid supplementation on growth response, gut morphology and microbial load in broiler chickens. Slovak Journal of Animal Science. 54(4): 176-185.

Panda, A.K., Rama Rao, S.V., Raju, M.V.L.N. and Shyam Sunder, G. 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Australasian Journal of Animal Sciences. 22(7): 1026-1031.

Pistova, V., Arpasova, H. and Hrncar, C. 2016. The effect of the humic acid and garlic (*Allium sativum*) on performance parameters and carcass characteristic of broiler chicken. Journal of Central European Agriculture. 17(4): 1168-1178.

Reitman, S. and Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 28(1): 56-63.

Saleh, A.A., Yassin, M., El-Naggar, K., Alzawqari, M.H., Albogami, S., Soliman, M., Shurky, M., Farrag, F. and Kirrella, A.A. 2022. Effect of dietary supplementation of humic acid and lincomycin on growth performance, nutrient digestibility, blood biochemistry and gut morphology in broilers under clostridium infection. Journal of Applied Animal Research. 50(1): 440-452.

Samudovska, A.H., Bujnak, L. and Zigo, F.2022. Carcass characteristic, caecal metabolites and dropping quality in broiler chickens fed diets containing a humic substances. Asian Journal of Agriculture and Food Sciences. 10(6): 133-138.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Method, 8th Edn. The lowa state university press, Ames, Lowa, USA.

Taklimi, S.M., Ghahri, H. and Isakan, M.A. 2012. Influence of different levels of humic acid and esterified glucomannan on growth performance and intestinal morphology of broiler chickens. Journal of Agricultural Science. 3(5): 663-668.

Zhang, A.R., Pirzado, S.A., Liu, G.H., Chen, Z.M., Chang, W.H., Cai, H.Y., Bryden W.L. and Zheng, A.J. 2020. Dietary supplementation with sodium humate improves egg quality and immune function of laying hens. Journal of Applied Animal Nutrition. 8(2): 93-99.