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Varun D Naik
Department of Poultry Science, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru, Karnataka, India

HC Indresh
Department of Poultry Science, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru, Karnataka, India

Krishnamurthy TN
Department of Poultry Science, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru, Karnataka, India

Wilfred Ruban
Department of Livestock Products Technology, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru, Karnataka, India

Corresponding Author:
Varun D Naik
Department of Poultry Science, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru, Karnataka, India

Effect of supplementing humic substance on growth performance in broilers

Varun D Naik, HC Indresh, Krishnamurthy TN and Wilfred Ruban

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Abstract
The study investigated the effects of supplementing broiler diets with humic substances on growth parameters. A total of 120 day-old broiler chicks were divided into four groups: a control group (T_1) fed a standard diet based on Bureau of Indian Standards (2007), and three experimental groups receiving diets with 0.02% humic substance (T_2), 0.04% humic substance (T_3), and 0.02% bacitracin methylene disalicylate (T_4). The results revealed significant improvements in body weight, feed intake, and feed conversion ratio with 0.04% humic substance and 0.02% bacitracin methylene disalicylate compared to the control group, with no notable impact on survivability. Interestingly, the effects of 0.04% humic substance were similar to those of 0.02% bacitracin methylene disalicylate. Therefore, 0.04% humic substance emerges as a promising alternative to antibiotic growth promoters for enhancing broiler growth performance.

Keywords: Humic substance, Growth performance, Antibiotic growth promoter, Broilers

Introduction
In recent years, the poultry industry has experienced significant growth due to advances in breeding, disease control, and management practices, contributing substantially to the global supply of high-quality proteins through meat and eggs and boosting farmers' revenue. However, this success has been accompanied by a concerning practice of sub-therapeutic use of antibiotics in poultry farming, traditionally aimed at enhancing growth and health in poultry by controlling pathogenic bacteria, modulating immunity, and exerting anti-inflammatory effects (Niewold, 2007) [14]. This dual scenario raises important questions about the sustainability and long-term implications of antibiotic use in the poultry industry, highlighting the need for a careful balance between growth objectives and public health concerns. Scientific evidence underscores the concerning consequence that unscientific antibiotic use is leading to the emergence of bacterial resistance (Apata, 2009) [2], with resistant bacteria detected in various environments, posing potential threats to human health (Zhang *et al*., 2020) [21]. In response to the escalating concern over antibiotic resistance, the European Union implemented a comprehensive ban on the use of growth-promoting antibiotics in animal production in 2006, followed by the United States in 2017, acknowledging the potential public health risks associated with antibiotic resistance (Salim *et al*., 2018) [18].

An emerging alternative in poultry nutrition involves incorporating humic substances into broiler diets. These substances, derived from the decomposition of organic matter, possess unique properties that can enhance nutrient digestibility, promote growth, and contribute to overall poultry health. This study aims to explore the potential of humic substances as growth promoters in broiler diets, aiming to uncover their mechanisms of action and the benefits they may offer. Humic substances are organic compounds formed from the decomposition of organic matter, characterized by long molecular chains and high molecular weight. Constituting the primary component of soil organic matter, they can be categorized into three principal fractions: humic acids (HA) (acid-insoluble fraction), fulvic acids (soluble in both alkali and acid), and humin (insoluble in both alkali and acid) (Abd El-Hack, 2016) [1].

With significant roles in poultry productivity, humic substances possess essential attributes related to their chemical composition, proteins, water solubility, antibacterial properties, and immune-stimulating effects. They can alter the intestinal microflora by increasing the counts of beneficial bacteria, as demonstrated by Schepetkin *et al*. (2003) [19]. Additionally, research by Taklimi *et al*. (2012) [20] suggests that humic acids (HA) can influence the crypt depth and villi height in the jejunum of broilers. Furthermore, humic substances have been observed to have a protective effect on the intestinal mucosa, displaying anti-inflammatory, adsorbent, antitoxic, and antimicrobial properties. The diverse structures and functional groups present in humic substances contribute to properties such as colloidal, spectral, electrochemical, and ion exchange, thereby imparting substantial adsorption capacity.

Mudronova *et al*. (2020) [12] observed a decrease in enterobacteria and an increase in lactic acid bacteria in broilers supplemented with humic substances, indicating a promotion of beneficial gut microbiota. Showing that humic substances can affect microbial metabolism, influencing carbohydrate and protein metabolism, and contribute to the reduction of pathogenic viruses and bacteria (Rath *et al*., 2005) [17].

Materials and Methods

Venkateshwara Hatcheries Pvt. Ltd. provided 120 day-old commercial broiler chicks for this investigation, and Novel Links, Srigandada Kaval, Bengaluru provided the humic substance. The chicks were first evaluated on the basis of their weight upon acquisition, and then they were split into four experimental groups at random. There were three duplicates in per group, and each replicate included 10 chicks. Based on the guidelines provided by the Bureau of Indian Standards (BIS) in 2007 [4], the basal diet (T_1) was created. For Treatment 2 (T_2), 0.02% humic material was added to the baseline diet. For Treatment 3 (T_3), 0.04% humic material was added to the baseline diet. Additionally, 0.02 percent antibiotic BMD (bacitracin methylene disalicylate) was added to the baseline diet for Treatment 4 (T_4).

Up to the age of six weeks, the chicks were kept under regular management procedures and grown in a deep litter system. The birds were vaccinated according to a standard vaccination schedule. Water and food were given to the animals whenever they needed them during the trial. The KVAFSU Institutional Animal Ethics Committee in Bidar, Karnataka, gave its approval to the study.

Growth parameters

During the experimental period, comprehensive data on growth performance parameters were meticulously recorded. These parameters included weekly body weight measurements, quantities of feed consumption, feed conversion ratio, and survivability rates.

Body weight (weekly cumulative)

In the scientific study, the investigation centered on monitoring the growth rate of individual birds through regular measurements of their body weights. These weight assessments were carried out in the early morning, before the birds were fed, and were conducted on a weekly basis. Data on cumulative body weight were collected at weekly intervals throughout the study's duration, with a final measurement taken at the conclusion of the trial.

Feed consumption (weekly cumulative)

In the research, the study meticulously recorded the average weekly cumulative feed consumption for each replication. This process involved calculating the weekly feed consumption by subtracting the remaining or unconsumed feed at the end of the week from the total amount of feed supplied during that particular week. The unconsumed feed was then added to the feed consumption for the subsequent week to arrive at the cumulative feed consumption. This method enabled the researchers to accurately track the birds' feed intake over time.

Feed conversion ratio (Weekly Cumulative)

In the study, the feed conversion ratio (FCR) was determined by calculating the ratio of feed consumed in kilograms to body weight in kilograms for each experimental group on a weekly basis, as well as cumulatively.

Survivability

Mortality in each group was recorded, and the study also noted the percentage of mortality in each treatment throughout the experiment's duration.

Results

Body weight

The average body weights (g/bird) of the birds in treatment groups T_1, T_2, T_3, and T_4 were 1731.18, 2117.67, 2124.47, and 2327.80, respectively, at the conclusion of the sixth week. An ANOVA analysis showed that there was a significant ($p<0.05$) variation in the treatment groups' average body weight. The average body weight of groups T_3 and T_4 was considerably ($p<0.05$) greater than that of groups T_1 and T_2. Body weight did not significantly change ($p>0.05$) between groups T_3 and T_4, or between T_1 and T_2.

Feed consumption

At the conclusion of the fifth week, the average feed intake (g/bird) for each treatment group was 2733.58 (T_1), 2761.53 (T_2), 2846.63 (T_3), and 2839.43 (T_4). ANOVA revealed a significant ($p<0.05$) variation in the weekly cumulative feed consumption across the treatment groups at the conclusion of the fifth week. Groups T_3 and T_4 consumed significantly more feed cumulatively ($p<0.05$) than groups T_1 and T_2. However, there was no appreciable difference in feed consumption ($p>0.05$) between treatment groups T_1 and T_2 or T_3 and T_4.

The overall average feed intake (g/bird) at the end of the sixth week was 3935.63 (T_1), 3927.23 (T_2), 3888.01 (T_3), and 3888.56 (T_4). According to statistical analysis, feed intake did not significantly differ between the treatment and control groups ($p>0.05$).

Feed conversion ratio (FCR)

At the conclusion of the sixth week, the birds in each of the four treatment groups had cumulative feed conversion ratios of 1.900 (T_1), 1.889 (T_2), 1.703 (T_3), and 1.709 (T_4). At the conclusion of the sixth week, the ANOVA showed a significant ($p<0.05$) difference in the cumulative feed conversion ratio of the birds across the treatment groups. Compared to groups T_1 and T_2, the treatment groups T_3 and T_4 had a considerably ($p<0.05$) higher cumulative feed conversion ratio. The cumulative feed conversion ratio did not change significantly ($P > 0.05$) between treatment groups T_3 and T_4, as well as T_1 and T_2.

Survivability

The impact of humic substance on percent survivability in broilers were studied and found that the survivability (%) values were 100 percent in all groups (T_1, T_2, T_3 and T_4). The statistical analysis revealed that there is no significant difference in survivability among different treatment groups.

Discussion

From the first week of the experiment to its conclusion, there was a significant difference ($p<0.05$) in the body weight of the birds fed with humic material as compared to the control group.

The current study's findings concur with those of Jaduttova *et al.* (2019) [7], who assessed the impact of incorporating humic compounds into feed at concentrations of 0.8% and 1.0%. The experimental group supplied with 1 percent humic substance had a considerably greater mean body weight than the control group, as indicated by the results ($p<0.05$).

The benefits of humic substance are responsible for the positive outcome. These benefits include strengthening the immune system, preventing the growth of mold and pathogenic bacteria, and creating a thin protective layer over the gastrointestinal tract's epithelial layer. This improved nutrient absorption led to an increase in body weight and cumulative weight gain in broilers (Abd El-Hack. 2016) [1].

Marcincakova *et al*. (2015) [11] disagrees with the present study since they found that supplementing humic substances in the feed at 0.6 percent concentration on broilers revealed no significant influence of humic substances on the body weight of the broilers.

There was a significant difference ($P \leq 0.05$) in the feed consumption of the birds fed with humic substance compared to the control group from first week till fifth week of the experiment.

The findings of the present results were in agreement with Dominguez *et al*. (2021) [4] who conducted a study to assess the influence of humic substances extracted from worm compost on broiler production parameters. The results indicated that feed intake increased in the humic substance supplemented group from 1–42 days.

Humic substance enhances feed intake and nutrient digestibility by promoting the elongation of villi. As villus length increases, so does the surface area available for nutrient absorption, consequently enhancing growth performance. Additionally, humic substances can help maintain a healthy gut microbiota and form a protective barrier against the invasion of microbes and harmful substances, preventing their entry into the intestine (Taklimi *et al.*, 2012) [20].

The findings of the present results were in disagreement with Kocabagli *et al*. (2002) [9] who investigated the growth-promoting effects of humic substances using the commercial preparation Farmagulator dry Humate (FH) on broiler chickens. In their study, FH was added to the diets at a concentration of 2.5 kg per ton of feed. The researchers concluded that there were no statistically significant differences ($p>0.05$) in overall feed consumption among the dietary groups receiving humate.

From the second week of the trial to its conclusion, the feed conversion ratio of the birds fed with humic material differed significantly ($p<0.05$) from the control group.

The present results align with the investigation carried out by Dominguez *et al.* (2021) [4], which assessed the impact of humic substances obtained from worm compost on metrics related to broiler production. According to their research, the group fed humic substances consumed less feed, gained weight at a rate comparable to that of the group provided antibiotic growth promoter, and had a much higher feed conversion ratio ($p<0.05$).

They concluded that humic substance has the capacity to enhance nutrient absorption and utilization, contributing to overall growth and physiological improvements in broilers. The increase in live weight without a proportional increase in feed quantity contributed to the overall better FCR. Which is attributed it to the stabilizing effect of humic substance on the gut microbiota and its ability to enhance nutrient utilization. This, in turn, contributed to a more favorable FCR (Abd El-Hack. 2016) [1].

The results of this study disagreed with those of Karaoglu *et al.* (2004) [8], who looked at how different amounts of extra humate affected growth performance. According to the results, humate had no discernible impact on the feed conversion ratio ($p>0.05$).

Until the completion of the trial, there was no discernible difference ($p>0.05$) in the survivorship of the birds in the groups fed humic material as compared to the control group.

The results of this study are in line with those of Pistova *et al.* (2016) [16], who investigated the impact of a supplement containing humic acid and herbal additives on broiler chicken production metrics. They found that the group of chicks administered the supplement had no mortality.

They explained it away as the result of humic substance's capacity to lower intestinal pH and prevent the formation of microbial infections. According to Islam *et al*. (2005) [6], humic acid has been shown to have antibacterial, antithyroidal, antiviral and anti-inflammatory properties in animals, which strengthens the immune system.

Table 1: Effect of supplementing humic substance on weekly cumulative body weight (g / bird / week) (Mean ± SE) in broilers.

Experimental group	Weeks					
	I	II	III	IV	V	VI
T_1	140.90 ± 2.99^b	339.53 ± 1.76^c	708.23 ± 3.78^b	1118.67 ± 2.41^d	1607.40 ± 5.70^b	1731.18 ± 12.03^b
T_2	144.50 ± 2.52^b	350.13 ± 3.06^b	713.80 ± 3.09^b	1130.33 ± 5.12^c	1618.07 ± 7.20^b	2117.67 ± 12.88^b
T_3	170.37 ± 2.11^a	387.97 ± 2.98^a	791.20 ± 4.66^a	1247.30 ± 0.95^a	1856.80 ± 8.43^a	2124.47 ± 14.96^a
T_4	171.07 ± 2.15^a	390.97 ± 3.14^a	785.80 ± 4.70^a	1232.33 ± 0.57^b	1842.47 ± 16.72^a	2327.80 ± 15.65^a

^{a,b,c,d}Means in the same column with no common superscript differ significantly ($P \leq 0.05$)

Table 2: Effect of supplementing humic substance on weekly cumulative feed intake (g / bird / week) (Mean ± SE) in broilers.

Experimental group	Weeks					
	I	II	III	IV	V	VI
T_1	106.02 ± 2.38[b]	383.83 ± 3.40[b]	953.50 ± 6.08[b]	1658.44 ± 2.75[b]	2733.58 ± 5.22[b]	3935.63 ± 20.42
T_2	110.37 ± 3.20[b]	398.24 ± 4.13[b]	954.20 ± 5.27[b]	1676.25 ± 0.74[b]	2761.53 ± 9.00[b]	3927.23 ± 23.29
T_3	135.72 ± 1.02[a]	435.07 ± 3.79[a]	1019.79 ± 5.88[a]	1723.01 ± 4.12[a]	2846.63 ± 14.32[a]	3888.01 ± 6.05
T_4	135.32 ± 1.28[a]	438.94 ± 2.15[a]	1010.11 ± 6.53 [a]	1709.02 ± 13.53[a]	2839.43± 24.47[a]	3888.56 ± 35.51

[a, b] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 3: Effect of supplementing humic substance on weekly cumulative feed conversion ratio (Mean ± SE) in broilers.

Experimental group	Weeks					
	I	II	III	IV	V	VI
T_1	1.116 ± 0.004	1.307 ± 0.005[a]	1.440 ± 0.003[a]	1.546 ± 0.003[a]	1.751 ± 0.005[a]	1.900 ± 0.003[a]
T_2	1.110 ± 0.015	1.306 ± 0.004[a]	1.427 ± 0.003[a]	1.545 ± 0.004[a]	1.756 ± 0.004[a]	1.889 ± 0.005[a]
T_3	1.084 ± 0.004	1.269 ± 0.003[b]	1.367 ± 0.004[b]	1.433 ± 0.004[b]	1.571 ± 0.005[b]	1.703 ± 0.003[b]
T_4	1.080 ± 0.002	1.272 ± 0.002[b]	1.365 ± 0.004[b]	1.440 ± 0.010[b]	1.580 ± 0.006[b]	1.709 ± 0.002[b]

[a, b] Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table 4: Effect of supplementing humic substance on survivability (%) in broilers.

Experimental group	Description of the treatment	Survivability percentage (%)
T_1	Basal diet	100 ± 0.00
T_2	Basal diet + 0.02% Humic substance	100 ± 0.00
T_3	Basal diet + 0.04% Humic Substance	100 ± 0.00
T_4	Basal diet + 0.02% Bacitracin disalicylate (BMD)	100 ± 0.00

Conclusion

According to the findings, body weight, feed intake, and feed efficiency improved with the addition of 0.04 percent humic substance and 0.02 percent bacitracin methylene disalicylate (BMD). However, humic substance may not have affected survivability, suggesting that it performed similarly to an antibiotic.

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