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Effect of Dietary Inclusion of Black Cumin (*Nigella sativa*) on Histo-Morphometrical Parameters of the Small Intestine in Broiler

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ABSTRACT

This study aimed to evaluate the effects of black cumin seed (BCS) supplementation on intestinal morphology and growth performance in broiler chickens during the finishing period. A total of 225 one-day-old Lohman meat (Indian River) broiler chicks, with an average body weight of 40 ± 0.50 g, were divided into five groups, with three replicates of 15 birds each. The treatments were T0 = control (basal feed), T1 = basal feed + antibiotic, T2 = basal feed + 0.5% BCS, T3 = basal feed + 1% BCS, and T4 = basal feed + 1.5% BCS. Birds were reared under standard management for 35 days, during which growth performance parameters (body weight, feed intake, feed conversion ratio) were recorded weekly. At the end of the trial, three broilers from each group were randomly necropsied, and samples of the small intestine (duodenum, jejunum, and ileum) were fixed with 10% neutral buffered formalin solution and stained with Hematoxylin and Eosin (H&E). The results indicated that average body weight gain, feed intake, and feed conversion ratio significantly improved with dietary inclusion of 1% and 1.5% BCS compared with the control and antibiotic groups. The dietary treatments influenced the morphology of small intestinal villi. Birds fed with 1.5% BCS presented higher villus height (duodenum, jejunum, ileum), villus surface area (duodenum), and villus height \square crypt depth (duodenum, jejunum, ileum) compared with control and other treatment groups ($p < 0.05$). In the duodenum, total goblet cell count was higher ($p < 0.05$) in all of the supplemented groups when compared with non-supplemented birds. Moreover, supplementation with 1.5% significantly decreased epithelial thickness and the number of intestinal goblet cells compared with other supplemented and control treatments ($p < 0.005$). These findings suggest that dietary supplementation with 1.5% BCS has a beneficial effect on intestinal morpho-physiology of broilers grown at normal conditions in an open-sided environment.

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Introduction

Nigella sativa L. (black cumin), an aromatic plant, has been used as a natural remedy for many ailments for over 2000 years. It is grown in various parts of the world, especially in the Middle East and South Asia, to support health. Black cumin seeds (BCS) have antiparasitic, anthelmintic, antidiabetic, analgesic, bronchodilator, renal protective, spasmolytic, and antihypertensive properties (Koç *et al.*, 2019; Selvaraju *et al.*, 2019). The grinded seeds of *N. sativa* have beneficial effects on metabolism, growth, and meat quality in broilers and also improve the performance of layers and its egg quality (Abu-Dieyeh *et al.*, 2008; Kishan *et al.*, 2023; Kumar & Patra 2017). There is general agreement that BCS has no adverse effects (up to 30 g/kg) on poultry performance and can be supplemented to mitigate the deleterious effects of hot climatic conditions (Hafez *et al.*, 2016). Supplementing poultry diets with BCS, BCS oil, and BCS meal improved growth performance, biochemical and hematological responses, and mortality rate (Al-Mufarrej, 2014; Akon *et al.*, 2019; Cimrin *et al.*, 2023). Feeding different forms and levels of BCS has also been reported to alleviate heat stress and is considered a natural antioxidant for poultry (Selvaraju *et al.*, 2019).

Functional anatomical and histological characteristics of the avian gastrointestinal tract are critical to their feed conversion efficiency (Adibmoradi *et al.*, 2006; Alshamy *et al.*, 2018). To facilitate maximal absorption of dietary components, the intestinal mucosa is highly convoluted and specialized. The epithelium is folded into villi and the epithelial cells have apical microvilli, forming a brush border observed by optical microscopy. These infoldings increase the small intestinal surface area for nutrient absorption by about 600-fold, thereby increasing nutrient absorption capacity (Alshamy *et al.*, 2018). Intestinal morphology (villus height and crypt depth) changes in response to exogenous agents. Deeper crypts indicate faster tissue turnover as they contain progenitor cells. Intestinal mucins are high-molecular-weight glycoproteins secreted by goblet cells. In chickens, mucins are expressed extensively by goblet cells in the colon and small intestine (Tollba *et al.*, 2003). Obviously, the improvement of poultry growth performance depends on intestinal health and consequently on microbiome composition (Popescu *et al.*, 2020; Rezaian *et al.*, 2012). An appropriate microbiota is favored by different vegetal additives. In this context, a method to improve animal performance is the use of vegetal feed additives, which have beneficial effects in poultry production and animal health and nutrition, possibly through increased feed intake and enhanced digestive secretions. These also have antimicrobial, antiviral, antioxidant, and immunomodulatory properties. A large number of previous studies have suggested an increase in the production performance of chickens fed with BCS. A number of experiments have been conducted to investigate the possibility of introducing black cumin seeds (BCS) as natural feed additive to poultry diets for better performance (Abbas, *et al.*, 2010; Abu Dieyeh *et al.*, 2008; Aydogan *et al.*, 2020; Erener *et al.*, 2010). However, no comprehensive published data are available on the effects of BCS on intestinal morphology in broiler chickens. The aim of this work was to evaluate the effect of black cumin used as a growth promoter on the morphology of the duodenum, jejunum, and ileum of broiler chickens during the finishing period.

Materials and Methods

Experimental Site

Broilers were reared at the Poultry Farm of Sher-e-Bangla Agricultural University, and the histological evaluation of the small intestine was conducted at the Anatomy, Histology, & Physiology Laboratory under the Faculty of Animal Science and Veterinary Medicine of Sher-e-Bangla Agricultural University, Dhaka-1207.

Collection of experimental broiler chickens

A total of 225-day-old Lohman Meat (Indian River) commercial broiler chicks were collected from Kazi hatchery, Gazipur, Dhaka.

Experimental layout, birds and dietary treatments

A total of 225-day-old broiler chicks were divided into five experimental groups with 3 replications of 15 chicks each using a completely randomized block design. The treatments consisted of a control group fed the basal diet (no BCS); three groups fed the basal diet supplemented with BCS at levels of 0.50%, 1%, and 1.50%; and one group fed the basal diet supplemented with an antibiotic (Doxycycline at 1 g/2 L of drinking water). The "Lohman Meat (Indian River)" strain, with an average body weight of 40.00 ± 0.50 g, was purchased from Kazi Farm Limited Hatchery, Gazipur, Dhaka. Starter and grower commercial Kazi broiler feed were procured from the local market. Feed was supplied four times daily by following the Indian River Management Manual and ad libitum drinking water two times daily. The composition of the starter and grower diet, which was collected from Kazi Farm Limited Company, is given in Table 1. The collected chicks were transferred to the university poultry farm and held in electric brooders for 7 days, following standard brooding protocol. During the brooding period, only the basal diet was given; no BCS was used as a treatment. The chicks were given glucose water with vitamin C to drink for the first 3 hours to help overcome dehydration and transportation stress. Subsequently, small feed particles were supplied on the newspapers to start feeding for the first 24 hours. After 7 days, chicks from the brooders were randomly assigned to dietary treatments. The birds were vaccinated on the proper schedule against Newcastle disease, Infectious bronchitis, and Infectious bursal disease (Table 2). Vitamin B complex, Vitamin A, D3, and E were used against deficiency diseases. Electromin and Vitamin C are also used to protect birds from heat stress.

Plant Material

Collection of BCS were obtained from a local market of Dhaka. The BCS were supplied to the birds after mixing with feed according to the desired treatments

Table 1. Chemical composition of the basal diet (starter and grower)

Parameter	Starter diet (0-7 days)	Grower diet (8-35 days)
Protein (%)	21.0	19.0
Fat (%)	6.0	6.0
Fiber (%)	5.0	5.0
Ash (%)	8.0	8.0
Lysine (%)	1.20	1.10
Methionine (%)	0.49	0.47
Cysteine (%)	0.40	0.39
Tryptophan (%)	0.19	0.18
Threonine (%)	0.79	0.75
Arginine (%)	1.26	1.18

Table 2. The vaccination schedule

Age of birds	Vaccine	Name of vaccine	Route of administration
2 days	Infectious Bronchitis + Newcastle Disease	CEVAC BIL Vaccine	One drop in one eye
9 days	Gumboro	CEVAC IBD L Vaccine	One drop in one eye
16 days	Gumboro	CEVAC IBD L Vaccine	Drinking water
19 days	Newcastle Disease	CEVAC NEW L Vaccine	Drinking water

Performance Parameters

The birds were individually weighed at weekly intervals (weeks 1 to 5). Average body weight (ABW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were also calculated weekly (FI/BWG).

Intestinal Tissue sampling and histomorphological analysis

At the end of the feeding trial (i.e., day 35), 3 birds from each group were randomly selected and slaughtered for small intestinal histo-morphological examination. About 3 cm lengths of duodenum (midpoint of the pancreatic loop), jejunum (midpoint of jejunum) and ileum (5 cm after Meckel's diverticulum) were removed. Forty-five duodenal, jejunal, and ileal samples (3 from each group) were collected from the birds directly after slaughter and fixed in 10% neutralized formalin. The fixed tissue samples were transported to the Department of Anatomy, Histology and Physiology, Faculty of Animal Science and Veterinary Medicine, SAU, Dhaka where they were further processed. The tissues were then dehydrated with increasing concentrations of ethyl alcohol (70%, 80%, 90%, 95% and 100%), cleared in xylene and embedded in paraffin. The paraffin blocks were then cut into 4 μ m-thick sections per broiler using a microtome, stained with hematoxylin and eosin (H&E), and examined under a light microscope (Euromex). Representative fields were photographed, and digital images were captured for morphometric analysis. A computer morphometric program, ImageJ software, was used for morphometric intestinal variables, villus height, crypt depth, and villus height: crypt depth ratio was evaluated in the duodenum, jejunum, and ileum. Villus height was measured from the tip of the villus to the villus crypt junction, and the crypt depth was measured from the base of the crypt up to the

region of transition between the crypt and villus. The absorptive surface area of the villus was estimated as described by Prakatur et al., (2019). By considering a villus as a cylindrical structure villus absorptive surface area was calculated using the formula: Villus absorptive surface area = $2\pi \times (\text{average villus width}/2) \times \text{villus height}$ (Hartono et al., 2021).

Statistical analysis

The total data were compiled, tabulated, and analyzed in accordance with the study's objectives. Excel Program was practiced for preliminary data calculation. The collected data were subjected to statistical analysis using one-way ANOVA in SPSS (version 16.0). Differences between means were tested using Duncan's multiple comparison test, LSD and significance was set at $p < 0.05$.

Results and Discussion

Growth performances

The results of average body weight gain (ABWG), FI and FCR are presented in Table 3. The ABWG was higher ($p < 0.05$) in 1.5% BCS (2351g) group compared to the control group (1991g). Significant ABWG differences among different trial groups of BCS are consistent with the previous studies (Khan, *et al.*, 2012; Yatoo, *et al.*, 2012; Al-Mufarrej 2013; Beg *et al.* 2016). Greater ABWG by BCS may be attributed to better nutrient absorption from the intestine (Kumar et al., 2017). Another possible reason for BCS's positive effect on ABWG in broiler chickens may be its antimicrobial activity. Data presented in Table 3 showed that the result of different treatments on final feed consumption (grams per broiler chicken) was significant ($P < 0.05$). The highest average feed consumption was found in 1.5% BCS (3085g), and the lowest result was in the control (2861 g) group. The feed consumption increases in addition of BCS in feed with significant difference among the treatments that agrees previous studies (Al-Mufarrej 2013; Saleh 2014; Khalaji, *et al.*, 2011; Abbas *et al.*, 2010; Erener *et al.*, 2010; Ismail 2011; Yatoo *et al.*, 2012). We hypothesize that dietary BCS may deteriorate feed efficiency in broiler chickens in a dose-dependent relationship. In this study, supplementation of BCS significantly improved FCR in broiler that is may be due to the better intensive management, lower mortality rate and the positive effect of BCS in the ration, which agrees with previous studies (Hartono *et al.*, 2021; Durrani *et al.*, 2007; Abu Dieyeh *et al.*, 2008; Erener *et al.*, 2010., Yatoo, *et al.*, 2012., Jahan *et al.*, 2016; Ghasemi *et al.*, 2014) showed supplementation of BCS significantly improved FCR in broiler. In contrast Nasir and Grashorn (2010) and Abbas and Ahmed (2010), reported that chicks fed diets containing 1 and 2% BCS showed a non-significant effect on FCR. The lack of effect of the BCS supplement might relate to the composition of basal diets, management and husbandry conditions, and the feed efficiency of the birds was at their maximum, leaving no or very little room for improvement by BCS inclusion.

Table 3. Effect of Black cumin and antibiotics on average body weight gain (ABWG), feed intake (FI), Feed Conversion Ratio (FCR), and survivability rate of 5-week-old broilers fed different levels of Black Cumin seed.

Treatments	Feed Intake	Body weight gain	FCR			
	Mean	SE	Mean	SE	Mean	SE
T ₀	2861.65 ^c	0.63	1991.46 ^d	0.64	1.43 ^a	0.00
T ₁	2998.79 ^{bc}	1.41	2131.64 ^c	0.66	1.41 ^{ab}	0.00
T ₂	3209.77 ^a	94.88	2351.82 ^a	0.80	1.39 ^{ab}	0.05
T ₃	3084.28 ^{ab}	0.77	2242.64 ^b	0.45	1.38 ^{ab}	0.00
T ₄	3085.78 ^{ab}	22.90	2351.82 ^a	0.80	1.32 ^b	0.00

Here, T₀ = Control (basal feed), T₁ = Basal feed + Antibiotic, T₂ = Basal feed + 0.50% BCS, T₃ = Basal feed + 1% BCS, T₄ = Basal feed + 1.5% BCS ^{abcd} Values bearing different letters within each column differ significantly ($P < 0.05$)

Intestinal morphometrical parameters

The effect of BCS on villus height, epithelial thickness, goblet cell number, crypt depth, and the ratio of crypt depth to villus height of different sections of the small intestine of experimental birds is shown in Table 4. Villi in the duodenum, jejunum and ileum of birds fed the diets supplemented with increasing levels of BCS were found to be correspondingly longer ($p < 0.05$) are shown in the Figures (1-3). Specially, the villus height tended to be higher ($P < 0.05$) in 1.5% dietary BCS groups than that of other groups. Feeding BCS significantly decreased epithelial thickness in different sections of the small intestine ($P < 0.05$) and this reduction was more obvious in 1.5% dietary levels of BCS. The goblet cell numbers in the villi of duodenum, jejunum and ileum of birds fed the diets supplemented with BCS were lower ($P < 0.05$) than control.

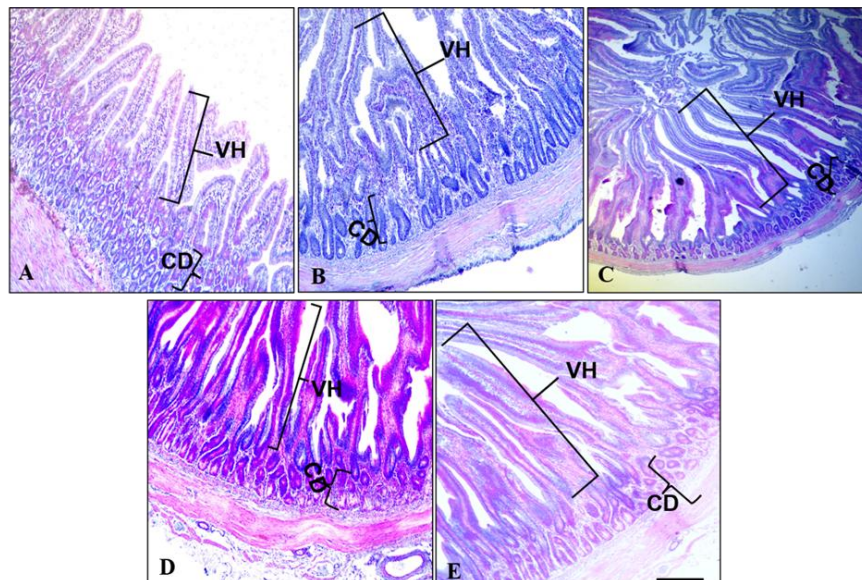


Figure 1. Villous length and crypt of duodenum A. Control, B: Antibiotic, C: 0.5% BCS, D: 1%, E: 1.5% BCS. In control, the villus structures are short and thickened compared to the birds fed BCS. CD: Crypt depth, VH: Villi height. H & E staining. Scale bars represent 50 μ m

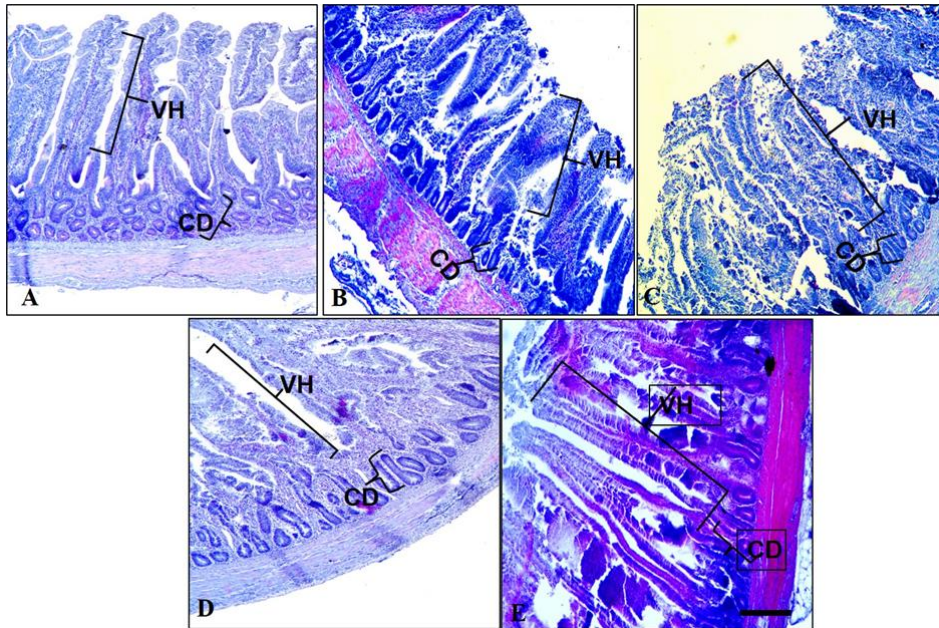


Figure 2. Villous length and crypt of jejunum A. Control, B: Antibiotic, C: 0.5% BCS, D: 1%, E: 1.5% BCS. In control, the villus structures are short and thickened compared to the birds fed BCS. CD: Crypt depth, VH: Villi height. H & E staining. Scale bars represent 50 µm

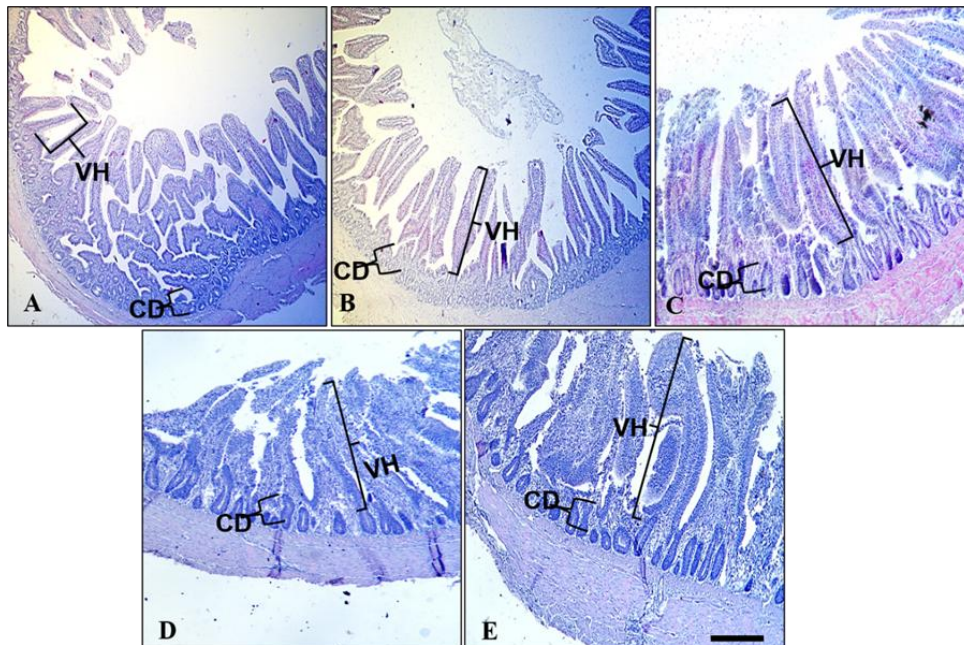


Figure 3. Villous length and crypt of ileum A. Control, B: Antibiotic, C: 0.5% BCS, D: 1%, E: 1.5% BCS. In control, the villus structures are short and thickened compared to the birds fed BCS. CD: Crypt depth, VH: Villi height. H & E staining. Scale bars represent 50 µm

Table 4. Intestinal morphometric parameters in broiler chickens supplemented with BCS

Parameter	T ₀	T ₁	T ₂	T ₃	T ₄
Duodenum					
Villous height (µm)	1752.9 ^c	1770.8 ^a	1959.5 ^a	1966.9 ^c	1966.9 ^{bcd}
Crypt depth (µm)	150.9 ^c	157.3 ^a	162.8 ^{ab}	163 ^{cd}	163 ^{bcd}
Villus height: Crypt depth	11.63 ^a	11.26 ^c	12.04 ^a	12.17 ^{cd}	12.17 ^{bcd}
Villous surface area (mm ²)	0.39 ^b	0.59 ^c	0.9981 ^a	1.0281 ^{cd}	1.281 ^{bcd}
Epithelial thickness (µm)	45.1	44.4	33.1	32	32
Goblet cell number	10.7	9.9	6.3	5.5	5.5
Jejunum					
Villous Height (µm)	808.1 ^c	838.2 ^a	946.4 ^a	952.5 ^c	952.5 ^{bcd}
Crypt depth (µm)	104	128.1	121	125	125
Villus height: Crypt depth	6.01 ^a	6.25 ^c	7.81	8.11 ^{bc}	8.34 ^{cd}
Villous surface area (mm ²)	0.358	0.523	0.725	0.741	0.741
Epithelial Thickness (µm)	39.7	37.7	32.2	30.64	30.64
Goblet cell number	10.4	10.3	7.6	6.6	6.6
Ileum					
Villous Height (µm)	741 ^c	849 ^c	924.11 ^{ab}	931.30 ^{ac}	935.12 ^{acd}
Crypt depth (µm)	93.1	125.2	119.7	124	124
Villus height: Crypt depth	6.84	7.74	7.98	7.97	7.97
Villous surface area (mm ²)	0.28	0.32	0.45	0.47	0.47
Epithelial thickness (µm)	36.55	34.29	25.2	22.9	22.9
Goblet cell number (mm)	11.1	10.7	7.5	6.9	6.9

Here, T₀ = Control (basal feed), T₁ = Basal feed + Antibiotic, T₂ = Basal feed + 0.50% BCS, T₃ = Basal feed + 1% BCS, T₄ = Basal feed + 1.5% BCS Results are given as means ± SEM

^{a,b,c,d} Means within a row with different superscripts are significantly different ($P < 0.05$)

In all sections of the small intestine, BCS inclusion increased ($P < 0.05$) crypt depth in a dose related manner and reduced villus height: crypt depth ratio in duodenum, jejunum and ileum compared with other treatments ($P < 0.05$) as shown in **Table 5**. The villus surface area in duodenum showed higher value ($P < 0.05$) in 1.5% BCS group (1.28 mm²) when compared to control (0.39 mm²), antibiotic (0.59 mm²), 0.5% (0.99 mm²), and 1% (1.0 mm²) BCS groups. Previous study by Fan et al., (1997) reported that increased villus height and villus height to crypt ratio are directly correlated with an increased epithelial turnover and longer villi are associated with activated cell mitosis, while shortening of villus and deeper crypts lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, and reduced performance. In agreement with our findings Hartono (2021) reported that the supplementation of black cumin through drinking water significantly increase ($P < 0.05$) the average sizes of villi height, width and villi area and gland diameter of small intestine

(duodenum, jejunum, ileum) and large intestine (colon) of broiler chickens. Another study by Boka (2014) found an increase in the villus height-to-crypt depth ratio after dietary supplementation with 2% BCS. In contrast to our findings, Aydogan (2020) and Kumar (2017) did not find significant effects on villous height, crypt depth, or their interrelation. Discrepancies among studies using BCS may result from differences in BCS dose rates.

Conclusion

In conclusion, the present study showed that the addition of BCS to feed mixtures has a significant protective effect on gut tissue in chickens, as reflected in better morphometric measures of intestinal villi and crypts across all experimental groups compared with the control group. Changes in intestinal morphology, as described above, can lead to improved nutrient absorption, reduced gut secretion, and better overall performance. These findings suggest that dietary supplementation with BCS has a beneficial effect on intestinal morpho-physiology as a natural growth promoter for broilers.

Competing Interest

The authors declared there is no conflict of interest.

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