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The effects of black cumin (*Nigella sativa* L.) seed on performance, intestinal morphology, ileum microbial count, and nutrient digestibility of broilers

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ABSTRACT

This study evaluated the effects of different levels of black cumin (*Nigella Sativa* L.) seed vs. virginiamycin and vitamin E on broilers' performance, intestinal morphology, ileal digestibility, and microbial count. A total of 420-day-old broilers (Cobb 500) were allotted to seven treatments, each replicated four times from 1 to 49 days of age. Treatments included: control, virginiamycin (200 mg/kg diet), vitamin E (150 mg/kg diet), and black cumin seed powder (5, 10, 15, and 20 g/kg of diet). Results showed that virginiamycin or black seed levels did not influence birds' body weight. Including vitamin E over the recommendation reduced body weight at 23 days of age ($P \leq 0.05$). virginiamycin supplementation increased ($P \leq 0.05$) feed intake from 1 to 10 days of age. In the jejunum, birds fed a diet containing vitamin E or different levels of black seed showed higher villus height and villus area. However, birds that received virginiamycin had significantly improved villus height to crypt depth ratio. Lactobacillus count increased in virginiamycin and 20 g/kg black seed groups ($P \leq 0.05$). In conclusion, including 20 g/kg black cumin seed in broilers' diet can improve performance, intestinal morphometric indices, and Lactobacillus count.

Keywords: antibiotic, vitamin E, black cumin, gut microbiota composition, villus height

INTRODUCTION

It is well known that feeding antibiotics is crucial in improving poultry performance. However, their beneficial potential has been scrutinised due to their residue in meat and egg products and the phobia of developing antibiotic-resistant microbes in humans (Nasir, 2009; Adil et al., 2011). Therefore, many countries and regulatory organisations are pushing to prohibit, restrict, or at least regulate antibiotic application in animal feed, forcing the feed industry to search for alternative candidates such as organic acids, probiotics, prebiotics, enzymes, and medicinal herbs and their essential oils (Guler et al., 2005; Abaza et al., 2008).

The black cumin (*Nigella sativa*) seed, a well-known traditional medicinal remedy, belongs to the Ranunculaceae family, growing mainly in the Mediterranean region (Ozer et al., 2020). Black cumin seed contains approximately 216, 406, 45, 84, 249, and 38 g/kg crude protein, ether extract, ash, crude fiber, nitrogen-free extract, and moisture, respectively (Takruri and Dameh, 1998).

In addition, it is a rich source of iron, copper, zinc, phosphorus, calcium, thiamin, niacin, pyridoxine, and folic acid (Takruri and Dameh, 1998). Linoleic acid (55.6%), oleic acid (23.4%), and palmitic acid (12.5%) are the primary fatty acids found in black cumin seed oil (Nickavar et al., 2003). During recent years numerous functional properties such as anti-aflatoxicosis (Rasouli-Hiq et al., 2017), anticancer (Nagi and Mansour, 2000; Khan et al., 2011), antidiabetic (Ghods et al., 2024), antimicrobial (Erner et al., 2010), analgesic (Hajhashemi et al., 2004), anti-inflammatory (Hajhashemi et al., 2004; Miguel, 2010), antioxidant (Miguel, 2010) and digestion enhancing capacity (Guler et al., 2005; Nasir, 2009; Beitawi et al., 2009; Nasir and Grashorn 2010; Miraghaee et al., 2011) has been claimed for black cumin seeds or its essential oil extracts. Most of the functional properties of black cumin seed have been attributed to its high profile of bioactive components such as p-cymene, carvone, limonene, thymoquinone, α-thujene, trans-anethole, and longifolene (Nickavar et al., 2003; Singh et al., 2005). Para-cymene and thymoquinone are major bioactive components in black cumin seed essential oil (Hajhashemi et al., 2004).

Though the chemical composition of black cumin seed has been investigated (Ramandan, 2007), but its potential as an alternative for growth promoters in chicks' diets has been investigated to a lesser extent (Alimohamadi et al., 2014). Therefore, the present work aimed to determine the effect of various levels of black cumin seed (*Nigella sativa*) powder on the growth performance, blood and serum traits, carcass characteristics, intestinal morphology, apparent ileal digestibility, ileum microbial population and antioxidant capacity in broilers.

MATERIALS AND METHODS

Black cumin seed preparation and analysis

Black cumin seed samples were purchased from a local supplier in Bolbanabad, Dehgolan, Kurdistan, Iran. The air-dried seeds were ground into a fine powder using a laboratory mill. The grounded powder's crude protein, ether extract, crude fiber, ash, calcium, and phosphorus contents were determined using laboratory methods (AOAC, 1990).

Essential oil extraction and analysis

The essential oil of air-dried black cumin seed powder was extracted using hydro-distillation techniques (3 hours) in a Clevenger-type apparatus. Then, the obtained oil was dried using anhydrous sodium sulfate and stored in a sealed glass vial below four °C refrigerator. GC/MS analysis of the obtained essential oil sample was performed at Central Laboratory of University of Kurdistan, Sanandaj, 416, Kurdistan, Iran (7890A Network GC system/ 5975C Inert XL, mass selective detector at 70 eV and 20 °C, Agilent Technologies Company, Sanata Clara, California, USA). Helium is the carrier gas at a 0.8 ml/ minute flow rate. Identification of essential oil components was based on an automated comparison of obtained spectra with the National Institute of Standards and Technology, NIST05 mass spectral database (NIST 05). The percentage of each component's peak area was determined relative to the sum of all component's peak areas and expressed as a percentage.

Birds, housing and feeding

This experiment was conducted in the Department of Animal Science, University of Kurdistan, Sanandaj, Kurdistan, Iran. A total of 420 one-day-old meat-typed chickens of a commercial strain (Cobb 500) were used to evaluate the effects of different black cumin seed powder levels on performance, relative weight of gastrointestinal organs, small intestine morphology, and some blood and serum components. The birds were weighed as a group on arrival and then distributed into seven dietary treatments. Each dietary treatment consisted of four-floor pen (102 × 140 cm^2) replicates containing 15 chicks. The pens were covered with wood shavings as litter, each with a suspended tube feeder and a plastic bell drinker. Birds were brooded at 32 °C during the first week, and after that, the temperature was reduced by 3 °C every week until the temperature reached 23 ± 2 °C. During the first days of experiments, birds were reared under a continuous 24-hour lighting program, then reduced to 23-hour lighting throughout the experimental period. Feed and water were provided, as well as ad libitum. The experiment was carried out as a completely randomized design. Dietary treatments included: a maize-soybean meal basal diet as control; basal diet supplemented with 5, 10, 15, or 20 g/kg of grounded black cumin seed; control supplemented with either 200 mg/Kg of virginiamycin or 150 mg/Kg of vitamin E. The virginiamycin was obtained from a local veterinary pharmacy (Sanandaj, Kurdistan, Iran). Vitamin E (MicrovitT E Promix 50) was purchased from a premix company (Saral Co., Sanandaj, Kurdistan, Iran). The experimental diets were fed from 1-10, 11-22, and 23-49 days of age, respectively. The ingredient and nutrient composition of the basal diets are shown in Table 1.

Table 1. Composition (g/kg) and calculated nutrient contents of experimental diets

Ingredient	Starter (1-10 d)					Grower (11-23 d)					Finisher (23-49 d)				
	Black cumin seed (g/kg)					Black cumin seed (g/kg)					Black cumin seed (g/kg)				
Black cumin seed	0.0	5.0	10.0	15.0	20.0	0.0	5.0	10.0	15.0	20.0	0.0	5.0	10.0	15.0	20.0
Corn	544	542	540	539	537	566	561	562	560	558	619	617	615	613	611
Soybean meal (44%CP)	387	385	383	381	379	363	364	359	357	355	316	314	312	310	307
Soybean oil	26.0	25.3	24.6	24.0	23.2	31.8	30.9	29.8	29.1	28.5	28.8	28.1	27.4	26.8	26.1
Dicalcium phosphate	16.6	16.6	16.5	16.5	16.4	16.3	16.2	16.2	16.2	16.1	14.4	14.4	14.4	14.3	14.3
$CaCO_3$	10.4	10.2	10.0	9.8	9.6	9.2	9.0	8.8	8.6	8.4	8.5	8.3	8.2	8.0	7.8
Common salt	3.7	3.7	3.7	3.7	3.6	3.7	3.7	3.7	3.7	3.7	3.2	3.2	3.2	3.2	3.2
Vitamin mixture[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral mixture[2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	4.0	4.0	4.0	4.0	4.0	3.2	3.2	3.2	3.2	3.2	2.9	2.9	2.9	2.9	2.9
L-Lysine	2.8	2.8	2.8	2.8	2.8	2.3	2.3	2.4	2.4	2.4	1.9	1.9	1.9	1.9	1.9
Calculated analysis[3]															
ME (Kcal/kg)	2950	2950	2950	2950	2950	3000	3000	3000	3000	3000	3050	3050	3050	3050	3050
Crude protein	220	220	220	220	220	210	211	210	210	210	192	192	192	192	192
Methionine	6.6	6.6	6.6	6.6	6.6	5.8	5.8	5.8	5.8	5.8	5.3	5.3	5.3	5.3	5.3
Methionine + Cysteine	9.8	9.8	9.8	9.8	9.8	8.9	8.9	8.9	8.9	8.9	8.2	8.2	8.2	8.2	8.2
Lysine	12.8	12.8	12.8	12.8	12.8	11.9	11.9	11.9	11.9	11.9	10.5	10.5	10.5	10.5	10.5
Calcium	9.0	9.0	9.0	9.0	9.0	8.4	8.4	8.4	8.4	8.4	7.6	7.6	7.6	7.6	7.6
AvailablePhosphorus	4.3	4.3	4.3	4.3	4.3	4.2	4.2	4.2	4.2	4.2	3.8	3.8	3.8	3.8	3.8
Sodium	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.4	1.4	1.4	1.4	1.4

[1] Provided per kg of diet: vitamin A 9000 IU, vitamin D3 (Colecaciferol) 200 IU, vitamin E (from Dl-α-tocopheryl acetate) 18 IU, vitamin K3 (from menadione dimethyl pyrimidinol) 2 IU, thiamin 1.8 mg, riboflavin 6.6 mg, nicotinic acid 10 mg, pantothenic acid 30 mg, pyridoxin 3 mg, folic acid 1 mg, vitamin B12 6 mg, folic acid 0.015 mg, cholin chloride 500 mg and antioxidant 100 mg.

[2] Provided per Kg of diet: manganese 100 mg, zinc 84.7 mg, iron 50 mg, copper 10 mg, iodine 1 mg, selenium 0.2 mg.

All diets were formulated to cover the nutrient requirements of NRC (1994).

Performance measurements

The bird's body weight was recorded on arrival and then on 11, 23, and 49 days of age. Feed intake was also measured on a pen basis during 1 to 11, 12 to 23, and 24 to 49 days growth periods. The weight of dead birds was recorded daily and used to correct feed consumption. The feed conversion ratio was calculated as feed intake (gram) to weight gain (gram) in each growth period.

Blood and serum collection

At 27 and 47 days of age, after 2 hours of fasting, two birds per replicate were randomly selected, and blood samples were collected from a brachial vein, centrifuged, and then the sera separated and collected in test tubes. Then, the serum albumin and protein concentrations were measured using a laboratory auto-analyzer (Abbott Alcyon 300 Model) and diagnostic colorimetric kits (Pars Azmoon Company, Tehran, Iran). To determine hemoglobin (Hb) values and to estimate the red blood cell (RBC) count, blood samples were collected in heparinized tubes at 27 and 47 days of age. Hemoglobin (Hb) values were measured using microhematocrit and cyanmethemoglobin methods. The RBC count was determined using a hemocytometer.

Carcass traits and gastrointestinal organs

At 27 and 47 days of age, the birds previously chosen for blood sample collection were slaughtered. The carcass, proventriculus, abdominal fat, liver, pancreas, gizzard, bursa of Fabricius, and spleen were collected, weighed, and their relative weight to body weight determined, with data expressed as a percentage of live body weight.

Intestinal morphology

At 27 days of age, 3 cm tissue segment from the middle sections of the duodenum, jejunum, and ileum of birds previously slaughtered were taken and, after removal of digesta and washing with physiological serum, fixed in formalin (10%) and dehydrated, cleared, impregnated with paraffin. The processed tissue segments were embedded in paraffin wax. Sections were cut (6 µm) from the waxed tissue using an SRM200 model microtome, then cleared of wrinkles by floating on warm water (50 °C) before mounting on slides. Two consecutive slices were mounted on each slide. The slides were stained by hematoxylin and eosin stain and then analyzed by light microscope to determine morphometric indices such as villus height, crypt depth, villus height to crypt depth ratio, and villus surface area (Gunal et al., 2006). The mean values of 10 villi per slide were used as the average for further analysis.

Apparent ileal digestibility

The total ileum contents of slaughtered birds at 47d were collected and stored at -20 °C until analysis. The organic matter of the dried samples was determined (AOAC 1995), and the ileum digestibility of dry matter, organic matter, and protein was calculated using chromium oxide (Cr_2O_3) as an external marker (Fenton and Fenton, 1979).

Microbial culture assay

About 10 cm from the middle part of the ileum of each slaughtered bird at 27 days of age was was taken, transferred on ice to the laboratory, and their contents were used for microbial cultures. The serial dilutions (10^{-3} to 10^{-8}) of samples were made with saline, and 250 µl of each solution was cultured on Mckongey agar (media for coliform culture) and Man rogosa and sharpe agar (media for lactobacillus culture) manufactured by Merck Germany. The Mckongey media plates were incubated at 37 °C for 24 h. Plates were put in desiccators that contained one sheet of gas pack to create an anaerobic environment for lactobacillus bacteria. Desiccators with contents were incubated at 37 °C for 48 h. Bacteria were enumerated by visual count of colonies using the best plates. The microbial enumeration was expressed as a $\log_{10}$ colony forming unit per gram (Manafi et al., 2016).

Statistical analysis

All collected data were subjected to analysis of variance as a completely randomized design using the General Linear Model procedures of SAS Institute (2001). Duncan's New Multiple Range Test was used to compare means differences at ($P \leq 0.05$).

RESULTS

The nutrient analysis of black cumin seed samples showed that they contained 934.4, 221, 276, 54, 53.2, 16.8, and 7.0 g per kg of dry matter, crude protein, ether extract, crude fiber, ash, calcium, and total phosphorus, respectively.

Analysis of the essential oil

The black cumin seed sample yielded 0.4% (v/w) of a yellowish essential oil with an aromatic odor. As shown in Table 2, thirty-six bioactive components were identified from collected essential oil, representing 99.99% of the total oil components detected. Para-cymene (23.12%), thymoquinone (22.25%), dinopolnop (19.59%), trimethyl hydroquinone (5.34%), and α-thujene (3.88%) were among the major components identified.

Performance

The effects of different dietary treatments on broiler chicks' average body weight (ABW) and body weight gain (BWG) are presented in Table 3. The results showed that birds' ABW at 11 and 23 days of age and BWG during 1 to 10 and 11 to 23 days periods were influenced by dietary treatments ($P \leq 0.05$). However, different treatments did not affect ABW at 49 days of age and BWG during 23 to 49 and 1 to 49 days' periods ($P > 0.05$). Results indicated that ABW was not influenced when virginiamycin or different black cumin seed powder levels were added to the control diet. However, birds that received a diet containing vitamin E over the current recommendation had significantly ($P \leq 0.05$) lower ABW compared to the control at 23 days of age.

The effects of different dietary treatments on average feed intake (AFI) and feed conversion ratio (FCR) are presented in Table 4. The AFI of broilers was influenced by experimental diets from 1 to 10 and 23 to 49 days ($P \leq 0.05$). As shown in Table 4, the birds fed diets containing virginiamycin had significantly ($P \leq 0.05$) higher AFI from 1 to 10 d compared to the birds fed control, vitamin E, or black cumin seed (5, 10, and 15 g/kg) contained diets.

Table 2. Essential oil constituents of grinded black cumin seed (*Nigella sativa* L.)

Components	%
P- cymene	23.12
Thymoquinone	22.25
Dinopolnop	19.59
Trimethyl hydroquinone	5.34
α-Thujene	3.88
9-Tetradecenol,Z	3.04
Longifolene	2.55
D- Silvestrene	1.55
Carvacrol	1.39
1-Chloro-1-decyloxyilolane	1.33
α-Terpinene	1.27
β-Pinene	1.02
cis-Linoleic acid	0.99
γ-Terpinene	0.98
α-Pinene	0.88
Palmitic acid	0.85
1,5,9-Decatriene, 2,3,5,8-tetramethyl	0.73
α-Gurjunene	0.72
dl-Alpha, 4-dihydroxy-3-methoxyphenylacetic acid	0.69
Linoleic acid ethyl ester	0.66
Acetophenone	0.63
Benzaldehyde, 4-fluoro-3-hydroxy	0.63
Longipinene	0.60
α-Phellandrene	0.58
4-Carene	0.56
β-Thujene	0.47
Isoledene	0.44
Butyric acid, hexadecyl ester	0.42
Benzimidazole-4-carbonitrile	0.41
ψ-Cumohydroquinone	0.41
4-Terpineneol	0.39
2-Tridecanone	0.37
Cyclododecene	0.31
Linoleic acid ethyl ester	0.28
α-Caryophyllene	0.26
Salicylaldehyde	0.23
Total	99.99

Table 3. Effects of experimental groups on average body weight (ABW) and body weight gain (BWG) of broiler chicks

Experimental groups	ABW(g)			BWG (g)			
	11d	23d	49d	1-10d	11-22d	23-49d	1-49d
Control	218^{ab}	689^{ab}	2430	174^{ab}	472^{ab}	1743	2387
Virginiamycin	226^{a}	701^{a}	2394	181^{a}	474^{ab}	1705	2371
Vitamin E	210^{b}	655^{c}	2466	165^{b}	444^{b}	1872	2480
5 g/kg BC[1]	208^{b}	690^{ab}	2411	163^{b}	482^{a}	1787	2366
10 g/kg BC	216^{ab}	664^{bc}	2426	171^{ab}	448^{b}	1803	2423
15 g/kg BC	211^{b}	695^{ab}	2468	166^{b}	483^{a}	1693	2423
20 g/kg BC	220^{ab}	706^{a}	2492	175^{ab}	486^{a}	1786	2447
SEM[2]	1.66	5.16	22.10	1.63	4.20	22.83	19.86
Probability	0.04	0.01	0.67	0.03	0.01	0.43	0.65

[1] BC: Black Cumin
[2] SEM: Standard Error of Mean
[a,b,c] Values within a column without a common superscript are significantly different (P < 0.05)

However, this difference disappeared during the rest of the rearing period. There were no differences between FCR among treatments in the present experiment (P > 0.05).

Blood and serum constituents

The effects of dietary treatments on hemoglobin (Hb) values, red blood cell (RBC) count, serum albumin, and protein concentrations at 27 and 47 days of age are presented in Table 5. As shown, different treatments did not affect blood and serum traits (P > 0.05).

Carcass traits and gastrointestinal organs

The effects of dietary treatments on internal organs at 27 and 47 days of age are presented in Table 6. At 27 days of age, the relative weights of proventriculus, spleen, bursa of Fabricius, and abdominal fat percentage to body weight were not influenced by different treatments (P > 0.05). However, birds fed diets containing vitamin E or 20 g/kg black cumin seed showed significantly (P ≤ 0.05) higher carcass dressing percentage at 27 days than the other groups. Birds fed vitamin E, or high levels of black cumin seed-containing diets, had significantly (P ≤ 0.05) higher gizzard and pancreas relative weights to body weight at 27 days. The relative weight of gastrointestinal organs to body weight at 47 days was not influenced by dietary treatments (P > 0.05).

Intestinal morphology

The effect of dietary treatments on morphometric indices of different segments of the small intestine, such as villus height, crypt depth, villus height to crypt depth ratio, and villus area at 27 days of age, are shown in Table 7. In the duodenum, only villus height to crypt ratio was significantly influenced by dietary treatments (P ≤ 0.05), in which birds fed a diet containing 10 g/kg black cumin seed showed significantly (P ≤ 0.05) higher ratio compared to the control. In jejunum segments, birds fed vitamin E, or different levels of black cumin seed had significantly higher villus heights than the control (P ≤ 0.05). However, birds fed vitamin E contained diet showed the lowest crypt depth compared to the control or birds fed different levels of black cumin seed-contained diets (P ≤ 0.05). In addition, compared to the control, all other treatments showed significantly higher villus height to crypt depth ratio. As shown in Table 7, villus area significantly increased when birds were fed by diet containing vitamin E or different levels of black cumin seed (P ≤ 0.05).

Table 4. Effects of experimental groups on average feed intake (AFI) and feed conversion ratio (FCR) in broiler chick

Experimental groups	AFI (g)				FCR (g/g)			
	1-10d	11-22d	23-49d	1-49d	1-10d	11-22d	23-49d	1-49d
Control	233^{b}	796	3560^{ab}	4547^{ab}	1.36	1.68	2.01	1.88
Virginiamycin	249^{a}	805	3281^{bc}	4312^{bc}	1.37	1.64	1.95	1.83
Vitamin E	236^{b}	771	3629^{ab}	4607^{ab}	1.42	1.69	1.98	1.89
5 g/kg BC[1]	230^{b}	792	3661^{a}	4660^{ab}	1.40	1.64	2.04	1.91
10 g/kg BC	234^{b}	768	3281^{a}	4748^{a}	1.36	1.71	2.10	1.88
15 g/kg BC	229^{b}	776	3224^{c}	4149^{c}	1.37	1.65	1.95	1.84
20 g/kg BC	237^{ab}	827	3748^{a}	4773^{a}	1.35	1.70	1.99	1.88
SEM[2]	1.71	6.22	49.61	44.76	0.007	0.01	0.01	0.01
Probability	0.03	0.13	0.005	0.01	0.06	0.48	0.33	0.55

[1] BC: Black Cumin
[2] SEM: Standard Error of Mean
[a,b,c] Values within a column without a common superscript are significantly different (*P* < 0.05)

Table 5. Effects of experimental groups on red blood cell count, Hb (g/dl), total protein (TP) and albumin content (AL) at 27 and 47 days of age

Experimental groups	Blood				Serum			
	27 days		47 days		27 days		47 days	
	RBC ($*10^6/\mu l$)	Hb (g/dl)	RBC ($*10^6/\mu l$)	Hb (g/dl)	TP (g/dl)	AL (g/dl)	TP (g/dl)	AL (g/dl)
Control	2.89	20.24	2.54	14.55	3.37	1.48	3.42	1.35
Virginiamycin	2.47	21.54	2.70	16.53	2.89	1.13	3.35	1.45
Vitamin E	3.12	27.56	3.20	18.89	3.01	1.27	3.41	1.39
5 g/kg BC[1]	3.79	24.59	2.68	13.05	3.14	1.42	3.42	1.59
10 g/kg BC	3.16	35.48	2.92	15.97	3.15	1.40	3.32	1.33
15 g/kg BC	2.90	28.51	2.58	15.69	3.37	1.46	3.76	1.55
20 g/kg BC	2.54	25.24	2.85	19.36	3.25	1.34	3.76	1.53
SEM[2]	0.10	1.48	0.08	0.7	0.08	0.04	0.07	0.03
Probability	0.57	0.08	0.52	0.16	0.59	0.19	0.42	0.31

[1] BC: Black Cumin
[2] SEM: Standard Error of Mean

Table 6. Effects of experimental groups on carcass characteristics (%) at 27 and 47 days of age

Experimental groups	Dressing	Proventriculus	Gizzard	Liver	Spleen	Pancreas	Bursa of Fabricius	Abdominal fat
	Carcass characteristics (%) at 27 days							
Control	53.68^{c}	0.50	2.05^{b}	2.69^{d}	0.08	0.30^{b}	0.12^{b}	1.70
Virginiamycin	55.77^{bc}	0.48	2.10^{ab}	3.79^{ab}	0.08	0.29^{b}	0.12^{b}	1.88
Vitamin E	62.24^{a}	0.60	2.61^{a}	4.21^{a}	0.12	0.46^{a}	0.14^{b}	1.74
5 g/kg BC[1]	54.48^{bc}	0.53	2.39^{ab}	3.06^{cd}	0.09	0.29^{b}	0.12^{b}	1.90
10 g/kg BC	54.45^{bc}	0.59	2.64^{a}	2.97^{cd}	0.10	0.41^{a}	0.18^{ab}	1.56
15 g/kg BC	58.87^{ab}	0.53	2.40^{ab}	3.35^{bcd}	0.10	0.39^{ab}	0.21^{a}	1.68
20 g/kg BC	62.19^{a}	0.59	2.55^{a}	3.44^{bc}	0.10	0.46^{a}	0.18^{ab}	1.77
SEM[2]	0.83	0.01	0.06	0.12	0.005	0.01	0.008	0.06
Probability	0.0004	0.11	0.02	0.001	0. 32	0.001	0.02	0.95
	Carcass characteristics (%) at 47 days							
Control	64.09	0.28	1.34	1.93	0.08	0.21	0.14	1.55
Virginiamycin	61.97	0.38	1.51	1.83	0.12	0.23	0.11	2.11
Vitamin E	63.43	0.33	1.31	1.88	0.08	0.21	0.15	1.58
5 g/kg BC[1]	64.34	0.34	1.44	1.66	0.09	0.20	0.13	1.67
10 g/kg BC	61.47	0.35	1.58	1.98	0.1	0.23	0.13	1.65
15 g/kg BC	62.07	0.36	1.58	1.86	0.09	0.20	0.11	1.82
20 g/kg BC	63.58	0.34	1.38	1.75	0.08	0.18	0.11	1.48
SEM[2]	0.47	0.01	0.03	0.05	0.002	0.006	0.006	0.07
Probability	0.60	0.17	0.06	0.70	0.19	0.50	0.85	0.37

[1] BC: Black Cumin
[2] SEM: Standard Error of Mean
$^{a, b, c, d}$ Values within a column without a common superscript are significantly different (P < 0.05)

Table 7. Effects of experimental groups on villus height, crypt depth, villus height to crypt depth ratio and villus area at 27 days of age

Experimental groups	Villus height	Crypt depth	Villus height to Crypt dept ratio	Villus area (mm^2)
		Duodenum (µm)		
Control	1052	257	3.35^{c}	0.18
Virginiamycin	1196	248	3.43^{bc}	0.23
Vitamin E	1408	235	5.97^{ab}	0.23
5 g/kg BC[1]	936	197	4.78^{bc}	0.18
10 g/kg BC	1353	211	7.20^{a}	0.26
15 g/kg BC	1341	292	4.69^{bc}	0.29
20 g/kg BC	1297	256	4.49^{bc}	0.16
SEM[2]	61.65	8.95	0.37	0.01
Probability	0.39	0.08	0.02	0.08
		Jejunum (µm)		
Control	506^{c}	230^{ab}	1.68^{d}	0.07^{c}
Virginiamycin	555^{bc}	251^{bc}	2.33^{c}	0.09^{bc}
Vitamin E	729^{ab}	206^{c}	3.02^{ab}	0.11^{ab}
5 g/kg BC[1]	814^{a}	336^{a}	2.88^{b}	0.14^{a}
10 g/kg BC	731^{ab}	263^{b}	2.81^{bc}	0.13^{ab}
15 g/kg BC	793^{a}	267^{b}	3.49^{a}	0.11^{ab}
20 g/kg BC	815^{a}	278^{b}	2.79^{bc}	0.14^{a}
SEM[2]	32.09	8.81	0.12	0.005
Probability	0.005	0.004	<0.0001	0.002
		Ileum (µm)		
Control	686^{abc}	202	3.47	0.11
Virginiamycin	628^{bc}	168	3.54	0.12
Vitamin E	581^{c}	206	3.25	0.11
5 g/kg BC[1]	759^{ab}	196	3.53	0.14
10 g/kg BC	713^{abc}	208	3.50	0.13
15 g/kg BC	701^{abc}	180	3.70	0.14
20 g/kg BC	818^{a}	240	2.71	0.13
SEM[2]	20.07	8.59	0.14	0.006
Probability	0.03	0.46	0.82	0.77

[1] BC: Black Cumin
[2] SEM: Standard Error of Mean
[a,b,c] Values within a column without a common superscript are significantly different (*P* < 0.05)

Virginiamycin supplementation to the control diet had no significant effects on most morphometric indices of jejunum but significantly improved villus height to crypt depth ratio. In the ileum, most of the morphometric indices were not influenced by dietary treatments compared to the control ($P > 0.05$), though birds fed a diet containing 20 g/kg of black cumin seed had higher villus height compared to the birds fed vitamin E contained diet ($P \leq 0.05$).

Apparent ileal digestibility

As shown in Table 8, ileum dry matter, organic matter, and protein digestibility at 47 days of age were not significantly influenced by different dietary treatments ($P > 0.05$).

Table 8. Effects of experimental groups on apparent ileal digestibility (%) at 47 days of age

Experimental groups	Ileum digestibility (%)		
	Dry matter	Organic matter	Protein
Control	74.5	75.2	70.98
Virginiamycin	74.0	76.4	70.43
Vitamin E	73.7	75.7	70.02
5 g/kg BC[1]	74.6	75.4	71.22
10 g/kg BC	74.1	75.2	70.51
15 g/kg BC	71.2	73.3	67.38
20 g/kg BC	74.5	76.6	70.30
SEM[2]	0.10	0.37	0.46
Probability	0.33	0.38	0.40

[1] BC: Black Cumin
[2] SEM: Standard Error of Mean

Microbial count

The effects of dietary treatments on the microbial population of ileum contents (CFU/g) at 27 days of age are presented in Table 9. As shown, different dietary treatments did not influence the ileum coliform count ($P > 0.05$). However, Lactobacillus count significantly increased when virginiamycin, or the highest level of black cumin seed (20 g/kg), was supplemented to the basal diets.

Table 9. Effects of experimental groups on microbial count at 27 days of age

Experimental groups	Ileum microbial population (CFU/g) at 27 days	
	Lactobacillus	Coliform
Control	8.13^{b}	7.76
Virginiamycin	8.80^{a}	7.40
Vitamin E	8.43^{ab}	7.74
5 g/kg BC[1]	8.28^{b}	7.46
10 g/kg BC	8.39^{ab}	7.95
15 g/kg BC	8.32^{ab}	7.31
20 g/kg BC	8.80^{a}	6.99
SEM[2]	0.06	0.11
Probability	0.03	0.30

[1] BC: Black Cumin
[2] SEM: Standard Error of Mean
[a,b] Values within a column without a common superscript are significantly different ($P < 0.05$)

DISCUSSION

The lack of significant effects following the addition of virginiamycin to the basal diet on broiler chicks' performance is in disagreement with reports on growth-enhancing properties of virginiamycin (Ahmadi, 2011). Feed antibiotics, especially in poor management conditions, may contribute to the general well-being of the birds and act as a growth promoter (Miyakawa et al., 2024). However, since the birds were reared under hygienic and stress-free circumstances in the present experiment, the lack of major positive influence of Virginmycin on birds' performance criterion is attributed to less effectiveness of feed antibiotics when birds reared under proper environmental and management conditions.

Supplementation of virginiamycin or 20 g/kg black cumin seed produced the highest body weight at 11 and 23 days, although the difference was insignificant with the control. The effects of black cumin seed on enhancing body weight can be attributed to the presence of high amounts of unsaturated fatty acids such as linoleic acid, oleic acid and linolenic acid (Kiani et al., 2020), the antimicrobial effects of thymoquinone (Randhawa et al., 2017), para-cymene (Marchese et al., 2017), carvacrol (Ben Arfa et al., 2006) or other effective compounds in the black cumin seed. This finding was in line with Beitawi and El-Ghousein (2008), who reported growth improvement effects of black cumin seed supplementation on broiler diets.

Adverse effects of excessive vitamin E on body weight at 23 days of age in the present study could be due to its interactions with other nutrients in the diet, including vitamin A, which may reduce the activity of the thyroid gland and consequently deteriorate chicks' performance (Nobakht et al., 2012). Vitamin A plays a significant role in modulating thyroid homeostasis because it increases iodine uptake (Capriello et al., 2022). Extra vitamin E may reduce vitamin A absorption by creating competition for absorption sites in the intestine and interactions for absorption, which leads to interference in the thyroid gland (Goncalves et al., 2015). It is generally accepted that vitamin E is a valuable, potent antioxidant agent. However, extra doses of Vitamin E, especially when the body is under stress status, may even decrease the activity of glutathione peroxidase, increase the production of free radicals, and harm the bird's immune balance (Murakami et al., 2007), which may explain the negative consequences of extra doses of vitamin E on broiler chicks' performance (Nobakht et al., 2012).

In fact, the observed improvements in carcass dressing percentage when vitamin E or the highest level of black cumin seed was added to the basal diets may indicate that both additives contributed to improvements in water holding capacity of the carcass, either via enhancing carcass antioxidant statues or due to the presence of high level of unsaturated fatty acids in black cumin seed (Guler et al., 2005; Yu et al., 2005; Erner et al., 2010). The adverse effects of an extra dose of vitamin E on the relative weight of the liver are in line with Nobakht et al. (2012) findings, which may partially relate to the body's need for detoxification of an extra dose of vitamin E via liver hypertrophy.

However, the present experiment findings on the positive influence of black cumin seed supplementation on carcass dressing percentage, and small intestine morphometric indices at 27 days of age, are in line with a general finding on medical herb benefits. It has been shown that the potential of bioactive constituents such as thymoquinone, p-cymene, carvacrol, etc. (Nagi and Mansour, 2000; Tariq, 2008) along with high amounts of unsaturated fatty acids (Yu et al., 2005) may contribute in general benefits have been observed when black cumin seed supplemented in broiler chicks' diet (Guler et al., 2005; Durrani et al., 2007; Erner et al., 2010; Saied et al., 2013).

The observed improvement in small intestine morphometric indices when black cumin seed was added to the basal diet, especially improvements in villus height/crypt depth and partial improvement of feed intake from 1 to 10 days of age, is a promising indication that the addition of black cumin seed may significantly enhance nutrient absorption and reduce small intestinal cell turnover. In facts, deeper crypt indicates a faster tissue turnover and a higher demand for new tissue, which may increase the demand for energy and protein to support tissue turnover (Miles et al., 2006).

Although our results had no significant effect on the coliform bacteria counts, adding the level of 20 g/kg of black cumin seed caused a numerical decrease in the coliform bacteria counts. Our findings support Kooti et al. (2016) and Fathi et al. (2023), who reported that black cumin seed had antimicrobial effect against a broad spectrum of bacteria, fungi, and pathogenic bacteria such as coliform and E. coli. This effect is attributed to the antibacterial activity of compounds such as para-cymene (Balahbib et al., 2021) and thymoquinone (Khan, 2018), which formed the most significant amounts in the essential oil. Khan et al. (2012) reported that the addition of black cumin seed (2.5 and 5%) had a similar reduction effect as the 0.1% of Terramycin on the total coliform bacteria and E. coli count, while it had no significant effect on the number of lactobacilli. The increase in the lactobacillus bacteria count as a result of the addition of virginiamycin and 20 g/kg of black cumin seed can be attributed to the fact that they were able to change the conditions of the digestive tract in favor of lactobacilli or beneficial bacteria.

CONCLUSION

In conclusion, the results indicate that including a high level of black cumin seed powder (20 g/kg) in broiler chicks' diet has the potential to improve meat-type chickens' performance, carcass characteristics, morphology indices, and lactobacillus count, though more work is needed to explore its antioxidant-enhancing benefits on bird meat quality.

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