



Research Article

Black Seeds (*Nigella sativa*) and Ginger Powder (*Zingiber officinale*) Effect on Growth Performance and Immune Response of Broiler Chickens

¹Huthail Najib, ²Ibrahim Al-Homidan, ²Moataz M. Fathi and ²Abdulaziz A. Al-Suhim

¹Department of Animal and Fish Production, College of Agricultural Sciences and Food, King Faisal University, 31982 Hofuf, Saudi Arabia

²College of Agriculture and Veterinary Medicine, Qassim University, 51452 Buraydah, Al-Qassim, Saudi Arabia

Abstract

Background and Objective: Antibiotic has been banned for animal use since 2006. Some studies provided evidences that some medicinal plants have antimicrobial properties and can replace antibiotics. This study was carried out to investigate the effect of adding 3 levels of black seeds and 3 levels of ginger root powder in broiler diets (starter and grower) on productive performance, immunity indicators and bacterial population of small intestine. **Materials and Methods:** A total of 700 day-old unsexed broiler chicks (Ross 308) were randomly allocated into 7 groups: A control group (corn-soybean basal diet with no supplement) and 6 dietary treatment groups receiving 10, 20 and 30 g kg⁻¹ of black seeds and 10, 20 and 30 g kg⁻¹ of ginger root powder. These chicks were distributed into 5 floor pens (replicates)/treatment (20 chicks each). **Results:** This study showed that black seed may play a potential role as an antioxidant in the components of blood more than ginger root powder does. Both black seed and ginger powder have a significant inhibitory effect on pathological bacteria in broiler's gut. **Conclusion:** Black seeds can be used as a feed additive to improve live body weight, antioxidant profile and gut microbiota of broilers. The recommended dosage of BS in the diets of broilers is 20 g kg⁻¹. Although, ginger powder did not affect growth performance and immune response, it can be used for improving antioxidant status and microbiota profile in broilers.

Key words: Black seeds, ginger powder, microbiota, immunity, anti-oxidant, broiler

Citation: Huthail Najib, Ibrahim Al-Homidan, Moataz M. Fathi and Abdulaziz A. Al-Suhim, 2020. Black seeds (*Nigella sativa*) and ginger powder (*Zingiber officinale*) effect on growth performance and immune response of broiler chickens. Asian J. Anim. Sci., 14: 1-8.

Corresponding Author: Huthail Najib, Department of Animal and Fish Production, College of Agricultural Sciences and Food, King Faisal University, 31982 Hofuf, Saudi Arabia Tel: 0096613504921884 Fax: 00966135896425

Copyright: © 2020 Huthail Najib *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics have been used for several decades to increase the profitability of poultry projects. However, the continuous use of antibiotics leads to increase the resistance of many intestinal pathogenic bacteria, deteriorate bird's immunity and accumulate residues in poultry products¹. Consequently, the use of antibiotics as growth promoters in animal feeds has been banned in Europe and many other countries since 2006. Accordingly, poultry producers seek to find alternative feed additives to improve their flock's productivity. Natural growth promoters represent alternative substances to antibiotics and artificial growth promoters. These natural materials include prebiotics, probiotics, symbiotic, yeast, enzymes, organic acids, betaine, carnitine, electrolytes and phytochemicals and plant extracts and etc.

Black seeds (BS) and ginger powder (GP) are frequently used in broiler production as an antimicrobial feed additive to enhance immunity and productive performance²⁻⁵. There is a general agreement that black seeds have no adverse effects (up to 30 g kg⁻¹) on the performance of poultry and could be supplemented to overcome the deleterious effects of hot climatic conditions⁶. Research in this area attributed the therapeutic benefit of the black seeds to thymoquinone, dithymoquinone, thymol, carvacrol, nigellone, selenium, D-tocopherol, transcucol and alphasitosterol^{7,8}.

Ginger powder (*Zingiber officinale*) is widely used in many countries as a food spice and has many therapeutic uses and play an effective role in stimulating digestion enzymes and affecting the activity of intestinal bacteria as well its role as an antioxidant substance. Ginger root contains several active chemical compounds including volatile oils such as zingiberene, curcumene, borneol, nerol, geraniol, geranyl, citronellyl acetate, α -terpineol and linalool and pungent compounds such as gingerols and shogaols⁹. The chemical composition of the two plants to my knowledge was not reported before. The chemical composition of both herbs is described below.

The current study was conducted to investigate the effect of supplementing broiler's diet with different levels of black seed (BS) and ginger root powder (GP) on growth performance, immune response, antioxidant status and gut microbiota.

MATERIALS AND METHODS

Birds, housing and dietary treatments: A total of 700 one day-old broiler chicks (Ross 308) were raised for 42 days in the

premises of Al-Qasim University of Saudi Arabia from March, 2016 to Mid April, 2016. Chicks were individually weighed and randomly divided into 7 different dietary treatments. Whole black seeds (*Nigella sativa*, BS) and ginger root powder (*Zingiber officinale*, GP) were added to the diet at different levels (0, 10, 20 and 30 g kg⁻¹ black seeds, 10, 20 and 30 g kg⁻¹ ginger powder). Each dietary group has 5 replicates (20 chicks/replicate). Feed and water were supplied *ad libitum*. All birds were raised under similar husbandry and environmental conditions. Supplemental lighting was provided on a continuous basis. Chicks were managed according to the guidelines suggested by the Ross Broiler Pocket Guide (2015, <http://eu.aviagen.com>). Two experimental diets were used in the current study. The starter diet was given to chicks from day 1-21 while grower diet was given for the remaining experimental period. These diets were formulated according to the NRC¹⁰ requirements. The composition of the experimental diets is given in Table 1. All procedures performed in the current experiment were approved by the Animal Ethics Committee of Al-Qasim University.

Samples of black seeds and ginger powder were analyzed based on the methods reported in AOAC¹¹. The results of the analysis were expressed as a percentage of the fresh sample. These results are shown in Table 2.

Growth performance: Upon arrival, all chicks were individually weighed to determine their initial weight and subsequently, distributed randomly into 7 dietary treatments. Body weight (g) was weekly determined until the end of the experiment (6 weeks). The feed conversion ratio was calculated for each replicate within each treatment as total feed consumption and total gain of body weight (0-42 days). Mortality rate was recorded daily for each replicate and the cumulative mortality percent was calculated. Feed consumption and feed conversion ratio were adjusted for mortality when appropriate.

Evaluation of immune response: Cell-mediated immune response *in vivo*, was assessed according to the method, in which 100 μ g of phytohemagglutinin (PHA-P) in 0.10 mL of sterile phosphate buffer saline (PBS) solution was used¹² (Sigma Chemical Co., St. Louis, MO). The effect of dietary treatments on the humoral immunity status of the chicks was determined by quantifying IgA, IgM and IgY globulins levels in blood plasma using quantitation kits (Gen Way Biotech Inc., San Diego, CA).

Table 1: Calculated analysis of the experimental diets (kg)

Ingredients	Black seeds (10 g kg ⁻¹)			Black seeds (20 g kg ⁻¹)			Black seeds (30 g kg ⁻¹)			Ground ginger (10 g kg ⁻¹)			Ground ginger (20 g kg ⁻¹)			Ground ginger (30 g kg ⁻¹)		
	Starter	Grower	Grower	Starter	Grower	Grower	Starter	Grower	Grower	Starter	Grower	Grower	Starter	Grower	Grower	Starter	Grower	Grower
Yellow corn	447.00	522.60	517.30	438.10	512.00	506.90	436.30	506.90	442.50	517.30	517.30	438.10	512.10	512.10	433.60	506.90	506.90	506.90
SBM, 48%	362.00	290.50	287.60	354.70	284.70	287.70	351.10	287.70	358.40	287.50	287.50	354.70	284.70	284.70	351.10	281.80	281.80	281.80
Wheat bran	120.00	120.00	118.80	117.60	117.60	116.40	116.40	116.40	118.80	118.80	118.80	117.60	117.60	117.60	116.40	116.40	116.40	116.40
Black seeds	0.00	0.00	10.00	20.00	20.00	30.00	30.00	30.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ginger	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	10.00	10.00	20.00	20.00	20.00	30.00	30.00	30.00	30.00
Vegetable oil	23.40	27.00	23.30	27.05	26.60	20.45	20.01	20.45	23.38	27.06	27.06	22.99	26.58	26.58	22.60	26.20	26.20	26.20
Methionine	2.20	1.80	2.17	1.79	1.76	1.74	2.13	1.74	2.12	1.78	1.78	2.18	1.76	1.76	2.17	1.74	1.74	1.74
Salt	4.60	3.00	4.50	2.97	2.94	2.91	4.46	2.91	4.50	2.97	2.97	4.51	2.94	2.94	4.46	2.91	2.91	2.91
Limestone	17.00	14.40	16.80	14.20	14.20	13.90	16.50	13.90	16.80	14.20	14.20	16.60	14.11	14.11	16.50	13.87	13.87	13.87
Pellet binder	0.00	1.00	0.00	0.90	0.90	0.90	0.00	0.90	0.00	0.90	0.90	0.00	0.90	0.90	0.00	0.90	0.90	0.90
Anti-coccidia	0.50	0.50	0.50	0.49	0.50	0.50	0.50	0.50	0.50	0.49	0.49	0.49	0.49	0.49	0.49	0.50	0.50	0.50
Broiler premix*	6.00	6.00	6.00	5.90	5.90	5.80	5.80	5.80	5.90	5.90	5.90	5.88	5.88	5.88	5.88	5.88	5.88	5.88
Di-calcium phosphate	17.30	13.20	17.10	13.00	12.90	12.80	16.80	12.80	17.10	13.10	13.10	16.95	12.94	12.94	16.80	12.90	12.90	12.90
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated nutrient composition (%)																		
Crude protein	23.05	20.27	23.00	22.97	20.23	20.22	22.92	20.22	22.92	20.16	20.16	22.78	20.050	20.050	22.65	19.94	19.94	19.94
ME (MJ kg ⁻¹)	12.77	13.43	12.81	12.85	13.50	13.53	12.89	13.53	12.78	13.42	13.42	12.78	13.430	13.430	12.78	13.42	13.42	13.42
Calcium	1.00	0.90	1.00	1.008	0.913	0.92	1.01	0.92	0.999	0.903	0.903	0.995	0.899	0.899	0.99	0.896	0.896	0.896
Available phosphorus	0.75	0.70	0.76	0.76	0.71	0.76	0.76	0.71	0.757	0.70	0.70	0.754	0.702	0.702	0.75	0.69	0.69	0.69
Total fat	4.76	5.33	5.00	5.33	5.90	6.18	5.17	6.18	4.80	5.37	5.37	4.84	5.41	5.41	4.89	5.45	5.45	5.45
Total fiber	4.96	5.06	5.13	5.10	5.19	5.26	5.62	5.26	4.97	5.08	5.08	4.98	5.08	5.08	4.99	5.08	5.08	5.08
Methionine	0.57	0.49	0.51	0.57	0.49	0.49	0.56	0.49	0.57	0.49	0.49	0.57	0.49	0.49	0.57	0.49	0.49	0.49
Meth+cyst	0.95	0.84	0.89	0.94	0.83	0.83	0.94	0.83	0.95	0.84	0.84	0.95	0.84	0.84	0.96	0.85	0.85	0.85
Lysine	1.26	1.07	1.25	1.24	1.05	1.05	1.23	1.05	1.26	1.07	1.07	1.27	1.08	1.08	1.27	1.08	1.08	1.08

*Multi-vitamin-minerals premix provide the following kg⁻¹ of diet: 2100 mg: Retinol acetate, 50 mg: Cholecalciferol, 30 mg: Tocopheryl acetate, 2.3 mg: Menadione, 1.8 mg: Thiamine, 5.5 mg: Riboflavin, 2.3 mg: Pyridoxine, 0.011 mg: Cobalamin, 27.6 mg: Nicotinic acid, 0.92 mg: Folic acid, 6.9 mg: Pantothenic acid, 0.092 mg: Biotin, 50 mg: Antioxidant (BHT), 8 mg: Copper, 0.35 mg: Iodine, 0.26 mg: Iron, 0.44 mg: manganese, 0.18 mg: Selenium, 44 mg: Zinc

Determination of antioxidant status: Total antioxidant capacity (mmol L⁻¹) was determined using commercial kit (Biodiagnostic[®]) for diagnostic and research reagents, Dokki, Giza, Egypt (www.bio-diagnostic.com)¹³.

Catalase activity (CAT) was assayed with a spectrophotometer at 510 nm using commercial kit (Biodiagnostic[®]) for diagnostic and research reagents, Dokki, Giza, Egypt, www.bio-diagnostic.com)¹⁴. Malondialdehyde (MDA) level was determined from MDA equivalent standard^{15,16}.

Bacterial count: Total aerobic bacteria, *Salmonella* sp. and *coliform* were analyzed in the intestinal digesta^{17,18}.

Statistical analysis: Summarized data for all response variables were subjected to combined analysis in Completely Randomized Design (CRD) where dietary treatments (TRT) were considered the main effect¹⁹. General linear models procedure in the PC-SAS[®] (SAS Institute was used to estimate the variations among the means²⁰. Variable means showing significant differences in the analysis of variance table were compared using Tukey's test¹⁹:

$$Y_{ik} = \mu + Tr_{ti} + e_{ik}$$

Where:

Y_{ik} = Response variable (measured attribute)

μ = General average

Tr_{ti} = Transaction effect

e_{ik} = Experimental error

RESULTS

Chemical analysis: The chemical analysis of the black seeds and the ginger powder shows that the black seeds excel ginger powder several times in its total fat content, raw fiber and raw protein, but ginger powder contains about 2.5 times that of the soluble carbohydrates compared to the black seeds (Table 2).

Growth performance: Growth performance at the final week of the study (week 6) is presented in Table 3. With the exception of 20 g kg⁻¹ black seeds treatment, most levels had a similar effect on body weight compared to the control. However, a highly significant improvement of final body weight was observed with 20 g kg⁻¹ BS, (p<0.002) compared to the control. Cumulative feed conversion was among the best with this treatment as well (p<0.009). Lower body weight and higher feed conversion was observed with the high

Table 2: Chemical analysis of black seeds and ginger powder

Chemical composition (%)	Black seeds	Ginger
Moisture	2.56	4.83
Dry matter	97.44	95.17
Crude ash	4.67	4.71
Organic matter	92.77	90.46
Crude protein	18.64	9.37
ME (MJ kg ⁻¹)*	16.84	13.13
Ether extract	33.54	9.13
Crude fiber	11.72	5.75
Soluble carbohydrates	28.87	66.20
Calcium**	0.700	0.600
Available phosphorus***	0.088	0.174
Crude ash/DM	4.79	4.95
Organic matter/DM	95.21	95.05
Methionine	0.367	0.464
Methionine+cystine	0.508	1.177
Lysine	0.429	1.160
Crude protein/DM	19.13	9.85
EE/DM	34.42	9.59
Crude fiber/DM	12.03	6.04
Soluble carbohydrates/DM	29.63	69.56

DM: Dry matter, EE: Ether extract, *Calculation was done based on the following Eq.²¹: ME (kcal kg⁻¹) = 32.95 (crude protein (%)+ether extract (%)) × 2.25+available carbohydrate (%)-29.20, **Calculation was reported by El-Tahir and Bakheet²², ***Adel and Prakash²³

Table 3: Effect of incorporating different levels of black seeds and ginger powder in the diets of broilers on growth performance parameters¹

Treatments	FBW	CFI	CFC	AM
Control (0 g kg ⁻¹)	1968.2 ^b	3995.06	1.95 ^{bc}	4.00 ^{ab}
Black seeds (g kg⁻¹)				
10	1934.5 ^b	3731.73	1.86 ^c	3.00 ^b
20	2090.0 ^a	4128.48	1.89 ^c	3.00 ^b
30	1925.8 ^b	3991.13	2.04 ^{abc}	4.00 ^{ab}
Ginger powder (g kg⁻¹)				
10	1953.0 ^b	3605.49	1.88 ^c	8.00 ^a
20	1919.2 ^b	3848.01	2.15 ^{ab}	7.00 ^{ab}
30	1965.2 ^b	3935.35	2.18 ^a	7.00 ^{ab}
SEM	29.14	11.01	0.07	1.28
p-value	0.002	NS	0.009	0.03

¹Means within each column, carrying different superscripts are significantly different, p<0.05, FBW: Final body weight at 42 weeks of age, CFI: Cumulative feed intake of the birds under each treatment, CFC: Cumulative feed conversion of the birds under each treatment, AM: Average mortality during the experimental period for each treatment

levels of ginger powder (Table 3). Birds on ginger powder suffered more mortality than other treatments (p<0.03).

Table 4 summarizes the immune response profile for humoral and cellular immunity. In this study, immunoglobulines IgA and IgY were not affected by the addition of black seeds or ginger powder. Moreover, a reduction was observed in IgM in supplemented groups compared with the control (p<0.007).

Black seeds and ginger powder effect on cellular immunity was evident in Table 4. Best immune response was found in birds fed black seeds after 24 and 48 h of injection. A consistent decrease in Toe web swelling beyond 24 h of injection was observed.

Table 4: Immune response profile for different dietary treatments

Treatments	Immune response profile					
	Humoral immunity immunoglobulin (IgA)			Cellular immunity toe-web swelling		
	IgA	IgM	IgY	24 h PI	48 h PI	72 h PI
Control (0 g kg ⁻¹)	0.34	3.25 ^a	3.04	0.51	0.34 ^{ab}	0.20 ^{ab}
Black seeds (g kg⁻¹)						
10	0.35	2.31 ^d	2.99	0.61	0.48 ^a	0.29 ^a
20	0.35	2.98 ^b	2.97	0.58	0.37 ^{ab}	0.20 ^b
30	0.33	2.87 ^b	2.90	0.56	0.35 ^{ab}	0.18 ^{ab}
Ginger powder (g kg⁻¹)						
10	0.35	2.56 ^c	3.06	0.44	0.27 ^{ab}	0.14 ^b
20	0.35	2.87 ^b	2.96	0.39	0.15 ^b	0.05 ^c
30	0.33	2.85 ^b	2.90	0.37	0.22 ^{ab}	0.11 ^{bc}
SEM	0.003	0.087	0.05	0.08	0.07	0.005
p-value	NS	0.007	NS	NS	0.02	0.03

^{a-d}Means within the same row are significantly differed, NS: Non-significant, PI: Post injection

Table 5: Antioxidant profile and total lipid for different dietary treatments

Treatments	Response criteria			
	Total antioxidant capacity (TAC)	CAT	Total lipid	MDA
Control (0 g kg ⁻¹)	31.83 ^{ab}	737.02 ^a	560.39 ^a	8.33 ^b
Black seeds (g kg⁻¹)				
10	41.66 ^{ab}	425.75 ^b	425.15 ^b	6.78 ^c
20	44.96 ^a	351.21 ^b	496.19 ^{ab}	7.06 ^c
30	27.37 ^{ab}	315.29 ^b	482.58 ^{ab}	7.53 ^{bc}
Ginger powder (g kg⁻¹)				
10	45.76 ^a	418.91 ^b	463.80 ^{ab}	9.75 ^a
20	20.18 ^b	341.85 ^b	410.18 ^b	6.81 ^c
30	37.60 ^{ab}	216.30 ^b	440.66 ^b	8.45 ^b
SEM	7.56	47.44	35.17	0.37
p-value	0.01	0.0001	0.08	0.0001

^{a-c}Means within the same row are significantly differed, p<0.05, MDA: Malondialdehyde, CAT: Catalase activity

Table 6: Effect of supplementing broiler diet with different levels of black seeds and ginger powder on total count of bacteria and number of *coliform*, *Salmonella*, *Campylobacter* and *Staphylococcus* bacteria in small intestine of broilers on day 42 of age¹

Treatments	Types of determined intestinal bacteria				
	Total count	<i>Salmonella</i>	<i>Staphylococcus</i>	<i>Campylobacter</i>	<i>Coliform</i>
Control (0 g kg ⁻¹)	6.29 ^a	5.40 ^a	5.51 ^a	5.44 ^a	4.50 ^a
Black seeds (g kg⁻¹)					
10	4.56 ^{bc}	3.55 ^{bc}	3.78 ^b	2.78 ^b	2.28 ^b
20	4.39 ^{bc}	3.43 ^{bc}	3.67 ^{bc}	2.64 ^c	2.06 ^{bc}
30	4.03 ^d	3.33 ^c	3.45 ^d	2.48 ^d	1.70 ^d
Ginger powder (g kg⁻¹)					
10	4.57 ^b	3.65 ^b	3.56 ^{cd}	2.72 ^{bc}	2.21 ^{bc}
20	4.36 ^c	3.59 ^b	3.42 ^{de}	2.48 ^d	1.98 ^c
30	3.84 ^e	3.51 ^{bc}	3.24 ^e	2.17 ^e	1.64 ^d
SEM	0.06	0.08	0.07	0.04	0.08
p-value	0.0001	0.0001	0.0001	0.0001	0.0001

¹Means within each column, carrying different superscripts are significantly different, p<0.001, NS: Not significant, p>0.05

Effect of treatments on MDA, was significant (p<0.0001), however, the highest effect was with the one received 10 g kg⁻¹ ginger powder. This treatment also has the highest total antioxidant activity and high catalase enzymes in the blood. Nevertheless, 10 and 20 g kg⁻¹ black seeds reported very high (TAC) compared to the control and other treatments except the 10 g kg⁻¹ ginger.

Effect of treatments on catalase activity (CAT) showed that both treatments recorded the lowest value of the enzyme in the blood compared to the control (Table 5).

The inclusion of the black seeds and ginger powder to the broiler diets, in general, resulted in a significant (p<0.0001) decrease in the total number of intestinal harmful bacteria, including *Salmonella*, *Staphylococcus*, *Campylobacter* and *coliform* form compared to the control (Table 6).

DISCUSSION

The positive effect of supplementing the broiler diet with black seeds on body weights in general was consistent with a study in which 1.5% of the black seeds gave the highest value compared to 2.5 and 3.5% and the control at 42 days²⁴. The birds on this treatment had insignificant higher feed consumption. Effect of black seeds on body weight was significantly higher with only 20 g kg⁻¹, while raising it to 100 g kg⁻¹ had a negative effect on body weight compared to the control over 4-7 weeks². Likewise, other study found that incorporating of black seeds in poultry diets increased growth performance, daily feed intake and feed efficiency²⁵. Adversely, effect of feeding 4% black seeds on broiler's body weight was the best²⁶. The non-significant effect of ginger levels on body weight of the chickens at 42 days in this study disagreed with a research which indicated that final body weight has increased linearly with increasing levels of ginger in the diet²⁷. Likewise other study noticed a significant increase in the final body weight when supplementing the broiler diet with ginger²⁸. However, our results were consistent with the work which indicated that ginger powder did not significantly affect the body weight gain for chicken at levels of ginger ranged from 0-2% of the diet²⁹ and with the study that found, inclusion of 2% ginger powder adversely affected broiler growth performance³⁰. Additionally, there was no significant difference among the dietary groups receiving 0, 7.5 and 15% of ginger root powder regarding body weight gain, feed intake and feed conversion ratio at 42 days of age⁴. Feed conversion ratio in this study for the whole growth period was the best with 10 g kg⁻¹ black seeds, while those of 30 g kg⁻¹ black seeds, 20 and 30 g kg⁻¹ ginger powder were the worst. However, it is clear from the values of Table 3 that the most efficient conversion was with 20 g kg⁻¹ black seeds. This could be due to the larger body weight of the birds fed 20 g kg⁻¹ BS. Agreeing with this results, a study in which an evidence was provided that feed conversion ratios were improved significantly by supplementing broiler diets with 1% black cumin seeds³¹. Mortality was also the best with 10 and 20 g kg⁻¹ black seeds in this study. However, Birds fed GP had almost double mortality than those under black seeds treatments. On the other hand, published reports indicated that the addition of ginger powder to broiler diets has significantly reduced the mortality rate compared to the control³²⁻³⁴. From the results of the studies mentioned above and the results of this study, it is assumed that higher mortality rate in ginger treatment may be due to some toxic compounds found in ginger powder. However, the last observation needs further investigation.

Results of the immune response in this study disagreed with the results of a study which provided evidence that immune compounds against ND and IBD in the broiler blood increased significantly when fed diets fortified with black seeds³⁵. Similar results obtained in a research using up to 1.4% with a significant increase in the weights of lymphatic organs³⁶. A more recent study recorded a significant increase lymphatic organs weights (Bursa and spleen) with levels of 0.2 and 0.4% black seeds in the feed³⁷. In the same context, recent report has pointed out that adding the black seeds to the broiler diets significantly increased the concentration of cell mediated immuno dynamic cells in blood with the addition of the black seeds at levels of 0.5, 1 and 2% in the feed. While a small number of studies recorded insignificant effect of the black seeds on immune compounds in blood³⁸.

As for the non-significant effect of ginger powder on the compounds and blood-immune cells in the current study, it was somewhat consistent with results of a study in which they found no significant effect of adding 0.4 g kg⁻¹ of ginger powder on antibodies against chicken flu or Newcastle disease and immunoglobulin³⁹. On the contrary, two other studies, each of which indicated that significant increase in blood antibodies against Newcastle ND as well as cell mediated immunity compound, called "Delayed Type Hypersensitivity" and weights of immunity organs, including bursa gland and spleen, when adding ginger powder extract in drinking water⁴⁰.

The effect of adding black seeds and ginger powder on the antioxidants profile is reported in Table 5. Although two studies, one using black seeds and other on ginger powder reported a positive effect on the activity of catalase enzyme however, the current study did not show such a notable effect on catalase activity which could be due to the fact that levels are not high enough to show this effect^{41,42}.

The inclusion of the black seeds and ginger powder to the broiler diets, in general, resulted in a significant ($p < 0.0001$) decrease in the total number of intestinal harmful bacteria. This result was in agreement with the study that showed a significant decrease in the preparation of *E. coli* and *Coliform* bacteria in chicken eggs fed on diets fortified with black seeds at levels 1.5, 2.5 and 5% compared to the control at the age of 39 days⁴³. Similar results were obtained^{35,44}.

The positive effect of seeds on the harmful bacteria was attributed to the effect of compounds found in the black seeds such as carvacrol, carvone and saponin and also the presence of compounds in ginger powder such as zingiberene and zingerol. These compounds reduce the growth and proliferation of these harmful bacteria by lowering the pH of the intestine, an environment that causes growth inhibition of intestinal bacteria^{32,45}.

It is suggested that 20 g kg⁻¹ BS can be used in broiler diets without jeopardizing the performance of the birds. Although, ginger powder did not positively affect growth performance and immune response, it can be used for improving antioxidant status and microbiota profile in broilers.

CONCLUSION

Results of this study showed that inclusion of black seeds improved live body weight, antioxidant profile and gut microbiota of broilers. Ginger powder, however, can be used to improve the antioxidant status of broilers and the gut health of the bird. Both spices may be used as a replacement to antibiotics. This may be of benefit to the producers. Further study may be needed to evaluate the economics of using black seeds and ginger powder in the diet of broilers

SIGNIFICANCE STATEMENT

This study discovered that the black seeds can be beneficial for the poultry producers. This study will also help the researchers to uncover the critical areas of ginger powder and black seeds that many researchers were not able to explore. Thus a new theory on feeding a replacement to antibiotics may be arrived at.

ACKNOWLEDGMENT

The authors are grateful to King Faisal University for providing the necessary fund (grant # 29980 dated 13/7/1438 H equivalent to April/10/2017 G) to complete the work of this research. Appreciation is also extended to Qassim University for facilitating the farm and lab work needed for this research.

REFERENCES

- Schwarz, S., C. Kehrenberg and T.R. Walsh, 2001. Use of antimicrobial agents in veterinary medicine and food animal production. *Int. J. Antimicrob. Agents*, 17: 431-437.
- Al-Homidan, A., A.A. Al-Qarawi, S.A. Al-Waily and S.E.I. Adam, 2002. Response of broiler chicks to dietary *Rhazya stricta* and *Nigella sativa*. *Br. Poult. Sci.*, 43: 291-296.
- Saleh, A.A., 2014. Nigella seed oil as alternative to avilamycin antibiotic in broiler chicken diets. *S. Afr. J. Anim. Sci.*, 44: 254-261.
- Habibi, R., G.H. Sadeghi and A. Karimi, 2014. Effect of different concentrations of ginger root powder and its essential oil on growth performance, serum metabolites and antioxidant status in broiler chicks under heat stress. *Br. Poult. Sci.*, 55: 228-237.
- Jahan, M.S., M. Khairunnesa, S. Afrin and M.S. Ali, 2015. Dietary black cumin (*Nigella sativa*) seed meal on growth and meat yield performance of broilers. *SAARC J. Agric.*, 13: 151-160.
- Hermes, I.H., F.A. Attia, K.A. Ibrahim and S.S. El-Nesr, 2009. Effect of dietary *Nigella sativa* L. on productive performance and nutrients utilization of broiler chicks raised under summer conditions of Egypt. *Egypt. Poult. Sci. J.*, 29: 145-172.
- Nasir, Z., A.R. Abid, Z. Hayat and H.I. Shakoor, 2005. Effect of Kalongi (*Nigella sativa*) seeds on egg production and quality in white Leghorn layers. *J. Anim. Plant Sci.*, 15: 22-24.
- Al-Saleh, I.A., G. Billedo and I.I. El-Doush, 2006. Levels of selenium, DL- α -tocopherol, DL- γ -tocopherol, all-*trans*-retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds. *J. Food Compos. Anal.*, 19: 167-175.
- Ravindran, P.N. and K.N. Babu, 2005. *Ginger: The Genus Zingiber*. CRC Press, Boca Raton, FL., ISBN: 9780849320217, Pages: 552.
- NRC., 1994. *Nutrient Requirements of Poultry*. 9th Rev. Edn., National Academy Press, Washington, DC., USA., ISBN-13: 978-0309048927, Pages: 176.
- AOAC., 2000. *Official Methods of Analysis of the Association of Official Analytical Chemists*. 16th Edn., AOAC International, Arlington, VA., USA.
- Ghazi, S., M. Habibian, M.M. Moeini and A.R. Abdolmohammadi, 2012. Effects of different levels of organic and inorganic chromium on growth performance and immunocompetence of broilers under heat stress. *Biol. Trace Elem. Res.*, 146: 309-317.
- Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic and V. Cosic, 2001. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.*, 54: 356-361.
- Aebi, H., 1984. Catalase *in vitro*. *Meth. Enzymol.*, 105: 121-126.
- Satoh, K., 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chim. Acta*, 90: 37-43.
- Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95: 351-358.
- McDonald, L.C., C.R. Hackney and B. Ray, 1983. Enhanced recovery of injured *Escherichia coli* by compounds that degrade hydrogen peroxide or block its formation. *Applied Environ. Microbiol.*, 45: 360-365.
- Van Horn, K.G., C.A. Gedris and K.M. Rodney, 1996. Selective isolation of vancomycin-resistant enterococci. *J. Clin. Microbiol.*, 34: 924-927.
- Steel, R.G.D. and J.H. Torrie, 1980. *Principles and Procedures of Statistics: A Biometrical Approach*. 2nd Edn., McGraw Hill Book Co., New York, USA., ISBN-13: 9780070609266, Pages: 633.
- SAS., 2002. *SAS System for Linear Models*. SAS Institute Inc., Cary, NC., USA.

21. Lodhi, G.N., D. Singh and J.S. Ichhponani, 1976. Variation in nutrient content of feedingstuffs rich in protein and reassessment of the chemical method for metabolizable energy estimation for poultry. J. Agric. Sci., 86: 293-303.
22. El-Tahir, K.E.H. and D.M. Bakheet, 2007. The black seed *Nigella sativa* linnaeus-a mine for multi-cures: A plea for urgent clinical evaluation of its volatile oil. J. Taibah Univ. Med. Sci., 1: 1-19.
23. Adel, P.R.S. and J. Prakash, 2010. Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). J. Med. Plant Res., 4: 2674-2679.
24. Islam, M.S., M.N. Siddiqui, M.A. Sayed, M. Tahjib-Ul-Arif, M.A. Islam and M.A. Hossain, 2016. Dietary effects of buckwheat (*Fagopyrum esculentum*) and black cumin (*Nigella sativa*) seed on growth performance, serum lipid profile and intestinal microflora of broiler chicks. S. Afr. J. Anim. Sci., 46: 103-111.
25. Kumar, P. and A.K. Patra, 2017. Beneficial uses of black cumin (*Nigella sativa* L.) seeds as a feed additive in poultry nutrition. World's Poult. Sci. J., 73: 872-885.
26. Shewita, R.S. and A.E. Taha, 2011. Effect of dietary supplementation of different levels of black seed (*Nigella sativa* L.) on growth performance, immunological, hematological and carcass parameters of broiler chicks. Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng., 5: 304-310.
27. George, O.S., S.G. Kaegon and A.A. Igboke, 2015. Effects of graded levels of ginger (*Zingiber officinale*) meal as feed additive on growth performance characteristics of broiler chicks. Int. J. Sci. Res., 4: 805-808.
28. Karangiya, V.K., H.H. Savsani, S.S. Patil, D.D. Garg, K.S. Murthy, N.K. Ribadiya and S.J. Vekariya, 2016. Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. Vet. World, 9: 245-250.
29. Zomrawi, W.B., K.A.A. Atti, B.M. Dousa and A.G. Mahala, 2013. The effect of dietary ginger root powder (*Zingiber officinale*) on broiler chicks performance, carcass characteristic and serum constituents. J. Anim. Sci. Adv., 3: 42-47.
30. El Tazi, S.M.A., 2014. Effect of feeding different levels of *Moringa oleifera* leaf meal on the performance and carcass quality of broiler chicks. Int. J. Sci. Res., 3: 147-151.
31. Guler, T., B. Dalkilic, O.N. Ertas and M. Ciftci, 2006. The effect of dietary black cumin seeds (*Nigella sativa* L.) on the performance of broilers. Asian-Aust. J. Anim. Sci., 19: 425-430.
32. Adeyemo, G.O., I.J. Olowookere and O.G. Longe, 2016. Effect of dietary inclusion of ginger (*Zingiber officinale*) dried with different methods on performance and gut microbial population of broiler chicks. Am. J. Exp. Agric., 11: 1-7.
33. Onu, P.N., 2010. Evaluation of two herbal spices as feed additives for finisher broilers. Biotechnol. Anim. Husbandry, 26: 383-392.
34. Mawahib, A.E., A.A. Elfadil, B.H. Eljack, T.E. Mohamed, A.Y. Abdelgadir, M.M. Megahed and R. Nasser, 2016. Effect of feeding garlic (*Allium sativum*) and ginger (*Zingiber officinale*) mixture on performance and immune response of broiler chicks. Sudan J. Sci. Technol., 17: 73-79.
35. Al-Beitawi, N. and S.S. El-Ghousein, 2008. Effect of feeding different levels of *Nigella sativa* seeds (Black cumin) on performance, blood constituents and carcass characteristics of broiler chicks. Int. J. Poult. Sci., 7: 715-721.
36. Al-Mufarrej, S.I., 2014. Immune-responsiveness and performance of broiler chickens fed black cumin (*Nigella sativa* L.) powder. J. Saudi Soc. Agric. Sci., 13: 75-80.
37. Toghyani, M., M. Toghyani, A. Gheisari, G. Ghalamkari and M. Mohammadrezaei, 2010. Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). Livest. Sci., 129: 173-178.
38. Ghasemi, H.A., N. Kasani and K. Taherpour, 2014. Effects of black cumin seed (*Nigella sativa* L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers. Livest. Sci., 164: 128-134.
39. Golshan, M.R., M. Toghyani and G. Ghalamkari, 2015. Evaluation of nettle (*Urtica dioica*) and ginger (*Zingiber officinale*) powder on serum antioxidants and immune responses of broiler chicks. Der Pharm. Lett., 7: 411-415.
40. Al-Yasiry, R.M.A., S.A.H. Jawad, K.J. Menati, S.A. Naji and I.H. Lokman, 2016. Effects of *Boswellia carterii* and *Boswellia serrata* in drinking water on the growth performance, hematology traits and immune response of broiler chicken. Res. Rev.: J. Food Dairy Technol., 4: 27-37.
41. Abd El-Hack, M.E., M. Alagawany, M.R. Farag, R. Tiwari, K. Karthik and K. Dhama, 2016. Nutritional, healthical and therapeutic efficacy of black cumin (*Nigella sativa*) in animals, poultry and humans. Int. J. Pharmacol., 12: 232-248.
42. Khan, R.U., S. Naz, Z. Nikousefat, V. Tufarelli, M. Javdani, M.S. Qureshi and V. Laudadio, 2012. Potential applications of ginger (*Zingiber officinale*) in poultry diets. World's Poult. Sci. J., 68: 245-252.
43. Khan, S.H., J. Ansari, A.U. Haq and G. Abbas, 2012. Black cumin seeds as phyto-genic product in broiler diets and its effects on performance, blood constituents, immunity and caecal microbial population. Ital. J. Anim. Sci., Vol. 11, No. 4. 10.4081/ijas.2012.e77.
44. Abu-Dieyeh, Z.H.M. and M.S. Abu-Darwish, 2008. Effect of feeding powdered black cumin seeds (*Nigella sativa* L.) on growth performance of 4-8 week-old broilers. J. Anim. Vet. Adv., 3: 286-290.
45. Mohammed, A., 2015. Effect of dietary ground ginger (*Zingiber officinale*) root additive on broiler performance. Ph.D. Thesis, University of Khartoum, Sudan.