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Immune-responsiveness, performance and blood chemistry of broiler chickens fed black cumin seed and/or turmeric

Sabry M. El-Bahr

A. Al-Ankari

Saad Shousha

Original Article

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Sabry M. El-Bahr[1,2*] A. Al-Ankari[3] Saad Shousha[1,4]

Abstract

Two hundred and fifty one-day old broiler chicks were used to evaluate the effect of black seeds and/or turmeric on performance and immune response to Newcastle Disease Virus (NDV), Infectious Bronchitis Virus (IBV) and Infectious Bursal Disease virus (IBDV) in broiler chickens of six weeks of age. The birds were divided into five groups (50 birds in each) with 5 replicates (10 birds each). Birds in the first group were fed a basal diet and served as a control. Birds of groups 2-5 were fed a basal diet mixed with black seed (20g/ kg basal diet), turmeric (20g/ kg basal diet), 2% BTM (10g from each plant/ kg basal diet), 4% BTM (20g from each plant/ kg basal diet), respectively. Body weight, total protein, albumin, globulins and antibody titer of NDV and IBVD significantly increased in the birds fed diets supplemented with 2 and 4% BTM followed by those of birds fed a diet supplemented with black seed alone and eventually those of birds fed a diet supplemented with turmeric alone compared to the control during 15-28 and 29-42 days of age. BTM was further distinguished from individual herbal administration by its ability to decrease the feed conversion ratio and in its zero mortality benefits. The examined doses of both plants either alone or in combination (BTM) were safe for birds as reflected in the undisturbed liver function tests. In conclusion, diets supplemented with 2 and 4% BTM can be used as opposed to those supplemented with 2% black cumin seed or turmeric alone. The lower (2%) BTM concentration has the same beneficial effect as the double dose (4% BTM). Therefore, for the economic and efficient production of broilers, instead of using 4% BTM or individual administration of 2% of each herb, 2% BTM can be used effectively.

Keywords: *Nigella sativa*, *curcuma longa*, viruses, antibody titer, biomarkers

[1]*Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia*
[2]*Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt*
[3]*Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia*
[4]*Department of Physiology, Faculty of Veterinary Medicine, Benha University, Egypt*
***Correspondence**: selbahar@kfu.edu.sa, sabryelbahr@hotmail.com* (S. M. El-Bahr)
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Introduction

High quality feed, strain selection and proper management participate in the production of maximum weight gain in broiler chickens (Nazeer *et al.,* 2002). Feed additives play a major role in poultry nutrition. These are described as substances that are non-nutritive in nature but enhance the effective use of nutrients under certain circumstances, promote growth and improve the efficiency of production (Cullison and Lowery, 1987). The use of herbal products has been reported as growth promotors, feed intake enhancers and immunostimulants (Briminkmeyer, 1996; El-Bahr, 2007; El-Bahr and Saad, 2008; Abdelwahab and El-Bahr, 2012; Ali *et al.,* 2014; Al-Mufarrej, 2013; Shihab, 2017). Black cumin seed *(Nigella sativa)* is a herbaceous plant, which is a member of the Ranunculocea family. Its seeds are black in color and are mainly used in confectionary, bread, cakes and other baked products (Stern, 2000). Turmeric *(Curcuma longa)* is a perennial herb that grows to a height of three to five feet and is cultivated extensively in Asia (India and China) and other countries with a tropical climate. *Curcumin*, is the active ingredient of turmeric and is a potent antioxidant and anti-inflammatory agent with hepatoprotective, anticarcinogenic and antimicrobial properties (Duvoix *et al.,* 2005; Aggarwal *et al.,* 2005). A maximum weight gain of 1485.5 g has been reported by adding 0.5% of black cumin seed to broilers' rations (Ihsan, 2003). The same dose of turmeric (0.5%) has enhanced the overall performance of broiler chickens (Al-Sultan, 2003). Recent study has reported that, 0.5% turmeric improved broilers' performance (Sedeghi and Moghaddam 2018). Black cumin seed (Al-Mufarrej, 2013; Seidavi *et al.,* 2020) and turmeric (Shihab, 2017) enhanced the immune-responsivenss of broiler chickens. The impact of the combined administration of black cumin seeds and turmeric has only been observed in fish (El-Bahr and Saad, 2008; Abdelwahab and El-Bahr, 2012) and rats (Alnazawi and El-Bahr, 2012; El-Bahr *et al.,* 2014). In this sense, researchers have shown that, hypolipidemic, hypocholesterolemic (Alnazawi and El-Bahr, 2012) and antidiabetic effects (El-Bahr *et al.,* 2014) in rats were achieved by the combined administration of black cumin seed and turmeric (1%). In Asian sea bass (*Lates calcarifer*) diets, the combined administration of both plants (BTM) at the same concentration (1%) comparatively improved growth performance and did not influence blood chemistry (Abdelwahab and El-Bahr, 2012). The inclusion of a black cumin seed and turmeric combination has been reported to be safe for the liver and kidneys at the dose examined as expressed in their biomarkers in fish (Abdelwahab and El-Bahr, 2012) and rats (Alnazawi and El-Bahr, 2012; El-Bahr *et al.,* 2014). Based on the detailed literature outlined above, the impact of the black cumin seeds and turmeric Mixture (BTM) on performance and immune-responsiveness of broilers chickens has not yet been investigated. Therefore, the current study aimed to evaluate the effect of black cumin seeds and/or turmeric on growth performance and immune response against Newcastle Disease Virus (NDV), Infectious Bronchitis Virus (IBV) and Infectious Bursal Disease virus (IBDV) in broiler chickens at six week of age.

Materials and Methods

Birds and experimental design: Two hundred and fifty -one day old broiler chicks both male and female (Ross 308; 40 ± 3g body weight) were housed in an open-sided poultry house at the Researches and Training Station, King Faisal University, Al-Ahsa, Saudi Arabia. The pens provided a floor area of 1.5m^2. The ambient temperature (25^{o}C ± 2) and relative humidity (50-60%) were monitored daily. Birds were randomly divided into five groups (50 birds in each) with 5 replicates (10 birds each). Birds in the first group were fed a basal diet and served as a control. Birds of groups 2-5 were fed a basal diet mixed with black cumin seed (20g/ kg basal diet), turmeric (20g/ kg basal diet), 2% BTM (10g from each plant/ kg basal diet), 4% BTM (20g from each plant/ kg basal diet), respectively. The birds in all groups were vaccinated with ND (La Sota) via drinking water at day 15 followed by a booster dose of Newcastle virus vaccine (La Sota) at day 25 and with IBD Gumbo L strain (Ceva-Hungary) at day 19. Ma5 vaccine was used via eye-drops for vaccination of one-day-old chicks against IB on day 1 and repeated on day 18 using the same vaccine (Shihab, 2017). Feed and water were provided ad libitum. All experimental procedures were maintained by and performed according to national guidelines and protocols, approved by the University Scientific Research Ethics Committee, King Faisal University, Saudi Arabia (Approval # DSR140251).The ingredient content and calculated nutrient analysis of starter, grower and finisher diets are shown in Table 1.

Plant materials: Black cumin seed *(Nigella sativa)* and turmeric *(curcuma longa)* were purchased from a local market in Al-Ahsa, Saudi Arabia and identified by botanists at the College of Agriculture and Food Sciences, King Faisal University, Saudi Arabia. The whole black cumin seeds and turmeric were crushed in a blender and mixed with the diet (20g/kg diet; 2%) while BTM (1:1 w w^{-1}) constituted either 20 g/kg diet (2%) or 40 g/kg diet (4%) of the basal diet.

Performance parameters: Body weight was determined at the end of the 2nd, 4th and 6th week and the average daily gain was calculated. Feed was weighed back at the end of the 2nd, 4th and 6th week and the average daily feed intake and feed conversion (gram feed: gram gain) was determined accordingly. Mortality was recorded daily during the experiment.

Immune response: Blood samples were collected from all birds of each group at the end of the 2nd, 4th and 6th week. Antibody titer of NDV, IBV and IBDV were detrmined using ELISA tests kits (IDEXX Laboratories, Inc. One IDEXX Drive Westbrook, Maine 04092 United States) according to the manufacturer's instructions.

Biochemical parameters: Blood samples were obtained from the wing vein of all birds of each group at14 day intervals using a 3 ml syringe and 23-gauge needle. Harvested sera were used for determination of the

activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) (Reitman and Frankel, 1957). In addition, serum values of total protein (Doumas *et al.*, 1981), albumin (Reinhold, 1953), globulin (Coles, 1974), blood urea nitrogen (Tabacco *et al.*, 1979), uric acid, creatinine (Todd and Henry, 1984), triacylglycerol (TAG); Gottfried and Rosenberg, 1973), total cholesterol (Zak *et al.*, 1954) and high and low density lipoprotein cholesterol (Lopes-Virella *et al.*, 1977) were also estimated.

Statistical analyses: The collected data was exposed to Analysis of Variance (ANOVA) using SPSS (version 16; IBM, Chicago, IL, USA) followed by Duncan's multiple comparison tests (Duncan, 1995) to compare the differences between dietary treatments where significant differences were observed (p<0.05).

Table 1 Ingredient composition and calculated nutrient analysis (gm/kg) of starter, grower and finisher broiler diets.

Ingredients	Diets		
	Starter	Grower	Finisher
Yellow corn grains	48.77	52	64.05
Soybean meal 44%	34.32	33	25.00
Corn gluten meal	6.75	6	3.34
Soybean oil	5.86	5.21	3.97
Limestone	1.50	1.24	1.26
Monocalcium phosphate	1.75	1.50	1.35
Choline chloride	0.10	0.10	0.10
Sodium chloride	0.35	0.35	0.35
[1]Vitamin and mineral premix	0.35	0.35	0.35
L-lysine HCl	0.14	0.14	0.17
DL-methionine	0.11	0.11	0.06
Nutrient specifications			
[2]ME Kcal/kg diet	2900	3000	3200
Minimum CP (%)	21	20	18.5
Minimum crude fat (%)	2.5	3.0	3.0
Ca (%)	1.00	0.9	0.77
Available P (%)	0.42	0.45	0.38
Methionine (%)	0.50	0.45	0.37
Lysine (%)	1.20	1.00	0.90

[1]Vitamin and mineral premix supplied each kg of feed with: Vitamin A 12000 IU; vitamin D_3 3000 IU; vitamin E 60 mg; vitamin C 100mg; vitamin K_3 2 mg; vitamin B_1 3 mg; vitamin B_2 8 mg; vitamin B_6 5 mg; vitamin B_{12} 0.03 mg; biotin 0.2 mg; pantothenic acid 15 mg; nicotinic acid choline 900; 30 mg; folic acid 2 mg; manganese 90 mg; iron 35 mg; copper 8 mg; iodine 2 mg; selenium 0.2 mg; cobalt 0.5 mg; zinc 70 mg; antioxidants 125 mg. [2]ME: metabolizable energy.

Results

Performance parameters: Body weight was significantly (p<0.05) higher in chicks fed diets supplemented with 2 or 4% BTM followed by those of birds fed a diet supplemented with black cumin seed alone and eventually those of birds fed a diet supplemented with turmeric alone compared to the control at 15-28 days, 29-42 and 1-42 days (Table 2). In relation to body weight gain compared to the control, there was no significant difference (p>0.05) among all experimental groups at 1-14 days of age, (Table 2). Feed intake was significantly (p>0.05) unchanged in birds fed a diet supplemented with black cumin seed and/or turmeric compared to the control during the whole experimental period (Table 2). Feed conversion ratio was significantly (p>0.05) unchanged in birds fed a diet supplemented with black cumin seed and/or turmeric compared to the control during 1-14 and 15-28 days of age. However, feed conversion ratio was significantly (p<0.05) reduced in birds fed a diet supplemented either with 2 or 4% BTM compared to those of birds fed black cumin seed or turmeric which remained comparable to the control (Table 2) during 29-42 and 1-42 days of age. During 1-14 days of age, zero mortality was recorded in birds fed diets supplemented with either 2 or 4% BTM compared to those of birds fed a diet supplemented with black cumin seed alone, turmeric alone and the control birds that all recorded a 2% mortality rate (Table 2). During 15-28 days of age, zero mortality was recorded in birds fed diets supplemented with either 2 or 4% BTM compared to those of birds fed a diet supplemented with black cumin seed alone, turmeric alone and the control birds that recorded 2, 2 and 4% mortality rate, respectively (Table 2). During 29-41 days of age, zero mortality was recorded in birds fed diets supplemented with either 2 or 4% BTM compared to those of birds fed a diet supplemented with black cumin seed alone, turmeric alone and the control birds that recorded 4, 4 and 6% mortality rate, respectively (Table 2). During the whole experimental period (1-41 days of age), zero mortality was recorded in birds fed diets supplemented with either 2 or 4% BTM compared to those of birds fed a diet supplemented with black cumin seed alone, turmeric alone and the control birds that recorded 8, 8 and 12% mortality rate, respectively (Table 2). Final body weight (1-42 days) was significantly (p<0.05) higher in chicks fed diets supplemented with 2 or 4% BTM followed by those of birds fed a diet supplemented with black cumin seed alone and eventually those of birds fed a diet supplemented with turmeric alone compared to the control (Table 2).

Immune response: Antibodies against NDV and IBV were significantly higher in the birds fed a diet supplemented with 2 or 4% BTM followed by those of birds fed a diet supplemented with black cumin seed alone and finally those of birds fed a diet supplemented with turmeric alone compared to the

control during 15-28 and 29-42 days of age (Table 3). On the other hand, no significant difference was noted for IBDV antibodies among any experimental groups including the control (Table 3).

Biochemical parameters: Total protein, albumin and globulin levels were significantly (p<0.05) increased in the serum of birds fed a diet supplemented with 2 or 4% BTM compared to those of birds fed a diet supplemented with either black cumin seed or turmeric alone which remained significantly (p>0.05) unchanged compared to the control (Table 4). ALT, AST, HDL-c, LDL-c, TAG, total cholesterol, BUN, uric acid and creatinine levels were significantly (p>0.05) unchanged in all experimental groups compared to the control (Table 4).

Table 2 Performance characteristics of broiler chickens supplemented with black cumin seed and/or turmeric at 1-14, 15-28, 29-42 and 1-42 days of age.

Time	Parameters	Groups					*p* values
		1	2	3	4	5	
1-14 days	Feed intake (g/bird/day)	36.7 ± 5.1	37.9 ± 5.2	36.6 ± 5.1	37.7 ± 4.1	38.0 ± 4.1	0.527
	Body weight gain (g/bird/day)	18.0 ± 8.0	17.7 ± 8.0	16.9 ± 7.5	19.0 ± 8.2	18.8 ± 7.1	0.646
	Feed conversion ratio	2.03 ± 0.1	2.14 ± 0.1	2.17 ± 0.1	1.98 ± 0.1	2.02 ± 0.1	0.984
	Mortality (%)	2%	2%	2%	0%	0%	
15-28 days	Feed intake (g/bird/day)	49.4 ± 5.1	50.0 ± 4.9	51.1 ± 5.2	54.0 ± 4.8	53.4 ± 3.9	0.901
	Body weight gain (g/bird/day)	25.5 ± 0.1^{d}	28.0 ± 0.5^{b}	27.0 ± 0.3^{c}	29.0 ± 0.7^{a}	29.4 ± 0.1^{a}	0.019
	Feed conversion ratio	1.94 ± 0.2	1.79 ± 0.1	1.89 ± 0.1	1.86 ± 0.1	1.82 ± 0.1	1.002
	Mortality (%)	4%	2%	2%	0%	0%	
29-42 days	Feed intake (g/bird)	55.5 ± 3.2	56.7 ± 4.5	56.1 ± 3.4	55.0 ± 4.5	55.2 ± 4.1	0.855
	Body weight gain (g/bird)	28.0 ± 0.7^{d}	30.0 ± 0.5^{b}	29.0 ± 0.0^{c}	32.5 ± 0.7^{a}	31.8 ± 0.6^{a}	0.026
	Feed conversion ratio	1.98 ± 0.1^{a}	1.89 ± 0.1^{a}	1.93 ± 0.1^{a}	1.69 ± 0.1^{b}	1.74 ± 0.1^{b}	0.054
	Mortality (%)	6%	4%	4%	0%	0%	
1-42 days	Total feed intake (g/bird)	1981.6 ± 50.1	2024.4 ± 30.2	2013.2 ± 30.1	2053.8 ± 30.2	2052.4 ± 30.1	0.896
	Total body weight gain (g/bird)	1001 ± 5.2^{d}	1059 ± 5.3^{b}	1020.6 ± 5.2^{c}	1127 ± 5.1^{a}	1120 ± 5.3^{a}	0.012
	Feed conversion ratio	1.98 ± 0.1^{a}	1.94 ± 0.1^{b}	1.99 ± 0.1^{a}	1.84 ± 0.1^{c}	1.86 ± 0.1^{c}	0.051
	Final body weight (g)	1046 ± 2.1^{d}	1104 ± 2.2^{b}	1065 ± 3.1^{c}	1172 ± 5.3^{a}	1165 ± 5.4^{a}	0.02
	Total mortality (%)	12%	8%	8%	0%	0%	

Values are mean ± standard errors of means (SEM) of five replicates of birds (n=10 for each replicate).

$^{a-d}$ values with different superscripts in the same row are significantly differed at $P < 0.05$. Group 1: Control; Group 2: black cumin seed treated birds(20g/ kg basal diet); Group 3: turmeric treated birds (20g/ kg basal diet); Group 4: birds treated with 2% black cumin seed and turmeric mixture (BTM; 10g from each plant/ kg basal diet for each plant. Group 5: birds treated with 4% black cumin seed and turmeric mixture (BTM; 20g from each plant/ kg basal diet for each plant).

Table 3 Titer of serum antibodies of broiler chickens supplemented with black cumin seed and/or turmeric at 1-14, 15-28, 29-42 and 1-42 days of age.

Time	Parameters	Groups					*p* values
		1	2	3	4	5	
1-14 days	NDV	515 ± 9.6	518 ± 8.9	516 ± 6.1	520 ± 7.7	522 ± 7.9	0.322
	IBV	273 ± 8.1	272 ± 7.8	283 ± 5.1	278 ± 7.6	280 ± 7.1	0.466
	IBD	1.9 ± 0.2	1.9 ± 0.2	1.8 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	0.641
15-28 days	NDV	1720 ± 10.2^{d}	1840 ± 11.7^{b}	1770 ± 11.1^{c}	1900 ± 10.9^{a}	1910 ± 11.0^{a}	0.026
	IBV	780 ± 9.9^{d}	850 ± 10.0^{b}	810 ± 9.5^{c}	950 ± 10.1^{a}	970 ± 10.0^{a}	0.013
	IBD	2.0 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	0.970
29-42 days	NDV	2200 ± 9.2^{d}	2450 ± 9.5^{b}	2600 ± 10.2^{c}	2700 ± 11.4^{a}	2680 ± 11.5^{a}	0.011
	IBV	1500 ± 6.4^{d}	1800 ± 7.7^{b}	1700 ± 10.0^{c}	2000 ± 10.2^{a}	2010 ± 10.2^{a}	0.011
	IBD	2.0 ± 0.2	1.9 ± 0.2	1.9 ± 0.1	1.9 ± 0.2	1.9 ± 0.2	0.889

Values are mean ± standard errors of means (SEM) of five replicates of birds (n=10 for each replicate).

$^{a-d}$ values with different superscripts in the same row are significantly different at $P < 0.05$. Group 1: Control; Group 2: black cumin seed treated birds(20g/ kg basal diet); Group 3: turmeric treated birds (20g/ kg basal diet); Group 4: birds treated with 2% black cumin seed and turmeric mixture (BTM; 10g from each plant/ kg basal diet for each plant. Group 5: birds treated with 4% black cumin seed and turmeric mixture (BTM; 20g from each plant/ kg basal diet for each plant).

Table 4 Serum biochemical parameters of broiler chickens supplemented with black cumin seed and/or turmeric at 1-14, 15-28, 29-42 and 1-42 days of age.

Parameters	Time	Groups					*p* values
		1	2	3	4	5	
Total protein (g/L)	1-14 days	30.5 ± 1.3	30.4 ± 1.1	30.5 ± 1.3	31.3 ± 2.1	31.2 ± 2.5	0.61
	15-28 days	20.1 ± 0.9^{d}	28.2 ± 1.2^{b}	24.1 ± 0.9^{c}	36.9 ± 1.6^{a}	36.2 ± 2.1^{a}	0.019
	29-42 days	20.3 ± 1.1^{d}	31.1 ± 1.5^{b}	24.3 ± 1.1^{c}	41.1 ± 1.7^{a}	37.2 ± 2.5^{a}	0.009
Albumin (g/L)	1-14 days	17.5 ± 1.0	16.4 ± 0.5	16.5 ± 0.4	17.3 ± 0.5	17.2 ± 0.9	0.883
	15-28 days	13.1 ± 0.4^{d}	16.2 ± 0.5^{b}	15.1 ± 0.3^{c}	19.9 ± 0.7^{a}	18.2 ± 0.7^{a}	0.015
	29-42 days	13.3 ± 0.2^{d}	16.1 ± 0.1^{b}	14.3 ± 0.2^{c}	21.1 ± 0.9^{a}	19.2 ± 1.0^{a}	0.011
Globulin (g/L)	1-14 days	13.0 ± 1.1	14.0 ± 0.2	14.0 ± 0.4	14.0 ± 0.6	14.0 ± 0.6	0.712
	15-28 days	07.0 ± 0.3^{d}	14.0 ± 0.2^{b}	09.0 ± 0.3^{c}	17.0 ± 0.8^{a}	18.0 ± 0.7^{a}	0.010
	29-42 days	07.0 ± 0.5^{d}	15.0 ± 0.4^{b}	10.0 ± 0.3^{c}	20.0 ± 1.0^{a}	18.0 ± 1.0^{a}	0.015
Triglyceride (mmol/L)	1-14 days	0.70 ± 0.12	0.72 ± 0.11	0.68 ± 0.12	0.69 ± 0.11	0.70 ± 0.12	0.987
	15-28 days	0.86 ± 0.11	0.85 ± 0.11	0.79 ± 0.13	0.89 ± 0.11	0.80 ± 0.12	0.880
	29-42 days	0.79 ± 0.12	0.69 ± 0.12	0.70 ± 0.12	0.80 ± 0.21	0.74 ± 0.13	0.606
Cholesterol (mmol/L)	1-14 days	2.81 ± 0.1	2.91 ± 0.1	2.87 ± 0.1	3.00 ± 0.2	3.43 ± 0.3	0.360
	15-28 days	2.76 ± 0.1	2.75 ± 0.1	2.69 ± 0.1	2.88 ± 0.1	2.87 ± 0.2	0.434
	29-42 days	3.00 ± 0.1	2.80 ± 0.2	2.77 ± 0.3	2.88 ± 0.2	3.12 ± 0.2	0.450
HDL-c (mmol/L)	1-14 days	1.43 ± 0.1	1.56 ± 0.1	1.61 ± 0.1	1.49 ± 0.03	1.34± 0.1	0.749
	15-28 days	1.39 ± 0.1	1.55 ± 0.1	1.54 ± 0.1	1.51 ± 0.01	1.40 ± 0.2	0.663
	29-42 days	1.40 ± 0.1	1.52 ± 0.2	1.50 ± 0.1	1.55 ± 0.3	1.42 ± 0.3	0.478
LDL-c (mmol/L)	1-14 days	1.33 ± 0.2	1.44 ± 0.2	1.47 ± 0.2	1.45 ± 0.2	1.40 ± 0.1	0.940
	15-28 days	1.30 ± 0.2	1.45 ± 0.2	1.43 ± 0.2	1.40 ± 0.1	1.50 ± 0.1	0.980
	29-42 days	1.29 ± 0.1	1.40 ± 0.2	1.44 ± 0.2	1.43 ± 0.1	1.50 ± 0.1	0.990
ALT (U/l)	1-14 days	12.5 ± 3.0	14.4 ± 2.0	13.9 ± 2.1	13.4 ± 2.1	10.6 ± 2.9	0.556
	15-28 days	10.7 ± 2.0	11.2 ± 1.9	10.9 ± 2.1	10.5 ± 1.4	10.3 ± 1.9	0.554
	29-42 days	11.6 ± 2.1	12.4 ± 1.6	10.9 ± 1.9	11.5 ± 0.9	10.5 ± 1.3	0.552
AST (U/l)	1-14 days	67.1 ± 2.4	70.7 ± 1.0	69.7 ± 2.0	72.6 ± 1.1	76.6 ± 0.9	0.768
	15-28 days	76.5 ± 2.1	78.4 ± 0.9	79.4 ± 1.8	80.5 ± 1.0	81.4 ± 1.1	0.890
	29-42 days	88.4± 1.6	90.3 ± 1.6	87.3 ± 2.0	90.7 ± 0.9	94.4± 1.3	0.655
Creatinine (µmol/L)	1-14 days	77.34 ± 9.4	79.24 ± 8.9	81.24 ± 2.8	80.12 ± 4.6	80.66± 5.4	0.561
	15-28 days	88.12 ± 9.6	80.65 ± 5.3	79.65 ± 4.9	80.37 ± 6.4	80.46± 5.1	0.800
	29-42 days	87.15 ± 8.1	90.76 ± 6.1	88.76 ± 5.0	92.65 ± 5.3	80.55 ± 5.4	0.990
Uric acid (µmol/L)	1-14 days	350.12 ± 7.4	350.44 ± 6.9	345.44 ± 5.0	360.43 ± 9.6	340.33 ± 9.4	0.989
	15-28 days	333.23 ± 7.0	355.45 ± 8.3	342.45 ± 9.1	345.50 ± 6.4	330.11 ± 8.1	0.640
	29-42 days	350.32 ± 2.2	367.44 ± 7.1	372.44 ± 9.2	370.63 ± 9.3	340.71 ± 9.4	0.652
Urea (µmol/L)	1-14 days	0.74± 0.02	0.72 ± 0.04	0.68 ± 0.1	0.69 ± 0.06	0.70 ± 0.02	0.463
	15-28 days	0.64 ± 0.02	0.66 ± 0.2	0.70 ± 0.3	0.70 ± 0.04	0.60 ± 0.07	0.260
	29-42 days	0.67 ± 0.02	0.65 ± 0.5	0.70 ± 0.4	0.68 ± 0.04	0.70 ± 0.02	0.389

Values are mean ± standard errors of means (SEM) of five replicates of birds (n=10 for each replicate).

[a-d] values with different superscripts in the same row are significantly different at $P < 0.05$. Group 1: Control; Group 2: black cumin seed treated birds(20g/ kg basal diet); Group 3: turmeric treated birds (20g/ kg basal diet); Group 4: birds treated with 2% black cumin seed and turmeric mixture (BTM; 10g from each plant/ kg basal diet for each plant. Group 5: birds treated with 4% black cumin seed and turmeric mixture (BTM; 20g from each plant/ kg basal diet for each plant).

Discussion

Performance parameters: In the current study, the control line reached an average body weight of 1046 ± 2.1 g. This body weight is lower than that reported in Ross dietary recommendations. This may be attributed to the feeding of these birds with a basal diet of mash ground feed without any additives. The supplemented basal diet was prepared in the Researches and Training Station, King Faisal University and not prepared in a feed mill, so the feeds were not pelleted or crumbled. In addition, basal diet was not treated with steam during processing. Furthermore, birds in the current study were housed in an open-sided poultry house and may have been affected by heat stress during the experiment. In the first 4 weeks, high feed conversion ratios could have been due to low feed intake. Low feed intake may have been due to environmental (high temperature, form of provided food and feed flavor) and/or physiological (volume of gastrointestinal tract and hormones) factors (Ferket and Gernat, 2006). In Al-Ahsa-Saudi Arabia, high temperature and relative humidity can induce physiological changes in the chicken's body. In order to neutralize the ambient heat, chicken energy is lost so that the state of the body remains in homeostasis. The use of energy to sustain this homeostatic state disrupts the diet's level of nutritional use in the meat so that the addition of tested herbs does not statistically make a real difference in the ratios of feed conversion and can be resonant with higher feed conversion ratios during the first 4 weeks of age (Candra and Putri, 2019). The positive effect of black cumin seed (Kumar and Patra, 2017) and turmeric (Guil-Guerrero *et al.*, 2017) supplementation on the performance of broiler chickens has been documented. The significant increase in body weight gain of birds fed a diet supplemented with 2% black cumin seed during 15-28 and 29-42 days of age is consistent with previous findings in broilers fed diets supplemented with 0.05% (Ali et al., 2014), 0.5% (Kumar *et al.*, 2017), 1% (Erener *et al.*, 2010; Toghyani *et al.*, 2010; Kumar *et al.*, 2017), 1.5% (Jahan *et al.*, 2015), 2.5 and 5% (Khan *et al.*, 2012) and in quails fed a diet containing 20% black cumin seed (Abd El-Hack *et al.*, 2016). Conversely, non-significant changes in the body weight gain of broilers fed diets supplemented with 0.7, 1.4, 2.1, or 2.8% black cumin seed have been

reported (Al-Mufarrej, 2013). In addition, the observed significant increase in body weight gain in birds fed a diet supplemented with turmeric 2% at the end of the 4th and 6th week agrees with previous findings in broilers fed a diet supplemented with 0.05% (Ali *et al*., 2014) or 0.25-0.5% (Hussein, 2013; Kafi *et al*., 2017; Sadeghi and Moghaddam, 2018) of turmeric. Conversely, previous findings (Gharejanloo *et al*., 2017) indicated that turmeric essential oil (0. 75 and 150mg/kg) did not affect the broilers' performance. The addition of turmeric (10, 20, or 30%) was not able to improve the broilers' performance (Wang *et al*., 2015; Isroli *et al*., 2017). In this research, the rise in broiler final body weight and body weight gain and reduction of feed conversion ratios with BTM supplements may be attributed to the effects of medical plant biological compounds on the improvement of antioxidant status and protein and fat metabolism (Windisch *et al*., 2008; Ali *et al*., 2014). Furthermore, medical plants supplementation promotes improved nutrient absorption in the diet and eventually leads to higher body weight, resulting in growth improvements and feed conversion ratios of broiler chickens (Windisch *et al*., 2008). Although this study has not examined digestibility and nutrient absorption, several researchers have stated that medicinal herbs affect the gastrointestinal tract of broilers, especially the small intestine, and increase the secretion of amylase and chymotrypsin and the absorption of villi in the intestine. It therefore increases the amount of nutrients and eventually improves performance (Pirmohammadi *et al*., 2016; Sedeghi and Moghaddam 2018). In broiler chickens, the observed superiority of black cumin seed over turmeric in terms of growing body weight gain has been recorded previously (Ali *et al*., 2014). In fish, the observed superiority of BTM over individual black cumin seed and turmeric supplementation has been reported (El-Bahr and Saad, 2008; Abdelwahab and El-Bahr, 2012). In the current study, the highest body weight gain was observed in birds fed a diet supplemented with BTM compared to the control followed by that of birds fed a diet supplemented with black cumin seed and finally that of birds fed a diet supplemented with turmeric. As a continuation of the distinguishing effect of BTM, the feed conversion ratio was significantly reduced in birds fed diets supplemented with BTM compared to that of birds fed diets supplemented with black seed alone, turmeric alone and the control. The reduction of feed conversion ratio is of high economic value since birds fed a diet supplemented with BTM can achieve a favorable market weight with a minimum consumption of food compared to birds fed a diet supplemented with black cumin seed alone, turmeric alone and the control birds. The observed zero mortality in birds fed 2 or 4 % BTM compared to that of birds fed diets supplemented with black cumin seed alone, turmeric alone or the control birds during one development cycle is another economic gain behind the advantage of BTM as a feed additive in broilers (6 weeks). Therefore, the use of BTM in the broiler diet from an economic point of view is recommended over individual black cumin seed or turmeric supplementation because BTM induces the highest body weight gain, the lowest feed conversion ratio and zero mortality during one production cycle.

Immune response: Similar to the growth performance results, the antibody titers of NDV and IBV were significantly increased in birds fed a diet supplemented with BTM followed by those of birds fed a diet supplemented with black cumin seed alone and finally those of birds fed a diet supplemented with turmeric alone compared to the control during 15-28 and 29-42 days of age. The enhancement of immune responsiveness by inclusion of black cumin seed (Al-Mufarrej, 2013) or turmeric (Shihab, 2017) in broiler diet has been recorded. Black seed improves the immune system by increasing lymphocyte production and inhibiting the creation of advanced dysplastic changes (Al-Jawfi *et al*., 2008). In addition, turmeric regulates the effectiveness of each of the T- cells, B-cells, Macrophages, hetrophils, natural killer cells and dentric cells (Shihab, 2017).

The efficacy of black cumin seed compared to turmeric in enhancing the antibody titer of NDV and IBV has been documented previously (Ali *et al*., 2014). The effectiveness of BTM in enhancing the antibody titers of NDV and IBV compared to that of black cumin seed or turmeric has been documented only in fish (El-Bahr and Saad, 2008). The current findings suggest the effectiveness of BTM in enhancing the antibody titers of NDV and IBV compared to that of individual supplementation with black cumin seed or turmeric in broiler chickens. Combining the beneficial effects of BTM on performance and immune response, it can be said that the zero mortality achieved by inclusion of BTM in broilers diet may be due to the observed efficacy of BTM compared to black cumin seed or turmeric in enhancing the antibody titers of viral infection (NDV and IBV).

Biochemical parameters: Similar to performance and immune response results, the concentrations of serum total proteins, albumin and globulin levels were significantly higher in birds fed a diet supplemented with BTM followed by those of birds fed a diet supplemented with black cumin seed alone and finally those of birds fed a diet supplemented with turmeric alone compared to the control during 15-28 and 29-42 days of age. Consistent with the current findings, previous studies reported that black cumin seed and turmeric induced a significant increase in total protein, albumin and globulin in the serum of broiler chickens (Alagawany *et al*., 2015) and fish (El-Bahr and Saad, 2008). In comparison, other studies reported non-significant changes in total protein, albumin and globulin levels in the serum of broiler chickens (Qasem *et al*., 2016), Japanese quails (Shokrollahi and Sharifi, 2018) and fish (Abdelwahab and El-Bahr, 2012) fed black cumin seed and/or turmeric enriched diets. Consistent with the current findings, earlier work (El-Bahr and Saad, 2008) reported the efficacy of BTM compared to black cumin seed or turmeric in increasing total protein, albumin and globulin in the serum of fish. The efficacy of BTM compared to individual black cumin seed and turmeric administration in increasing the concentrations of serum total protein, albumin and globulin of broiler

chickens confirmed its role in enhancing immune response as discussed above. The unchanged ALT, AST, BUN, uric acid and creatinine levels indicated that the studied herbs either alone or in a combination were safe for liver and kidney function of broiler chickens. Previous work has shown that the dosage of both herbs tested did not interfere with the liver and kidney function of fish (El-Bahr and Saad, 2008; Abdelwahab and El-Bahr, 2012) as reflected in biomarkers of renal and kidney function. In broiler rabbits, dietary supplementation of turmeric did not show significant changes in the liver and kidney function (Basavaraj *et al.,* 2011). However, high ALT and low AST levels were observed in the serum of broiler chicks fed black cumin seed (Shewita and Taha, 2011).

The current study concluded that the studied doses of black cumin seed and turmeric either alone or in combinations (2 and 4% BTM) improved the overall performance and immune response of broiler chickens. The efficacy of dietary black cumin seed and turmeric mixtures (2 and 4% BTM) has been stated to be the best in improving body weight gain, serum protein and antibody titers, and lowering the feed conversion ratio and mortality rate in broiler chickens, followed by that of dietary black cumin seed and ultimately dietary turmeric. The examined doses of both plants either alone or in combination (BTM) were safe to birds as reflected in the undisturbed liver functions tests. Diets supplemented with 2 and 4% BTM can therefore be used as opposed to those supplemented with 2% black cumin seed or turmeric alone. The lower (2%) BTM concentration has the same beneficial effect as the double dose (4% BTM). Therefore, for the economical and efficient production of broilers, instead of using 4% BTM or individual administration of 2% of each herb, 2% BTM can be used effectively.

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