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FULL LENGTH ARTICLE

Immune-responsiveness and performance of broiler chickens fed black cumin (*Nigella Sativa* L.) powder

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Abstract The main objective of this study was to determine the effects of various levels of dietary black cumin seed (BCS) on immune-responsiveness, broiler performance and lymphoid organs' ratio scores against NDV, IBV and IBDV vaccines. One hundred and sixty-one day-old broiler chicks (Rose 308) were randomly assigned into five groups with three replicates of seven birds each (21 chicks per group) and fed diets supplemented with 0.7%, 1.4%, 2.1% or 2.8% black cumin. All chickens were bled weekly for 35 days post-treatment. Body weights, thymus, bursa and spleen ratio scores were determined at 21 and 35 days while body weight was determined weekly. The results revealed non-significant differences in body weight between all groups compared to the control group. Antibody titers against NDV also exhibited non-significant differences at third, fifth and sixth weeks of the experiment between treated groups while they were significantly different from the control group. At the fourth week, group C showed significantly different antibody titers from other treated groups. However, antibody titers against IBV were significantly different in the fifth and sixth weeks against the control group. Similar results were recorded with IBDV vaccine compared to the control group while group C had significant differences in titers at 4, 5 and 6 weeks post-vaccination. Lymphoid organs, namely thymus, bursa and spleen showed non-significant differences between groups. This study showed that dietary supplement of black cumin seed at the level of 1% or 1.4% would enhance immune responsiveness in broiler chickens.

1. Introduction

Antibacterial feed additives have been used in the poultry industry for many years to improve the productivity in terms of weight gain and feed conversion ratio by helping to control infections in the gut mucosa. However, antibacterial additives were banned throughout the world from being used in animal and poultry feeds since resistance to these antibacterial agents was recorded with most of them (Wegener et al., 1998; Shea, 2003). Antimicrobial agents of plant origin such as essential oils, plants extracts and complete plant substances, have gathered significant consciousness as alternatives to the traditional antibacterial feed additives. On the other hand, feed antibiotics, which have been used for promoting growth in farm animals were shown to negatively affect profitability of the

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animals. Currently, the feed industry is researching various substitutes for antibiotics (Hertrampf, 2001; Humphrey et al., 2002). Black cumin (*Nigella sativa* L.), also known as black seed and grown in Asian and Mediterranean countries, is one of such alternatives that could be used as feed additives. A few studies showed that black cumin has antibacterial activity, indicating that it could be a substitute for conventional antimicrobial drug (El-Kamali et al., 1998; Mouhajir et al., 1999; Nair et al., 2005). (see Figs. 1 and 2)

The seeds of *N. sativa* L. have been used for centuries in the Middle East, Northern Africa, Far East, and Asia for the treatment of asthma (El-Tahir et al., 1993) and as an antitumor agent (El-Daly, 1998). In addition, the seeds have been reported to have many other biological properties including antiparasitic (Mahmoud et al., 2002), anti-diabetic (Al-Hader et al., 1993), and diuretic effects (Zaoui et al., 2000).

The effects of dietary black cumin or oils on the performance of poultry were studied and it was determined that black cumin and its oil extract affected feed intake and BW positively in the broilers (Halle et al., 1999; Guler et al., 2006; Ziad and Mohammad, 2008; Erener et al., 2010). Some authors showed that diets supplemented with 10% black cumin seed had no adverse effects on performance (Al-Homidan et al., 2002). Other studies indicated that the addition of ground black cumin seeds (BCS) in concentrations less than 0.25 up to 0.75% and 1% or 2% of the diet had undesirable effects on performance and carcass quality (Abbas and Ahmed, 2010; Majeed et al., 2010; Nasir and Grashorn, 2010). The seed was shown to be effective against total coliform count in the intestine of broilers (Erener et al., 2010). Oil extracted of BCS was shown to effectively inhibit *L. monocytogenes* (Nair et al., 2005; Arici et al., 2005; Ali et al., 2007).

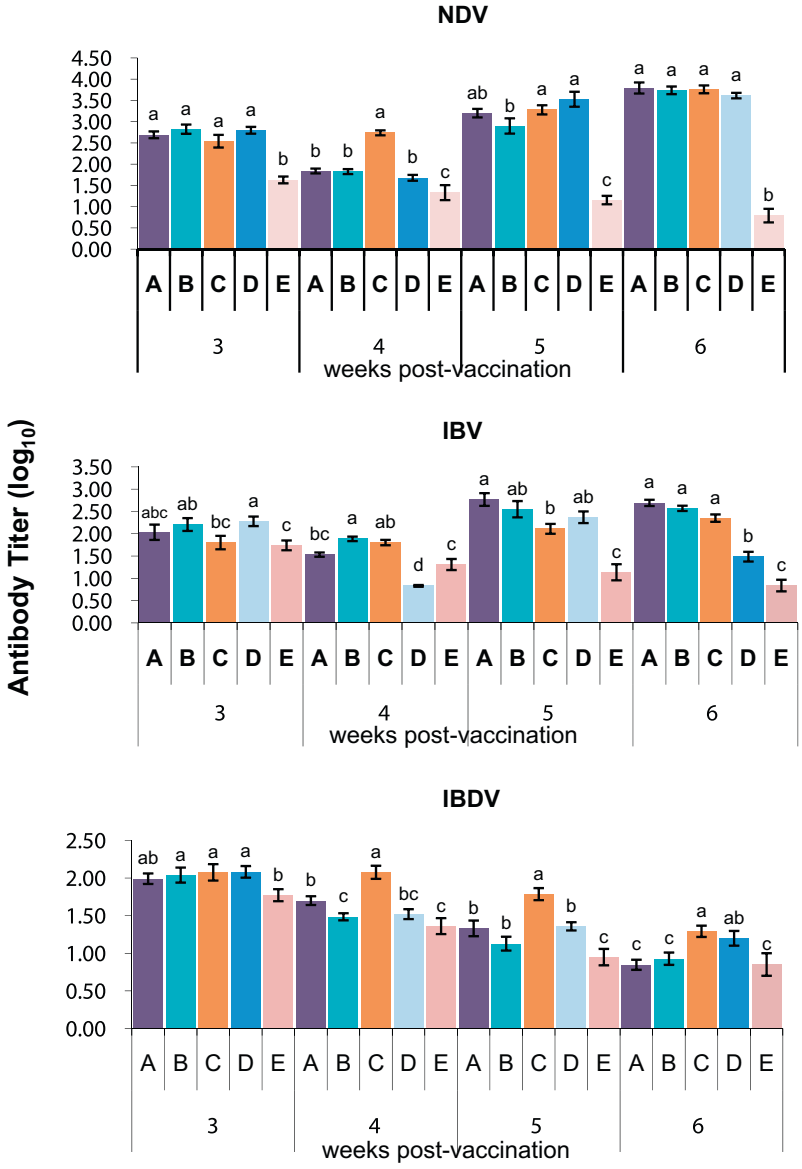

Figure 1 Newcastle disease virus, infectious bronchitis virus and infectious bursal disease, respectively virus-specific antibody titers in chickens ($n = 12$) immunized with 0.1 mL of virus activated vaccine. Blood samples were collected on 0, 7, 14, 21, 28, 35 and 42 days post-immunization for ELISA assay. Bars with different letters at the same time point are significantly different ($P < 0.05$).

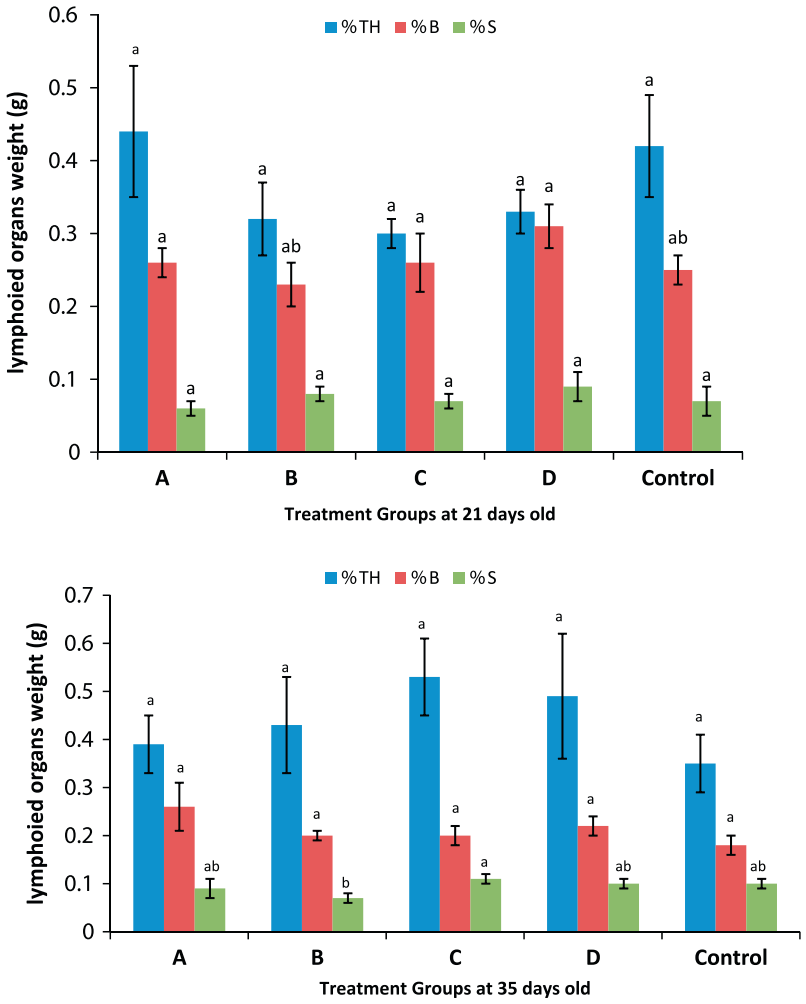

Figure 2 Effect of black cumin seed on lymphoid organs' weight at 21 and 35 days. Each bar represents all birds in the same group. Bars with different letters are significantly different.($P < 0.05$).

Studies to explore the effect of BCS on immune system of human volunteers using 1 g twice daily indicated enhanced immune functions such as improving helper T cells to suppressor T cells ratio as well as improving natural killer cell activity (El-Tahir et al., 1993). Dorucu et al. (2009) found that serum proteins and total immunoglobulin levels were significantly ($P < 0.05$) higher than those of the control group in rainbow trout fish. Antibody titer against ND and IBD was reported by Al-Beitawi et al. (2009) to be significantly enhanced by BCS. However, some workers reported that BCS had no significant effect on antibody titer against ND. However, there was significantly increased lymphoid organ ratio (Bursa and Thymus) with increasing BCS levels (Shewita and Taha, 2011).

BCS has various components that affect bacterial activity and inhibit aflatoxin production by fungi (Nasir and Grachorn 2006; Maraqa et al. 2007) However, there have been a limited number of studies associated with the effect of diets supplemented with black cumin on broiler chicken immune-responsiveness and lymphoid organs in poultry.

The objective of this study was to determine the effects of black cumin seeds on immune-responsiveness, lymphoid organs' ratio scores and performance of broiler chickens.

2. Materials and methods

2.1. Birds and husbandry

Three hundred 1-day-old Ross 305 chicks obtained from a local hatchery were assigned randomly to 30 cages with 10 birds per cage in brooding batteries. Each cage was equipped with a feeder and an automatic waterer. Birds were brooded at 33 °C during the first week with the brooding temperature being reduced to 3 °C/week until it reached approximately 24 °C by week four of age. Light was provided continually using incandescent lamps. All birds were vaccinated against ND and IB at one day and at 17 days of age. During the starter period (0–3 week) they received a commercial starter diet, and during grower period (3–6 week) they received a commercial grower diet provided by (ARASCO). Feed and water were provided *ad libitum*.

2.2. Preparation of black seed (N. sativa L.) for addition to feed

Black seeds were obtained from a local herb store in Riyadh, Saudi Arabia, and used in the diets after grinding, at the rate

of 2.8, 2.1, 1.4 and 0.7 g/kg as commercial broiler starter and finisher rations for groups A, B, C and D, respectively. Group F was kept as control.

2.3. Body weight measurements

Body weight was determined by cage at 1, 7, 14, 21, 28 and 35 days of age and the average daily gain was calculated. Feed was weighed back on 7, 14, 21, 28 and 35 days and the average daily feed intake and feed conversion (gram feed: gram gain) were determined weekly and during the period of 1–35 days. Mortality was recorded daily during the experiment.

2.4. Vaccination and immune response of chicks

All birds were vaccinated against ND and IB using live attenuated vaccines, according to the manufacturer's directions at day one with booster dose at 17 days of age (Fort Dodge Animal Health-USA). Blood samples were collected from 15 birds from each group at 7, 14, 21, 28 and 35 days of age. To detect the titer against ND and IB Elisa kits were used according to the manufacturer's instructions (IDEXX Laboratories, Inc. One IDEXX Drive Westbrook, Maine 04092 United States)

2.5. Lymphoid organs' collection

At ages 21 and 35 days, three replicate pens of two chicks (one male and one female) each were taken randomly (six birds/treatment) and slaughtered. At the time of slaughter, live body weight of each bird was obtained; the birds were killed by exsanguinations, and the spleen, thymus and bursa were collected and weighted.

2.6. Statistical analysis

Data were statistically analyzed by a two-way ANOVA using the General Linear Models procedure of SAS® (SAS Institute, 1996). However, actual percentage data are reported. Mortality data were very low and non significant among treatments, therefore they are not reported.

3. Results and discussion

3.1. Body weight evaluation

Tables 1–5 illustrate the effect of different levels of black cumin on body weight (BW), weight gain (WG) and feed intake (FI) (g/d per bird) in chickens. Diets supplemented with 0.7%, 1.4%, 2.1% or 2.8% black cumin had no significant effect on BW, WG while a significant effect ($P < 0.05$) on feed intake was observed only in the third week. However, feed conversion ratio (FCR) showed significant differences ($P < 0.05$) on the fifth week between control group and all groups fed on black cumin-supplemented diets (Table 4). Total gain (TG) total feed (TF) and TF: TG (g:g) were not significantly affected by all treatment compared to the control group. However, there was an increase in BW in all treated groups compared to the control at the end of experiment (Table 1). From this study, black cumin seed supplementation has caused no adverse effects with any of the levels used in the experiment. The effects of dietary black cumin on body weight and feed conversion ratio of the chickens are different from studies by others. El-Bagir et al. (2006) showed that dietary black cumin supplementation at the level of 1% or 3% significantly ($P < 0.01$) increased final BW of laying hens. However, other studies showed that addition of black cumin seeds into the diet significantly decreased BW of the chickens (El-Sheikh et al., 1998; Akhtar et al., 2003; Majeed et al., 2010). On the other

Table 1 Effect of dietary feeding treatment on broiler's body weight.

Treatment	Days					
	1	7	14	21	28	35
Body weight (g)						
Control	45.3	143.1	354.6	692	1068	1494
BCS 0.7%	45.2	139.6	355.2	654	1035	1527
BCS 1.4%	45.2	145.7	353.6	674	1097	1584
BCS 2.1%	45.1	150.2	379.4	696	1136	1660
BCS 2.8%	45.1	138.1	348.2	670	1084	1609
SEM	0.07	3.96	9.74	20	30	50.7
Source of variation						
Treatment	0.4272	0.2440	0.2237	0.5731	0.2441	0.1928

Table 2 Effect of dietary feeding treatment on broiler's weight gain.

Treatment	Weeks				
	Week 1	Week 2	Week 3	Week 4	Week 5
Weight gain (g/d)					
Control	13.96	30.22	48.18	53.70	60.98
BCS 0.7%	13.48	30.76	42.70	54.52	70.20
BCS 1.4%	14.34	29.74	45.70	60.40	69.60
BCS 2.1%	15.02	32.74	45.32	62.68	75.00
BCS 2.8%	13.40	30.02	46.02	59.14	75.02
SEM	056	1.02	2.12	2.98	3.77
Source of variation					
Treatment	0.2339	0.2712	0.5085	0.1951	0.0930

Table 3 Effect of dietary feeding treatment on broiler's feed intake.

Treatment	Weeks				
	Week 1	Week 2	Week 3	Week 4	Week 5
Feed intake (g/d)					
Control	26.58	47.62	84.88[a]	118.88	129.60
BCS 0.7%	27.10	45.94	74.56[b]	108.00	138.08
BCS 1.4%	29.24	49.24	73.04[b]	122.34	121.72
BCS 2.1%	29.58	50.62	80.74[ab]	126.38	144.60
BCS 2.8%	25.04	47.40	76.58[ab]	118.88	134.78
SEM	1.39	3.19	2.69	4.26	6.29
Source of variation					
Treatment	0.1583	0.8616	0.0344	0.0690	0.1516

Different letters (a-b) within a column indicate significant differences at the 0.05 level.

Table 4 Effect of dietary feeding treatment on broiler's feed: gain ratio.

Treatment	Weeks				
	Week 1	Week 2	Week 3	Week 4	Week 5
Feed: gain ratio (g:g)					
Control	1.67	1.57	1.77	2.25	2.13[a]
BCS 0.7%	1.75	1.49	1.76	2.00	1.99[ab]
BCS 1.4%	1.72	1.66	1.60	2.03	1.75[b]
BCS 2.1%	1.66	1.55	1.80	2.03	1.93[ab]
BCS 2.8%	1.65	1.58	1.68	2.03	1.81[b]
SEM	0.06	0.09	0.08	0.12	0.08
Source of variation					
Treatment	0.7620	0.7645	0.3962	0.5699	0.0351

Different letters (a-b) within a column indicate significant differences at the 0.05 level.

Table 5 Effect of dietary feeding treatment on broiler's total gain, total and feed: gain ratio.

Treatment	Total gain (g)	Total feed (g)	Total feed: gain ratio (g:g)
Control	1449	2853	1.97
BCS 0.7%	1482	2756	1.87
BCS 1.4%	1539	2769	1.80
BCS 2.1%	1615	3024	1.88
BCS 2.8%	1564	2819	1.81
SEM	50.7	81.26	0.06
Source of variation			
Treatment	0.1929	0.1779	0.3378

hand, the results of the present study showed that supplementation of the diet with black cumin did not negatively influence final BW of the broiler chickens significantly. In fact, there was some improvement though non-significant while feed intake and feed gain ratio increased significantly at the third and fifth weeks, respectively. Shewita and Taha (2011) showed that inclusion of *N. Sativa* in the diets of broiler chickens improved body weight and FCR at a lower dose while a higher inclusion rate showed no significant differences in comparison with the control group. The findings in the present study with regard to the performance are in agreement with those of Majeed et al. (2010) and Nasir and Grashorn (2010). In contrast to the present study, however, El-Nattat and El-Kady (2007) reported that supplementation of black cumin meal at the level of 17% into the diet decreased broiler chicken performance. Similar findings were reported by Abbas and Ahmed when they used BCS at a level of 1% or 2%.

3.2. Immune-responsiveness to vaccinal viruses

Means ± SD antibody titers against NDV, IBV and IBDV were determined using Elisa test kits (IDEXX Laboratories, Inc. One IDEXX Drive Westbrook, Maine 04092 United States). The data showed increase in antibody titer ($P < 0.05$) for NDV vaccine with the addition of BCS to broiler diets in the fourth weeks for group C compared to other groups (Fig. 1). However, antibody titer against IB was not significantly different between the groups except at the fourth and fifth weeks when group D had reduced antibody titer against IBV. Antibody titers against IBDV were significantly increased in group C at the fourth and fifth weeks in comparison to other groups. These results agree with those of Shewita and Taha (2011) who reported significant dose-dependent improvement in antibody titer against NDV. At the same time, Al-Beitawi et al. (2009) showed that antibody titers against NDV and IBDV increased significantly with BCS. Using BCS at the level of 40 g/k^{-1} enhanced antibody production against NDV and IBDV in broiler chickens (Durrani et al., 2007). Moreover, using black cumin oil significantly enhanced the immune system through increased lymphocyte production, and inhibited development of advanced dysplastic changes after topical application of DMBA (7,12-Dimethylbenz(a) anthracene) in hamsters to induce immune-suppression (Al-Jawfi et al., 2008). However, the total immunoglobulin levels were significantly ($P < 0.05$) higher than those of the control group when fed BCS based on that finding and the low cost of BCS addition with immune-simulative effect of BCS, Dorucu et al. (2009) recommended its use in fish feed to reduce mortalities caused by some pathogens. The present results disagreed with the finding of (Jang, 2011) who recorded no significant effects on immunity parameters except in heterophile: lymphocyte ratio.

3.3. Lymphoid organs' evaluation

The thymus at 21 day old chickens showed no significant difference in weight between all groups at that age with a similar effect shown in group A with the control. Other groups were lower but not significantly as compared with the control group. However, at 35 days of age there was no significant difference between all groups except that groups B, C and D have a higher percentage than groups A and control. Meanwhile, the bursa at 21 days showed no significant difference between all groups and the controls. At 35 days of age, the bursa also showed no significant difference between all groups post-vaccination. Similarly, there was no significant difference between spleen weight ratio with BW for other groups at 21 days of age and at 35 days of age. No significant difference was recorded between all groups for all lymphoid organs tested. El-Deek et al. (2009) reported no significant difference in lymphoid organs as an indication of immune response due to feeding different dietary levels of BCS whether in the spleen or bursa of Fabricius weight percentage and this result agrees with our finding. However, the findings of Shewita and Taha (2011) disagreed with the present results which showed improved dose dependant bursa and thymus weight.

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