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Effects of clove powder supplementation on performance, blood biochemistry, and immune responses in broiler chickens

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Abstract

The goal of this study was to evaluate the effects of clove *(Syzygium aromaticum)* supplementation levels on performance, blood parameters, and immune response of broilers. A total of 336 one-day-old broilers were assigned to one of eight treatments: 1% to 6% clove supplementation levels, a positive control (vaccinated) and a negative control (unvaccinated). Bodyweight gain, feed intake, feed conversion ratio, serum protein profile, and immune responses were measured weekly. Lymphoid organs were weighed at 21 and 35 days. Levels of dietary clove between 2% and 6% supplementation resulted in a gradual decrease in weight gain and feed intake with an increase in feed conversion ratio at two and three weeks, whereas those that received 4% to 6% supplementation had reduced weight gain and the 3% to 6% supplemented animals consumed the least amount of feed at four and five weeks. High levels of clove supplementation (4%, 5%, and 6%) resulted in reduced total serum protein and albumin and the greatest activity of aspartate aminotransferase at 35 days. Antibodies to viruses against which the birds had been vaccinated were not affected by different levels of clove supplementation compared with the positive control. However, they were increased in birds in the negative control group without vaccination. Relative weights of lymphoid organs were not affected by any treatments at 21 and 35 days. These results indicate that clove supplementation at levels greater than 2% can lead to negative effects on performance without improving the health of the liver and immune responsiveness of broilers.

Keywords: *Gallus domesticus*, serum biochemical, enzymes, antibody titers, lymphoid organs
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Introduction

Antibiotics have been used for many years in poultry feed to promote growth and to discourage or eliminate specific pathogenic microorganisms (Gadde *et al.*, 2017). However, the poultry industry is currently moving toward the reduced use of antibiotics because of increased concern about antibiotic-resistant bacteria and antibiotic residue in meat and eggs (Mashayekhi *et al.,* 2018). The European Union (EU) issued a ban on the use of antibiotics in animal feed in 2006 (Belal *et al.*, 2018). However, researchers contend that herbs, medicinal plants and spices may be alternatives to antibiotics for promoting animal health (Vijayasteltar *et al*., 2016; Al-Shammari *et al*., 2017). Cloves, cardamom, anise, ginger, black pepper, and cumin are a group of aromatic plants that are used widely due to their positive effects on growth and health of poultry, probably as a result of their immune stimulatory properties (Chowdhury *et al*., 2018; Kunnumakkara *et al*., 2018).

Cloves *(Syzygium aromaticum)* are considered one of the most versatile spices. They contain a large number of biologically active compounds, such as eugenol, eugenol acetate, and β-caryophyllene (Jimoh *et al.*, 2017). Eugenol is the most biologically active compound in cloves and makes up 70–80% of clove oil (Al-Shaikh & Perveen, 2017). Many studies have evaluated the effects of clove powder (CLP) on performance, immune response, blood parameters, and lymphoid organs in broiler chickens (Mustafa, 2016; Mahrous *et al.*, 2017).

In this study, the authors hypothesized that the use of various levels of CLP might have a differential effect on the performance, blood biochemistry and immune responses in broiler chickens. Therefore, the

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purpose of this study was to evaluate the effects of various levels of CLP on performance, immune responses, blood parameters, and lymphoid organs in broiler chickens.

Materials and Methods

Clove seeds were purchased from a herbal shop in Riyadh City, Saudi Arabia. It was ground into powder at the College of Food and Agriculture Sciences, King Saud University, Kingdom of Saudi Arabia. The CLP was then added at various levels of concentration in broiler diets. Thus, the CLP used in the study was derived from dry buds of clove.

Three hundred and thirty-six one-day-old mixed-sex broiler chicks (Ross 308) were obtained from a commercial hatchery. Based on similar bodyweight, all chicks were divided randomly into 48 replicates (seven birds per replicate). Feed and water were made freely available to birds during the entire experimental period. The birds were housed in battery-powered electrically heated replicates, with an initial temperature of 35 °C, which was gradually reduced to reach 22 °C at 21 days old. It was then maintained at this temperature for the remainder of the 35-day period. The corn-soya-based diets were formulated according to the recommendations of the National Research Council (NRC, 1994) and Ross management guide, including a starter (23% crude protein and metabolizable energy 3000 kcal/kg feed) from 0 to 14 days), and finisher (20% crude protein and metabolizable energy 3100 kcal/kg feed) from 15 to 35 days (Table 1). The diets were supplemented with six levels of CLP (1%, 2%, 3%, 4%, 5%, and 6%, based on their basal diet) to form six clove treatment diets along with a positive control (vaccinated birds without CLP) and a negative control (unvaccinated birds without CLP). All treatments and control diets were allocated to six replicates/cages of birds.

Table 1 Composition and calculated nutrient content (%) of starter and finisher diets for broiler chickens

Ingredients	**Experimental diet**	
	Starter (0–14 days)	**Finisher (15–35 days)**
Yellow corn	57.25	58.60
Soybean meal	30.00	30.80
Wheat bran	0.00	2.50
Corn gluten	6.00	0.00
Choline CL 60	0.05	0.05
Palm oil	2.19	0.00
Fat	0.00	4.40
Dicalcium phosphate	2.30	1.94
Limestone	0.70	0.57
Salt	0.40	0.21
Premix[a]	0.50	0.50
DL-Methionine	0.18	0.17
Lysine-HCL	0.32	0.14
Threonine	0.11	0.13
Calculated analysis		
Metabolizable energy (Kcal/kg)	3000	3100
Crude protein (%)	23	20
D-Lysine (%)	1.28	1.13
Total sulphur amino acids (%)	0.95	0.86
Calcium (%)	0.96	0.84
D-Threonine (%)	0.86	0.74
Non-phytate P (%)	0.47	0.41

[a] vitamin A: 2 400 000 IU; vitamin D: 1 000 000 IU; vitamin E: 16 000 IU; vitamin K: 800 mg; vitamin B1: 600 mg; vitamin B2: 1 600 mg; vitamin B6: 1 000 mg; vitamin B12: 6 mg; niacin: 8 000 mg; folic acid: 400 mg; pantothenic acid: 3 000 mg; biotin: 40 mg; antioxidant: 3000 mg; cobalt: 80 mg; copper: 2000 mg; iodine: 400; iron: 1 200 mg; manganese: 18 000 mg; selenium: 60 mg; zinc: 14 000 mg

The chickens were vaccinated by nasal instillation against Newcastle disease at 5 and 22 days of age (HB1 and LaSota strains). Similarly, they were vaccinated against infectious bronchitis (H120 strain). They were vaccinated against infectious bursal disease (D78) by nasal instillation at 14 days of age. The vaccinations were based on recommendations of the Ministry of Agriculture in Saudi Arabia.

The weight of chicks and their feed consumption were recorded weekly for each replicate. The average body weight gain (BWG) and feed conversion ratio (FCR) were calculated for each treatment group. The mortality rate was recorded daily.

At 7, 14, 21, 28, and 35 days, 3 ml blood was collected from the wing vein (brachial vein) from 18 birds per treatment and centrifuged at 3000 rpm for 15 min. Serum was separated and stored at -80 °C until analysis was performed. Serum total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured using reagent kits according to the manufacturer's instructions (Randox, Laboratories, Ltd., Crumlin, UK) via a spectrophotometric analyser (UDICHEM 310, United Diagnostics Industry, Dammam, Kingdom of Saudi Arabia). To determine serum globulin, albumin concentration was subtracted from serum total protein according to Albaadani et al. (2018). Enzyme-linked immunosorbent antibody assay (ELISA kits were used according to the manufacturer's instructions (IDEXX, Europe B.V., Hoofddorp, The Netherlands) for lymphoid organ measurements included antibody production against the vaccinated viruses (NDV, IBV, and IBDV).

At 21 and 35 days of age, six birds per treatment were randomly selected based on the average weight of birds subjected to the same treatment and slaughtered. The carcass was dissected to separate the lymphoid organs (thymus, bursa, and spleen) and weighed to calculate the percentage of the thymus, bursa, and spleen to live bodyweight.

The experiment was conducted in a completely randomized design. The following statistical model was used for the experiment:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: μ is the general mean
T_i is the effect of treatment ($i = 1, \ldots, 8$)
e_{ij} is the random error associated with Y_{ij} observation

The data were analysed using the general linear model procedure of SAS (SAS Institute Inc., Cary, NC., USA). The statistical differences between means were tested using Tukey's test (P <0.001). All values were expressed as statistical means ± standard error of the mean (SEM).

Results and Discussion

Effects of all dietary treatments on performance are presented in Table 2. In the current study, the inclusion of various levels of CLP from 2% to 6% resulted in a gradual decrease (P <0.0001) in bodyweight gain (BWG) and daily feed intake (FI) with a gradual increase (P <0.0001 and P =0.015, respectively) in FCR at two and three weeks old, compared with those of 1% CLP and the controls (positive and negative). However, the inclusion of CLP at 4% to 6% caused a decline in daily weight gain, as well as when 3% to 6% of CLP was added to feed intake, compared with other treatments, without any change in FCR in all dietary treatments at 4 and 5 weeks old.

An increased level of cloves, beyond 2% of the diet, led to a negative effect on performance, which may have been caused by an adverse effect on the palatability of the feed. However, the current results agree with those of Mohammadi *et al.* (2014), who found that broilers supplemented with clove oil (100 mg/kg, 300 mg/kg, and 500 mg/kg) did not grow linearly and had decreased growth performance and total feed intake with the addition 500 mg of cloves/kg of diet. Similarly, Agostini *et al.* (2012) used various levels of clove oil (100, 200, 1000, and 2500 mg/kg), which exhibited decreased growth performance at 1000 and 2500 mg/kg. In another study by Mukhtar (2011), clove oil at 200, 400, and 600 mg/kg in the diet of birds improved growth performance. In a recent study by Mahrous *et al.* (2017), no significant differences in growth performance (BWG and FCR) were observed when broiler chickens were fed clove bud supplements at a rate of 0.5 and 1.0 g/kg diet, but performance deteriorated in groups supplemented with higher levels of cloves (1.5 g/kg diet).

Table 2 Effects of clove powder supplementation on performance of broiler chickens

Days	Diet[1]								SEM[2]	*P*-value
	1	2	3	4	5	6	C+	C-		
Bodyweight gain (BWG, g/day)										
1–7	16.79^{ab}	16.31^{b}	11.93^{c}	11.46^{c}	9.98^{d}	9.50^{d}	17.85^{a}	16.96^{a}	0.456	<.0001
8–14	36.72^{a}	31.41^{b}	28.43^{b}	24.08^{c}	19.69^{d}	16.64^{d}	39.08^{a}	37.77^{a}	1.140	<.0001
15–21	58.58^{a}	48.83^{b}	42.70^{c}	36.94^{d}	30.46^{e}	31.59^{e}	60.37^{a}	61.69^{a}	1.822	<.0001
22–28	76.47^{bc}	82.74^{ab}	80.84^{ab}	68.98^{c}	56.90^{d}	55.69^{d}	82.48^{ab}	91.96^{a}	4.002	<.0001
29–35	85.07^{a}	88.60^{a}	82.00^{a}	71.58^{b}	67.77^{b}	56.47^{c}	88.72^{a}	92.94^{a}	3.595	<.0001
Feed intake (FI, g/day)										
1–7	20.8^{b}	19.5^{c}	18.6^{cd}	19.1^{c}	17.4^{d}	17.4^{d}	22.0^{a}	22.2^{a}	0.415	<.0001
8–14	47.7^{a}	42.0^{b}	39.3^{bc}	37.7^{c}	32.9^{d}	30.5^{d}	49.9^{a}	50.7^{a}	1.199	<.0001
15–21	82.7^{a}	72.7^{b}	66.3^{c}	58.1^{d}	48.4^{e}	46.1^{e}	84.45^{a}	86.0^{a}	1.867	<.0001
22–28	118.6^{abc}	117.0^{bc}	112.3^{c}	100.8^{d}	86.4^{e}	80.4^{e}	127.1^{a}	123.9^{ab}	2.823	<.0001
29–35	140.0^{ab}	146.8^{ab}	138.6^{b}	122.5^{c}	113.2^{c}	99.6^{d}	153.5^{a}	145.5^{ab}	4.529	<.0001
Feed conversion ratio (FCR)										
1–7	1.24^{d}	1.20^{d}	1.56^{c}	1.68^{bc}	1.75^{ab}	1.84^{a}	1.24^{d}	1.31^{d}	0.046	<.0001
8–14	1.30^{d}	1.34^{d}	1.39^{d}	1.57^{c}	1.68^{b}	1.84^{a}	1.30^{d}	1.33^{d}	0.036	<.0001
15–21	1.42^{bc}	1.49^{abc}	1.57^{ab}	1.57^{ab}	1.61^{a}	1.49^{abc}	1.42^{bc}	1.37^{c}	0.050	0.0151
22–28	1.59	1.42	1.40	1.47	1.53	1.47	1.54	1.39	0.070	0.4050
29–35	1.64	1.66	1.70	1.73	1.68	1.78	1.65	1.65	0.043	0.3056

[1] 1 to 6 indicates the percentage of clove powder in the diet, C+ indicates the positive control group (vaccinated and not receiving clove powder) and C- indicates the negative control group (unvaccinated and also not receiving clove powder)
[2] SEM: standard error of means for treatment effect
[a- e] Values within rows with different superscripts are significantly different (P <0.05)

Effects of dietary CLP on total protein, albumin, and globulin in serum of the broilers are shown in Table 3. Concentrations of serum total protein in all treatments were similar at 7 and 14 days old. However, the concentration of serum total protein was significantly reduced at day 21 (P =0.04) in groups that received high levels of CLP at 5% and 6% of diet, compared with birds in groups that received 1%, 2%, and 3% of CLP. Serum total protein concentration decreased significantly (P =0.03) in comparison with groups that received 1% CLP against the same treatment groups (5% and 6% CLP/kg of diet) at day 28. In addition, serum total protein concentration decreased significantly (P =0.02) in treatment groups that received high levels of CLP (4, 5, and 6% of diet) compared with birds in treatment groups that received 3% of CLP in the diet at day 35. However, birds in all treatment groups did not differ significantly compared with the control groups (positive and negative control). Birds that received 5% and 6% of CLP showed reduced albumin levels compared with the positive control. However, birds that received lower levels of supplementation had similar levels of serum albumin and did not differ from either control at 35 days of age. Albumin levels in all treatments were not influenced at younger ages (7, 14, 21, and 28 days of age). Levels of globulin were not affected by treatments at any age (P >0.10). These results agree with those of other investigators, who reported that total serum protein, albumin, and globulin levels of birds were not affected by 0.5% CLP compared with those of the control group (Tariq *et al.*, 2014). Furthermore, Chowdhury *et al.* (2018) used dietary clove bud oil at 0.6% and did not report a significant influence on serum total protein in broiler chickens.

Many medicinal herbs and plants have similar effects on the blood biochemical profile in poultry. For example, Hassan and Awad (2017) stated that 0.2%, 0.5%, and 0.8% dietary supplementation with thyme powder in broilers did not affect serum total protein, albumin, and globulin when compared with that of the control group. Saeid et al. (2010) reported that 0.4% and 0.6% of ginger had no significant effect on serum total protein, albumin, and globulin compared with that of the control group in broilers. However, Natsir et al. (2017) showed that a mix of garlic and Phyllanthus niruri at 1.2% of the broiler diets had no significant effects on serum globulin, whereas significant differences were observed in serum total protein and albumin compared with the control group. Furthermore, Jafari et al. (2011) reported that dietary garlic powder supplementation at the levels of 1% and 3% had no effects on serum globulin in broiler chicks, but it tended to decline with age.

Table 3 Effects of clove powder supplementation on serum total protein, albumin, and globulin in broiler chickens

Days	Diet[1]								SEM[2]	*P*-value
	1	2	3	4	5	6	C+	C-		
Total protein (g/dl)										
7	3.29	3.37	3.21	3.33	3.14	3.14	3.21	3.17	0.07	0.32
14	3.10	3.28	3.36	3.37	3.29	3.43	3.36	3.39	0.08	0.44
21	3.29[a]	3.35[a]	3.37[a]	3.24[ab]	3.01[b]	3.00[b]	3.10[ab]	3.16[ab]	0.08	0.04
28	3.55[a]	3.49[ab]	3.45[ab]	3.42[ab]	3.31[b]	3.34[b]	3.45[ab]	3.44[ab]	0.06	0.03
35	3.32[ab]	3.27[ab]	3.51[a]	3.11[b]	3.12[b]	3.06[b]	3.23[ab]	3.20[ab]	0.07	0.02
Albumin (g/dl)										
7	1.89	1.95	1.90	1.91	1.80	1.81	1.94	1.83	0.05	0.39
14	1.77	1.76	1.68	1.88	1.88	1.76	1.86	1.74	0.05	0.11
21	1.95	1.93	1.92	1.85	1.88	1.91	1.90	1.88	0.04	0.54
28	2.12	2.19	1.99	1.92	1.98	1.89	2.00	2.07	0.06	0.06
35	1.89[ab]	1.87[ab]	1.83[ab]	1.88[ab]	1.78[b]	1.77[b]	2.01[a]	1.95[ab]	0.04	0.02
Globulin (g/dl)										
7	1.37	1.39	1.72	1.56	1.24	1.27	1.39	1.37	0.13	0.42
14	1.46	1.33	1.89	1.51	1.58	1.49	1.44	1.73	0.17	0.37
21	1.35	1.41	1.41	1.36	1.01	1.11	1.40	1.24	0.14	0.31
28	1.44	1.42	1.53	1.53	1.47	1.37	1.52	1.32	0.10	0.43
35	1.27	1.45	1.42	1.49	1.12	1.23	1.18	1.43	0.15	0.13

[1] 1 to 6 indicates the percentage of clove powder in the diet, C+ indicates the positive control group (vaccinated and not receiving clove powder) and C- indicates the negative control group (unvaccinated and also not receiving clove powder)
[2] SEM: standard error of means for treatment effect
[a- b] Values within rows with different superscripts are significantly different (P <0.05)

Liver enzyme activity levels were not affected (P >0.05) by treatments at all times of collection (7, 14, 21, 28, and 35 days old), except that AST activity was greatest in birds that received high levels of cloves at 5% and 6% compared with all other treatments at 35 days old (Table 4). The AST enzyme is one of the biomarkers of liver functions. Thus, the high AST levels observed in treatment groups that received 5% and 6% levels of CLP per kg of diet may be an indication that CLP at those concentrations can adversely affect liver function, and damage often results in abnormally heightened levels of liver enzymes (Kaplan *et al*., 2003). There were no studies on the effects of inclusion of high levels of CLP in broiler chicken diets on serum biochemical parameters, including total protein, albumin, globulin, AST, ALT, and ALP. Many researchers have studied lower dietary levels of CLP compared with the current study. For example, Mahrous *et al.* (2017) used concentrations of CLP at 0.5, 1.0, and 1.5 g/kg in the diet of broiler chickens and found that ALT activity was not significantly affected at the third and fifth weeks. Mustafa (2016) showed no significant differences in AST, ALT, and ALP enzyme concentrations when broiler chickens were fed mixed essential oils (clove, anise, and caraway) at a rate of 200, 400, and 600 mg/kg feed. However, the ALP enzyme proportion was slightly decreased at 600 mg/kg.

In other species, dietary supplementation with cloves has had mixed effects. Hoseini et al. (2011) used 300, 500, and 700 mg/L of CLP and observed no effect on serum levels of AST, ALT, and ALP in beluga sturgeon (Huso huso). Moreover, Vijayasteltar et al. (2016) reported that CLP inclusion at 0.25, 0.50, and 1.0 g/kg in the diet of rats did not affect AST, ALT, and ALP activity. In another study, Velisek et al. (2005) used clove oil at 0.3, 0.5, 0.7, 0.9, and 1.1 g/L and reported unchanged AST, ALT, and ALP in carp.

Kazi et al. (2017) also used CLP at 1 g/kg and observed no effects on AST, ALT, and ALP activity in rabbits. Moreover, the actions of AST and ALT showed no significant differences for all treatments in rats fed clove extract at 250 and 500 mg/kg diet (Saeed et al., 2017). In another study, Mustafa (2016) showed no significant differences in AST, ALT, and ALP enzyme concentrations in broiler chickens fed mixed essential oils (cloves, anise, and caraway) at a rate of 200, 400, and 600 mg/kg feed. However, ALP activity was slightly decreased in broiler chickens fed 600 mg/kg feed compared with that of the other groups. An experiment conducted on the fish Labeo rohita by Asimi & Sahu (2016) indicated that the concentrations of ALT, AST, and ALP enzymes in liver and muscle were decreased in groups fed 0.5 and 1.0% clove extract compared with that of the control group. Furthermore, Gashlan & Al-Beladi (2017) observed a decrease in liver AST, AST, and ALP in rats fed clove oil at 300 and 600 mg/kg feed compared with the control group.

Table 4 Effects of clove powder supplementation on liver enzymes of broiler chickens

Days	Diet[1]								SEM[2]	*P*-value
	1	2	3	4	5	6	C+	C-		
ALT (IU/L)										
7	8.09	6.64	7.74	7.86	7.86	8.00	7.94	7.61	1.16	0.97
14	7.92	6.94	9.01	9.09	9.42	10.44	9.57	9.30	0.99	0.32
21	8.36	7.88	9.82	9.50	10.36	10.38	9.19	9.76	0.97	0.60
28	9.17	8.45	9.44	9.77	10.92	11.08	10.34	10.06	0.69	0.08
35	8.06	8.01	8.72	10.49	11.07	11.10	10.06	10.34	0.98	0.18
AST (IU/L)										
7	128.1	131.7	130.6	133.9	137.7	139.7	133.5	127.3	6.05	0.55
14	134.3	133.6	150.1	150.9	157.8	160.2	148.7	130.0	7.07	0.06
21	126.1	130.4	140.4	139.4	145.6	148.1	131.3	142.3	5.70	0.24
28	133.5	145.6	145.6	145.6	150.0	156.9	134.2	130.7	6.43	0.20
35	160.4[b]	170.3[b]	180.6[ab]	181.2[ab]	194.4[a]	193.3[a]	166.8[b]	166.2[b]	6.62	0.01
ALP (U/L)										
7	1374	1420	1464	1452	1480	1490	1416	1400	60.15	0.91
14	1400	1364	1336	1412	1471	1473	1345	1395	63.60	0.85
21	1291	1285	1366	1340	1398	1396	1338	1390	75.95	0.95
28	1190	1196	1248	1315	1309	1330	1238	1236	57.48	0.59
35	995	1082	1124	1159	1223	1232	992	1017	59.01	0.09

[1] 1 to 6 indicates the percentage of clove powder in the diet, C+ indicates the positive control group (vaccinated and not receiving clove powder) and C- indicates the negative control group (unvaccinated and also not receiving clove powder)
[2] SEM: standard error of means for treatment effect
[a- b] Values within rows with different superscripts are significantly different ($P < 0.05$)

Antibody titers against NDV vaccine at 28 days were significantly ($P < 0.05$) greater in 1%, 2%, and 3% CLP groups compared with both the positive and negative controls (Table 5). However, at 14 and 35 days old, greater ($P < 0.05$) antibody titers were observed among the vaccinated birds compared with the unvaccinated birds (negative control). Nonetheless, the various levels of CLP did not show significant effects on the titers of antibodies that are specific to NDV compared with the positive control in the first and third weeks old (Table 5).

Antibody titers against the IBV vaccine at 28 and 35 days showed a significant ($P < 0.05$) difference between the vaccinated birds and the unvaccinated birds, whereas the negative control had a lower antibody titer compared with that of other treatments. However, no significant ($P > 0.05$) differences were observed between the broilers that fed on CLP and the positive control group (Table 5).

Table 5 Effects of clove powder supplementation on specific antibody titers of broiler chickens

Days	Diet[1]								SEM[2]	*P*-value
	1	2	3	4	5	6	C+	C-		
Newcastle disease virus										
7	3.97	3.96	3.99	3.89	3.85	3.95	3.93	3.80	0.09	0.24
14	3.59[a]	3.53[a]	3.49[a]	3.43[a]	3.33[a]	3.46[a]	3.43[a]	3.00[b]	0.11	0.001
21	2.57	2.68	2.55	2.55	2.48	2.30	2.50	2.18	0.11	0.08
28	2.12[a]	1.11[a]	2.12[a]	2.01[ab]	1.82[b]	2.78[b]	1.76[b]	1.13[c]	0.10	<.0001
35	3.62[a]	3.60[a]	3.62[a]	3.57[a]	3.57[a]	3.41[a]	3.28[a]	1.00[b]	0.12	<.0001
Infectious bronchitis disease virus										
7	2.86	2.85	2.89	2.90	2.87	2.78	2.89	2.50	0.11	0.07
14	2.36	2.29	2.17	2.27	2.11	2.19	2.22	2.15	0.09	0.18
21	1.74	1.74	1.76	1.66	1.67	1.55	1.66	1.33	0.07	0.49
28	2.06[a]	2.00[a]	1.99[a]	2.03[a]	2.01[a]	1.91[a]	1.98[a]	1.06[b]	0.06	<.0001
35	2.33[a]	2.40[a]	2.28[a]	2.31[a]	2.20[a]	2.28[a]	2.27[a]	0.96[b]	0.10	<.0001
Infectious bursal disease virus										
7	3.17	3.20	3.12	3.13	3.14	3.12	3.11	3.11	0.10	0.89
14	2.49	2.54	2.28	2.14	2.27	2.30	2.30	2.27	0.09	0.07
21	1.85	1.77	1.70	1.76	1.69	1.69	1.67	1.61	0.11	0.67
28	2.02[a]	1.98[ab]	1.91[abc]	1.95[ab]	1.88[abc]	1.86[bc]	1.77[c]	1.31[d]	0.05	<.0001
35	2.22[a]	2.21[ab]	2.19[ab]	2.02[ab]	2.23[a]	2.11[ab]	1.99[b]	1.12[c]	0.07	<.0001

[1] 1 to 6 indicates the percentage of clove powder in the diet, C+ indicates the positive control group (vaccinated and not receiving clove powder) and C- indicates the negative control group (unvaccinated and also not receiving clove powder)
[2] SEM: standard error of means for treatment effect
[a- b] Values within rows with different superscripts are significantly different (P <0.05)

The antibody titers against IBDV were significantly (P <0.05) increased at day 28 in treatments with 1%, 2%, and 4% CLP compared with the positive control group. There was also a significantly (P <0.05) increased antibody titer against IBDV in the 3%, 5%, and 6% CLP, and positive control groups compared with that of the negative control (Table 5).

These results agree with those of other investigators, such as Chowdhury *et al*. (2018), who noted that dietary inclusion of clove bud oil at 0.6% increased the antibody titers against NDV in broiler chickens at the age of 28 and 35 days. Furthermore, Gandomani *et al*. (2014) found that antibody titers against NDV and IBDV were improved in laying hens fed diets supplemented with 0.2 and 0.4% CLP compared with those of the control group. In addition, Mehr *et al*. (2014) showed that dietary clove oil at 0.45 g/kg significantly increased immunoglobulin M and T cells. Similarly, Bello *et al*. (2016) reported that clove is a potent immunomodulatory agent that can improve B cell functions, humoral responses, and immunoglobulin (IgA, IgG, IgM) levels. Furthermore, Mahrous *et al*. (2017) found that the levels of serum immunoglobulin (IgA, IgG, and IgM) were significantly (P <0.05) increased in broiler chickens fed diets supplemented with 1.0% and 1.5% CLP. On the other hand, the exact mechanism by which clove and other phytogenic feed additives stimulate the immune responses in chickens and other animals is mostly unknown (Nimmerjahn & Ravetch, 2010). It is possible that clove and other herbs act as additional bonds with immunoglobulin molecules at the Fc receptors, which stimulate the immune response (Ahmed *et al*., 2013). In contrast to the results of this study, Najafi & Torki (2010) noted that antibodies against NDV did not differ significantly in broiler chickens fed a diet supplemented with 200 mg/kg of clove oil for 42 days. Also, Mehr *et al*. (2014) found that dietary inclusion of clove oil at 0.15, 0.30 g/kg did not affect antibody titers against NDV and avian influenza in broiler chickens. Moreover, Poorghasemi *et al*. (2017) reported that antibody titers against NDV and IBDV were not affected by dietary lemon balm supplementation in broiler chickens. Additionally, Toghyani *et al*. (2011) stated that antibody titers against avian influenza virus and NDV were not significantly different in broiler chickens fed diets supplemented with 2 g/kg and 4 g/kg of cinnamon bark powder. Similarly, Soltan *et* *al*. (2008) showed that the antibody titer against NDV was not significantly different in broiler chickens fed diets supplemented with anise seed compared with that of the control group.

Relative weights of lymphoid organs are presented in Table 6. There were no significant treatment effects on the relative weights of bursa, spleen, and thymus at 21 and 35 days old. These results agree with those of Chowdhury *et al.* (2018), who observed that CLP supplementation at 0.6% concentration did not affect the relative weights of the lymphoid organs. In contrast, Gandomani *et al*. (2014) found that a rise in clove bud powder content resulted in increased relative weight of the spleen of laying hens. This inconsistency may be attributed to the differences in the sources of the clove plants, the levels of CLP, and diet composition or environmental conditions.

Table 6 Effects of clove powder supplementation on the relative weight of lymphoid organs of broiler chickens

Organ weight	Diet[1]								SEM[2]	*P*-value
	1	2	3	4	5	6	C+	C-		
At 21 days of age										
Thymus%	0.41	0.41	0.41	0.39	0.40	0.40	0.41	0.37	0.019	0.810
Bursa%	0.23	0.21	0.23	0.20	0.20	0.19	0.19	0.19	0.018	0.582
Spleen%	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.004	0.571
At 35 days of age										
Thymus%	0.40	0.44	0.43	0.44	0.43	0.43	0.39	0.36	0.025	0.282
Bursa%	0.19	0.16	0.18	0.17	0.16	0.15	0.17	0.16	0.016	0.650
Spleen%	0.10	0.09	0.09	0.10	0.10	0.10	0.09	0.09	0.006	0.325

[1] 1 to 6 indicates the percentage of clove powder in the diet, C+ indicates the positive control group (vaccinated and not receiving clove powder) and C- indicates the negative control group (unvaccinated and also not receiving clove powder)
[2] SEM: standard error of means for treatment effect

Conclusion

An increased level of cloves (greater than 2%) had a negative effect on performance of broiler chickens, which may be attributed to an adverse effect on the palatability of the feed without affecting the health of the liver and immune responsiveness. Further research is essential to clarify the mechanism behind this effect.

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Authors' Contribution

SI supervised the study. SI and EH conceived and designed it. EH conducted the experiment and analysed the samples and the data. EH, MM, and HH contributed in writing and revised the manuscript. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

Conflict of Interest Declaration

There is no conflict of interest.

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