In ovo injection of black cumin (*Nigella sativa*) extract on hatching and post hatch performance of thermally challenged broiler chickens during incubation

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ABSTRACT The objective of this study was to investigate the effects *in ovo* injection of black cumin (BC) extract on chick's quality and response of thermally challenged broiler chickens. A total of 700 hatching eggs of broiler chickens (Marshall) were assigned to 7 treatments of 100 eggs each and incubated using the conventional protocol (37.8°C) for the first 10 d and then exposed to a high temperature (39.6°C) for 6 h daily from day 10 until day 18 of the incubation. At embryonic day 17.5, the eggs were randomly allotted to 7 treatment groups, viz.: eggs without *in ovo* injection (WA), eggs injected with 0.9% saline solution (SA), 3 mg ascorbic acid (AA), 2 mg BC (TB), 4 mg BC (FB), 6 mg BC (SB), and 8 mg BC (EB) extracts. Experiment was laid out in a Completely Randomized Design. After hatching, the chicks were reared separately according to *in ovo* treatments for 8 wk. Data were collected on hatchability, chick quality, internal organs, growth performance, plasma superoxide dismutase, malondialdehyde, and triiodothyronine (T_3). The results showed that the hatchability of the eggs in the AA group was similar to that of SB eggs and higher than that of the other treatment groups. The intestinal weights of SB and EB birds were significantly higher ($P < 0.05$) than those of TB, SA, and WA. The final weights of the birds of SB and AA were higher ($P < 0.05$) than those of other treatments. The feed conversion ratio of the birds of TB and FB was comparable to that of EB and WA but higher than that of SB and AA. At hatch, the creatinine of the birds in SA and WA was similar to that of EB, FB, and TB but higher ($P < 0.05$) than that of AA and SB. Also, the plasma malondialdehyde, T_3, and superoxide dismutase of SB and AA birds were better ($P < 0.05$) than those of the control groups. Overall, it was concluded that 6 mg of BC extract improved the antioxidant status and posthatch performance of thermally challenged broiler chickens.

Key words: physiology, *in ovo* injection, broiler, incubation, antioxidant

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INTRODUCTION

Several attempts have been made to improve the thermo-tolerance of chickens to adapt to heat stress conditions such as heat acclimation, including the use of thermal conditioning (Yahav and Hurwitz 1996; Yahav and Plavnik, 1999; Willemsen et al., 2010; Nyuiadzi et al., 2020). During the last few days of incubation, chicken embryos grow faster, and their rate of nutrient utilization increases (Elwan et al., 2019). However, the nutrients in the eggs are not sufficient at this critical period (Straus and Takemoto, 1991; Foye et al., 2006). The fast growth of chicken embryos is connected with the production of free radicals that are capable of causing oxidative stress (Deeming and Pike, 2013). Oxidative stress results when birds are exposed to thermal stress, disrupting the redox balance of antioxidant and proantioxidant at the expense of antioxidant,

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causing cellular/tissue damage (Barrett et al., 2017). As the antioxidant defense is crucial for the optimal performance of the birds (Surai et al., 1996), efficient antioxidant capability in the embryonic tissues is important to protect the developing embryo against peroxidative damage during embryogenesis and also in readiness for the increase in oxidative stress during postembryonic development (Sies, 1993; Surai et al., 1996) to ensure optimal growth. Embryos may experience thermal stress at the stage of hatching as a result of the heat produced from metabolism and incubator (Tullett, 1990). Ameliorating the antioxidant status of developing embryos can enhance their development, particularly at the late phase of incubation. Thus, the use of *in ovo* feeding of antioxidant constituents may be of immense benefits to the embryo at prehatch and posthatch growth phases (Yigit et al., 2014).

In the recent decades, attention has been given to the use of phytogenic additives as antioxidant constituents and growth promoters from spice, herbs, and their products (Wenk, 2003; Pathak et al., 2016; Oke et al., 2017; Oke 2018) as a result of the several benefits obtained from them. Black cumin (*Nigella Sativa L.*) is an aromatic Ranunculaceae herbaceous plant, and its seeds have been used around the world for its pharmacological actions and medicinal properties for over 2,000 yr. The black cumin seeds have been reported to contain pharmacologically active constituents such as dithymoquinone, carvacrol, thymohydroquinone, thymol, and thymoquinone (Nasir et al., 2005; Al-Saleh et al., 2006). About 24% of the essential oil of black cumin seeds is thymoquinone which has antihistaminic, antibacterial, anti-inflammatory, and antioxidant properties (Arslan et al., 2005).

The use of *in ovo* feeding has been receiving attention in the recent years because beneficial biochemical and physiological balances, including improved oxidative protection, can be offered to the embryos during incubation (Malheiro et al., 2012). The technique is effective in offering nutrient directly to the embryos at a cheaper cost than dietary additives (Kadam et al., 2013). *In ovo* injection technology can improve the hatching quality and subsequent growth of broiler chickens (Ipek et al., 2004). This technique can be used to deliver bioactive compounds with antioxidant properties to the developing embryos. Several nutrients, including L-carnitine, carbohydrates, nucleotides, amino acids, creatine, egg white, peptides, electrolytes, vitamins, and plant extracts, have been investigated *in ovo* (Kucharska-Gaca et al., 2017). Several plant extracts, such as moringa, tomato, garlic, savory, and thyme extracts, have been used (Fazli et al., 2015; Saki and Salary, 2015; N'nanle et al., 2017).

As avian embryos are subjected to thermal stress by the excessive production of environmental and metabolic heat during the later hatching phase (Tullett, 1990), *in ovo* injection of black cumin may be of tremendous benefits by improving the chick quality and the posthatch performance. Mortality of hatchlings may also be improved. The injection of the extract may be of benefits also to the hatchling as they migrate from chorioallantoic respiration to pulmonary respiration. To our knowledge, there is no information on the *in ovo* injection of black cumin extract on thermally challenged chickens. The aim of this study was therefore to investigate the *in ovo* injection of black cumin extract on chick quality, response of thermally challenged broiler chickens.

MATERIAL AND METHODS

Ethical Approval

This research was carried out in accordance with the Institutional Animal Ethics Committee guidelines of the Federal University of Agriculture, Abeokuta, Nigeria. The hatching eggs and the chicks in the experiment were provided proper care and management without unnecessary discomfort.

Experimental Animals and Management

A total of 700 hatching eggs of Marshall broiler chickens were assigned to 7 treatments of 100 eggs each, set in an incubator (Avida G4; Chick Master) with a temperature of 37.8°C and relative humidity of 60% from day 1 to 10 and then exposed to a high temperature (39.6°C) for 6 h daily from day 10 until day 18 of the incubation period. On the 18th d of incubation, eggs were transferred from the setter tray to the hatcher basket, with an incubation temperature of 37.5°C and relative humidity 65 to 70%.

Black Cumin Seed Extraction

Nigella sativa dry seeds, purchased from a reputable dealer, were cleaned, dried, and mechanically grounded to the powdered form, and this was soaked in a liquid containing 96% ethanol and distil water at a ratio of 50:50 for 72 h. The homogenized mixture obtained was filtered thoroughly using sterile filter paper, and the pure solution obtained was evaporated with rotary evaporator to render the extract alcohol free, and the extract was kept in refrigerator at 4°C until used (Musa et al., 2004).

In Ovo Feeding Procedure

At day 17.5 of incubation, the eggs were randomly allotted to 7 treatment groups, viz.: eggs without *in ovo* injection (WA), eggs injected with saline solution (0.9% saline) (SA), eggs injected with ascorbic acid (3 mg/egg) (AA), eggs injected with 2 (TB), 4 (FB), 6 (SB), and 8 mg (EB) of black cumin extract. Saline solution was injected into the eggs to serve as a negative control because a previous study (Mcgruder et al., 2011) showed that there was no difference in hatching traits and posthatch performance between eggs injected with saline solution and intact eggs. Also, *in ovo* ascorbic acid was adopted as a positive control in this study because of the fact that it has been conventionally

used to improve hatching and growth of broiler chickens (Nowaczewski et al., 2012; El-Senousey et al., 2018; Zhu et al., 2019).The extract and ascorbic acid were homogenously dissolved in saline solution. The extract was injected into the air chamber of incubated eggs with evidence of a living embryo. The dose of ascorbic acid was adopted following the recommendation of Zakaria and Al-Anezi (1996). A hole was carefully drilled through the shell above the air chamber by using a needle, the eggs were injected through the air sac with 0.5 mL of solution using a 24G hypodermic needle (25-mm long), and the pinpoint hole was sealed using wax (Zhai et al., 2011). The eggs were placed back in the hatcher at the temperature of 37.5°C and relative humidity of 65 to 70%.

Post Hatch Chicks' Management and Feeding

The hatched chicks were reared according to the *in ovo* treatments on a deep litter system, with the floors of the pens covered with 4-cm-deep wood shavings. Rearing temperature of 32°C was used during the first week and then reduced by 2 to 3°C/wk until it got to 24°C. The birds were fed diets formulated to meet NRC (1994) nutrient recommendations for different ages. Feed and fresh water were available for *ad libitum* consumption. The posthatch experiment lasted for 56 d (the average market age of broiler chickens in our region). The birds were subjected to acute heat stress of 35°C for 60 min, to assess their thermotolerance at day 56 of age.

Data Collection

Embryonic Development Index and Chick Quality

Egg Weight Initial weights of eggs were measured before incubation with the aid of a sensitive scale.

Hatchability The percentage hatchability was calculated as the number of chicks hatched per the fertile eggs after candling.

Chick Weights At hatch, the weights of chicks were measured on an electronic weighing balance and expressed in grams.

Chick to Egg Ratio This was calculated from the weight of the eggs and the chicks that hatched from them.

Internal Organs and Breast Muscle Eight chicks per treatment group were euthanized by cervical dislocation, just after hatching. The birds were dissected, and the liver, heart, pectoral muscles, small intestine, left lung, and residual yolk were carefully removed and weighed; the weights were expressed as percentage of the chick weights.

Chicks' Quality Parameters After 510 h of incubation, all hatched chicks were individually weighed and examined macroscopically to identify the different characteristics associated with chick quality, using the score proposed by Tona et al. (2003). The chick length was obtained as described by Wolanski et al. (2007). Chick quality was determined on the basis of physical parameters. These included activity, feathering, condition of eyes, conformation of legs, condition of the navel area, yolk retraction, and status of the membrane.

Growth Performance Feed intake was determined by subtracting the feed left over from the feed offered to birds daily:

$$\text{Feed intake} = \text{Quantity of feed offered} - \text{Quantity of feed left over}$$

$$\text{Average feed intake (g/bird)} = \frac{\text{Feed intake}}{\text{Number of birds per replicate}}$$

Body weights of the birds were taken weekly:

$$\text{Average body weight gain (g/bird)} = \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{Number of birds per replicate}}$$

Feed conversion ratio (FCR) was obtained by dividing the feed intake by the weight gain of the birds:

$$\text{FCR} = \frac{\text{Feed consumed (g)}}{\text{Body weight gain (g)}}$$

Plasma Antioxidants and Thyroid Hormone Blood samples were collected from 2 chicks from each replicate at hatch and week 8 via the jugular vein, into ethylenediamine tetra-acetic bottle, and then centrifuged (3,000 × *g*) for 15 min at room temperature to separate the plasma. Plasma samples were kept at −80°C until analyzed. The concentrations of total superoxide dismutase (**T-SOD**) activity and malondialdehyde (**MDA**) levels in plasma were analyzed using the commercial assay kits from Nanjing Jiancheng Biochemistry Reagent Co. (NJBC; Nanjing, Jiangsu, China; Bai et al., 2017) according to the instructions of the manufacturer. Plasma triiodothyronine (T_3) were also measured by radioimmunoassay (Gupta et al., 1997).

Serum Biochemistry and Haematological Parameters At day 56 of age, 2 birds from each replicate were randomly selected, and 3 mL of blood samples collected via the jugular vein into ethylenediamine tetra-acetic acid and plain bottles for the determination of hematological parameters (pack cell volume, red blood counts, hemoglobin, white blood counts, and the differential counts) and serum metabolites (alanine transferase, aspartate aminotransferase, total protein, albumin, globulin, triglyceride, creatinine, and glucose) according to standard procedures (Jain, 1986). Blood samples in the plain bottles were centrifuged (3,000 × *g*) for 15 min at room temperature to separate the plasma and serum and kept at −20°C.

Thermal Challenge at Slaughter Age At the 56th d, 2 birds per replicate were transferred to a preheated room (35°C) for acute heat stress experiment. The chickens

Table 1. Effect of *in ovo* injection of black cumin extract on hatchability and chick quality of thermally challenged broiler chickens.

Parameters	TB	FB	SB	EB	AA	SA	WA	SEM	*P* value
Hatchability (%)	86.48^{c,d}	87.75^{b,c}	91.93^{a,b}	82.57^{d}	93.53^{a}	85.23^{c,d}	86.07^{c,d}	0.778	0.0001
Egg weight (g)	69.46	69.32	70.09	70.25	69.62	69.63	69.73	0.194	0.8981
Chick weight (g)	49.99	49.12	50.72	51.08	50.43	47.93	47.26	0.517	0.3575
Chick/Egg ratio	0.72	0.71	0.72	0.73	0.72	0.69	0.68	0.007	0.3706
Chick length (cm)	11.25	11.50	12.50	11.75	12.00	11.00	11.00	0.166	0.1268
Rectal temp (°C)	38.88^{a,b}	38.28^{b}	38.33^{b}	38.70^{a,b}	38.58^{a,b}	39.08^{a}	39.00^{a}	0.072	0.0021
Respiratory[1]	61.25	56.25	57.50	65.00	57.50	70.00	71.25	2.220	0.3717
Activity	11.00	10.00	11.00	9.00	11.00	10.00	10.00	0.522	0.9539
Appearance	11.00	12.00	12.00	11.00	12.00	11.00	10.00	0.295	0.4984
Eye	11.00	11.00	11.00	12.00	12.00	11.00	11.00	0.295	0.9117
Leg	12.00	11.00	12.00	12.00	12.00	12.00	11.00	0.198	0.5577
Refracted yolk	9.00	12.00	11.00	9.00	11.00	6.00	6.00	0.940	0.4977
Navel area	12.00	11.00	12.00	12.00	12.00	11.00	11.00	0.238	0.6774
Remaining yolk	10.00	10.00	11.00	10.00	11.00	10.00	9.25	0.418	0.9463

Means within a row with different superscript letters differ significantly at $P < 0.05$.

Abbreviations: AA, 3 mg ascorbic acid; BC, black cumin; EB, 8 mg BC extracts; FB, 4 mg BC; SA, eggs injected with 0.9% saline solution; SB, 6 mg BC; TB, 2 mg BC; WA, eggs without in ovo injection.

[1]Rate/min.

were exposed to acute heat stress at the market age so as to simulate the sudden increase in ambient temperature in tropical environment when a sudden heat wave strikes.

Experimental Design

Data were subjected to analysis of variance in a completely randomized design with *in ovo* treatment as the fixed effect. Means differences were tested at 0.05 level of significance with Tukey correction for multiple comparisons. Data were analyzed with SAS (2008) statistical package.

RESULTS

Table 1 shows the effect of *in ovo* injection of black cumin extract on hatchability and chick quality of thermally challenged broiler chickens during incubation. There was no difference in the hatchability of the eggs of AA and SB, but the hatchability of AA eggs was higher than that of the other treatment groups. The eggs of TB, FB, SA, and WA had a comparable hatchability. There was no difference in most of the quality parameters of the chicks at hatch. The rectal temperature of the chicks was closely related across the treatment, but the rectal temperature of the chicks of FB and SB was lower than that of WA and SA.

Effect of *in ovo* injection of black cumin extract on internal organs and breast muscle of day-old chick is presented in Table 2. *In ovo* injection of black cumin did not influence the relative weights of pectoral muscle, intestinal length, proventriculus, gizzard, liver, residual yolk, and lungs of the chicks. However, heart and intestinal weights were affected. The intestinal weights of SB and EB birds were significantly higher than those of TB, SA, and WA but not different from those of the other treatments. The relative heart weights of the chicks from the SB group were higher ($P < 0.05$) than those of the WA group.

Table 3 shows the effect of *in ovo* injection of black cumin extract on performance of broiler chickens (4–8 wk). The final weights of the birds of SB and AA were similar but significantly higher than those of the other treatments. The final weights of the birds of FB and EB were comparable but higher than those of WA which was similar to those of SA and TB. The weight gain of the birds followed a similar trend. The feed intake of the birds of AA was similar to that of FB and SB. The feed intake of the birds of WA, SA, EB, and TB was similar to that of birds of FB and SB. The FCR of the

Table 2. Effect of *in ovo* injection of black cumin extract on internal organs and breast muscles of day-old chicks.

Parameters	TB	FB	SB	EB	AA	SA	WA	SEM	*P* value
Chick weight (g)	49.99	49.12	50.72	51.08	50.43	47.93	47.26	0.517	0.3575
Pectoral muscle %	3.61	2.90	4.11	5.33	3.59	2.58	2.37	0.336	0.2355
Intestinal weight %	1.59^{b}	1.92^{a,b}	2.44^{a}	2.52^{a}	1.93^{a,b}	1.39^{b}	1.48^{b}	0.123	0.0049
Intestinal length (cm)	8.50	8.50	9.00	11.00	9.00	8.50	8.00	0.355	0.4201
Proventriculus %	0.66	0.74	0.70	0.66	0.73	0.67	0.46	0.035	0.4707
Gizzard %	3.03	4.24	3.35	3.34	3.56	4.14	3.58	0.153	0.3399
Heart %	0.61^{a,b}	0.63^{a,b}	0.75^{a}	0.72^{a,b}	0.74^{a,b}	0.53^{a,b}	0.51^{b}	0.028	0.0173
Liver %	1.85	2.14	2.24	2.35	2.12	1.67	1.49	0.096	0.0798
Residual yolk %	14.50	14.77	13.85	11.03	13.15	15.27	16.34	0.654	0.5217
Left lung %	0.38	0.39	0.34	0.37	0.35	0.44	0.33	0.019	0.8922

Means within a row with different superscript letters differ significantly at $P < 0.05$.

Abbreviations: AA, 3 mg ascorbic acid; BC, black cumin; EB, 8 mg BC extracts; FB, 4 mg BC; SA, eggs injected with 0.9% saline solution; SB, 6 mg BC; TB, 2 mg BC; WA, eggs without in ovo injection.

Table 3. Effect of *in ovo* injection of black cumin extract on performance of broiler chickens at the finisher phase (day 28–day 56).

Parameters	TB	FB	SB	EB	AA	SA	WA	SEM	*P* value
IW(g)	1,112.50^{b,c}	1,125.00^{b,c}	1,337.50^{a}	1,270.00^{a,b}	1,137.50^{b,c}	1,062.50^{c}	1,037.50^{c}	23.257	0.0001
FW (g)	3,200.0^{b,c}	3,300.0^{b}	3,792.5^{a}	3,300.0^{b}	3,735.00^{a}	3,000.0^{b,c}	2,937.50^{c}	64.581	0.0001
BWG (g)	2,087.50^{b,c}	2,175.00^{b}	2,455.00^{a}	2,030.00^{b,c}	2,597.50^{a}	1,937.50^{b,c}	1,900.00^{c}	50.697	0.0001
FI (g)	4,963.8^{b}	5,215.3^{a,b}	5,321.8^{a,b}	4,887.0^{b}	5,722.5^{a}	4,959.5^{b}	4,835.3^{b}	68.017	0.0002
FCR (g/g)	2.38^{b}	2.40^{b}	2.17^{c}	2.41^{a,b}	2.20^{c}	2.57^{a}	2.55^{a,b}	0.030	0.0001

Means within a row with different superscript letters differ significantly at $P < 0.05$.

Abbreviations: AA, 3 mg ascorbic acid; BC, black cumin; BWG, body weight gain; EB, 8 mg BC extracts; FB, 4 mg BC; FCR, feed conversion ratio; FI, feed intake; FW, final weight; IW, initial weight; SA, eggs injected with 0.9% saline solution; SB, 6 mg BC; TB, 2 mg BC; WA, eggs without in ovo injection.

chickens of SA was similar to that of WA and EB but higher than that of other treatment groups. The FCR of the birds of TB and FB was comparable to that of EB and WA but higher than that of SB and AA whose FCR was similar.

Table 4 shows the effect of *in ovo* injection of black cumin extract on serum biochemical parameters of broiler chicks at hatch. The albumin, globulin, and total protein of the chicks were similar across the treatment groups. However, the creatinine of the birds in SA and WA was similar to that of EB, FB, and TB but higher than that of AA and SB whose values were comparable. The ALT of the birds of SA was higher than that of the other treatment groups. The ALT level in the birds of SA was higher than that of the other treatment groups but FB, SB, EB, and AA had similar level. AST of the birds of WA was higher than that of the other treatment groups, while it was higher in the birds of SA than that of TB which also was significantly higher than that of FB, SB, EB, and AA which had similar level. The serum glucose of the birds was closely related, but the birds of SA and WA had higher levels than that of SB. The blood glucose and triglycerides of the birds were somewhat similar, but the birds of SA and WA had higher levels than those of SB.

The effect of *in ovo* injection of black cumin extract on hematological parameters of broiler chicks at hatch is presented in Table 5. Apart from the red blood cell, all the other hematological parameters of the chicks were not influenced by the treatments. The red blood cell value of the chicks of EB was similar to that of TB, FB, SB, and AA but higher than that of SA and WA.

Effect of in ovo injection of black cumin extract on plasma MDA, SOD, and triiodothyronine (T_3) of newly hatched chicks is presented in Table 6. The plasma MDA of the birds of SA and WA was comparable and higher than that of the other treatment groups whose values were alike. The plasma SOD of the birds of SB was comparable to that of TB and AA and higher than that of WA and SA. The triiodothyronine of the chickens in AA was similar to that of TB and SB but higher than that of the other treatments. The birds of TB, FB, EB, SA, and WA had similar plasma T_3.

Table 7 shows the effect of *in ovo* injection of black cumin extract on plasma MDA, SOD, and triiodothyronine (T_3) of broiler chickens at market age. The plasma MDA of the birds of WA was comparable to that of SA and TB groups but lower than that of FB, EB, and AA. The plasma SOD of the birds of SB, FB, and AA was higher than that of SA and WA. The triiodothyronine of the chickens injected with black cumin extract was similar, but the level of T3 of FB birds was higher than that of SA and WA.

Table 8 shows the effect of *in ovo* injection of black cumin extract on hematological parameters of broiler chickens at market age. There was no difference in the hematological parameters of the birds across the treatments.

Table 9 shows the effect of *in ovo* injection of black cumin extract on serum biochemical parameters of broiler chickens at market age. The creatinine kinase and glucose of the birds were not affected after thermal challenge at market age. However, the ALT of the birds

Table 4. Effect of *in ovo* injection of black cumin extract on serum biochemical parameters of broiler chicks at hatch.

Parameters	TB	FB	SB	EB	AA	SA	WA	SEM	*P* value
Creatinine[1]	0.40^{a,b}	0.40^{a,b}	0.35^{b}	0.40^{a,b}	0.35^{b}	0.50^{a}	0.50^{a}	0.018	0.0207
ALT (U/L)	47.00^{c}	36.00^{c,d}	25.50^{d}	33.00^{d}	32.50^{d}	343.50^{a}	169.00^{b}	30.664	<0.0001
AST (U/L)	246.00^{c}	191.50^{d}	163.50^{d}	186.50^{d}	157.00^{d}	348.00^{b}	500.00^{a}	32.467	<0.0001
Total protein[1]	4.00	4.55	4.80	5.10	4.80	3.30	3.50	0.259	0.4581
Albumin[1]	1.50	1.55	1.70	1.35	1.65	1.05	1.10	0.076	0.0497
Globulin[1]	2.50	3.00	3.10	3.75	3.15	2.25	2.40	0.237	0.7471
Glucose[1]	187.50^{a,b}	181.00^{a,b}	158.50^{b}	182.50^{a,b}	162.00^{b}	208.50^{a}	212.00^{a}	5.724	0.0122
Triglycerides[1]	84.00^{a,b}	81.50^{a,b}	60.00^{b}	79.50^{a,b}	73.50^{a,b}	114.00^{a}	114.50^{a}	5.676	0.0127

Means within a row with different superscript letters differ significantly at $P < 0.05$.

Abbreviations: AA, 3 mg ascorbic acid; ALT, alanine transaminase; AST, aspartate aminotransferase; BC, black cumin; EB, 8 mg BC extracts; FB, 4 mg BC; SA, eggs injected with 0.9% saline solution; SB, 6 mg BC; TB, 2 mg BC; WA, eggs without in ovo injection.

[1]mg/dL.

Table 5. Effect of *in ovo* injection of black cumin extract on haematological parameters of broiler chicks at hatch.

Parameter	TB	FB	SB	EB	AA	SA	WA	SEM	*P* value
RBC ($\times 10^6/\mu L$)	$2.10^{a,b}$	$2.12^{a,b}$	$2.40^{a,b}$	3.31^{a}	$2.27^{a,b}$	1.79^{b}	1.71^{b}	0.15355	0.0386
WBC ($\times 10^9/\mu L$)	2.50	2.79	2.79	3.02	2.57	4.56	3.52	0.23295	0.1847
PCV (%)	32.50	34.50	35.00	34.50	37.00	30.00	31.00	0.8103	0.2363
Haemoglobin (g/dL)	11.00	11.50	12.45	12.00	13.15	10.80	10.65	0.30128	0.2861
Basophil (%)	0.00	0.00	0.50	0.50	0.50	0.00	0.00	0.1138	0.6813
Eosinophil (%)	1.50	1.00	1.00	1.00	1.50	1.00	1.50	0.26057	0.9958
Heterophil (%)	30.50	25.00	21.50	26.00	27.50	34.50	29.00	1.53504	0.4421
Lymphocyte (%)	65.50	71.00	74.00	70.00	68.50	60.50	64.50	1.63891	0.4076
Monocyte (%)	3.50	3.00	2.50	2.50	2.00	3.50	5.00	0.41744	0.6706

Means within a row with different superscript letters differ significantly at $P < 0.05$.

Abbreviations: AA, 3 mg ascorbic acid; BC, black cumin; EB, 8 mg BC extracts; FB, 4 mg BC; PCV, packed cell volume; RBC, red blood cells; SA, eggs injected with 0.9% saline solution; SB, 6 mg BC; TB, 2 mg BC; WA, eggs without in ovo injection; WBC, white blood cells.

of TB was similar to that of SA and WA and higher than that of FB, SB, EB, and AA. There was no difference in AST between the birds of SB and AA, but it was lower than that of the treatment groups. The total protein of the chickens in AA was not different from that of SB chickens but higher than that of the other treatment groups. There was no difference in triglyceride between birds of SA and WA, but it was higher than that of the other treatment groups.

DISCUSSION

The use of plant-derived extracts has shown to be residue free, natural, and less toxic, and they are thought to be ideal feed additives, in contrast to the use of synthetic inorganic chemicals in food animal production (Wang et al., 1998). The findings in this study indicate that the use of black cumin *in ovo* was beneficial to broiler chickens at hatch and posthatch growth. Despite the various studies on the dietary inclusion of black cumin in the diets of chickens in literature, there is a scarcity of information on the effect of *in ovo* injection of black cumin extract. The use of *in ovo* is a possible way of improving hatchability and posthatch performance of chickens (Bello et al., 2013; Li et al., 2016). The results of the hatchability in the present study suggest that the solutions injected were safe to the developing embryos as shown by the control groups. The extract influenced the hatchability of the birds in a dose-dependent manner. The higher hatchability in the eggs of AA in the present study is in agreement with the findings of Ipek et al. (2004) and Zakaria and Al-Anezi (1996). The similarity in the hatchability of AA and SB eggs in this study indicates the solution at this dose enhanced the hatching process. This improvement in the hatchability may support the earlier finding on the antioxidant properties of black cumin (Arslan et al., 2005). Moreover, the lower hatchability in TB and EB groups than that of SB is an indication that these levels were suboptimal. The lack of significant difference in the hatchability of SA and WA is in agreement with an earlier report (Mcgruder et al. (2011)).

The intestinal weight of the newly hatched chicks from the eggs of SB and EB was higher than that of SA and WA (negative control groups) in the present study. This suggests that the intestinal development of the chicks benefited from the antioxidant constituents of black cumin. This observation partially agrees with the observation of Itallo et al. (2019) who reported that the use of different levels of vitamin E *in ovo* resulted in higher intestinal weights of newly hatched chicks. The use of antioxidants on developing embryos has been reported to confer oxidative protection on the intestines and other organs from free radicals that could impair development before hatching (Surai et al., 1999). Indeed, antioxidant protection is an important mechanism on chick development at hatching time (Surai, 2002). The enhanced intestinal development of the chicks may explain the better performance of the birds in this treatment group. Bhanja et al. (2008) reported a higher relative weight of small intestine in glucose-injected embryos on the day of hatch (Bhanja et al., 2008). The lower intestinal weights of the chicks of TB than those of SB and EB in the present study indicate the dose-dependence effect of the extract on the gut development of the chicks. The similarity between the chicks of SA and WA corroborates the findings of Mcgruder et al. (2011) who reported that there was no

Table 6. Effect of *in ovo* injection of black cumin extract on plasma malondialdehyde (MDA), superoxidedismutase (SOD), and triiodothyronine (T_3) of newly hatched chicks.

Parameters	TB	FB	SB	EB	AA	SA	WA	SEM	*P* value
MDA (mg/mL)	0.70^{b}	0.68^{b}	0.65^{b}	0.74^{b}	0.67^{b}	1.53^{a}	1.46^{a}	0.106	0.0012
SOD (Unit/mL)	$1.49^{a,b,c}$	$1.93^{a,b}$	2.55^{a}	$1.38^{b,c}$	$2.52^{a,b}$	0.44^{c}	0.44^{c}	0.231	0.0006
T_3 (ng/mL)	$2.54^{a,b,c}$	$2.45^{b,c}$	$3.53^{a,b}$	$2.42^{b,c}$	3.96^{a}	1.72^{c}	1.51^{c}	0.239	0.0021

Means within a row with different superscript letters differ significantly at $P < 0.05$.

Abbreviations: AA, 3 mg ascorbic acid; BC, black cumin; EB, 8 mg BC extracts; FB, 4 mg BC; SA, eggs injected with 0.9% saline solution; SB, 6 mg BC; TB, 2 mg BC; WA, eggs without in ovo injection.

Table 7. Effect of *in ovo* injection of black cumin extract on plasma malondialdehyde (MDA), superoxidedismutase (SOD), and triiodothyronine (T_3) of broiler chickens at market age (day 56).

Parameters	TB	FB	SB	EB	AA	SA	WA	SEM	*P* value
MDA (mg/mL)	$0.68^{a,b,c}$	$0.66^{b,c}$	0.64^{c}	$0.66^{b,c}$	0.65^{c}	$0.71^{a,b}$	0.71^{a}	0.008	0.0034
SOD (Unit/mL)	$2.10^{a,b}$	4.00^{a}	3.71^{a}	$2.55^{a,b}$	3.39^{a}	0.55^{b}	0.56^{b}	0.385	0.0031
Total T3 (ng/mL)	$2.23^{a,b}$	3.29^{a}	$2.53^{a,b}$	$2.58^{a,b}$	$2.53^{a,b}$	2.03^{b}	1.94^{b}	0.129	0.0320

Means within a row with different superscript letters differ significantly at $P < 0.05$.

Abbreviations: AA, 3 mg ascorbic acid; BC, black cumin; EB, 8 mg BC extracts; FB, 4 mg BC; SA, eggs injected with 0.9% saline solution; SB, 6 mg BC; TB, 2 mg BC; WA, eggs without in ovo injection.

difference between the chicks from eggs injected with saline and uninjected eggs.

The higher weights of the broiler chickens of SB and AA in the present trial clearly illustrate that the bioactive compounds in black cumin influenced the growth of the birds. It is interesting to note that the final weights of the birds of AA and SB were comparable. There is a scarcity of information on the use of black cumin extract *in ovo*; however, dietary inclusion of black cumin improved body weights of birds (Osman, 2002; Guler et al., 2006; Abu-Dieyeh and Abu-Darwish, 2008; Ashayerizadeh et al., 2009; Ismail, 2011; Yatoo et al., 2012; Jahan et al., 2015). In fact, several bioactive compounds such as thymol, thymoquinone, p-cymene, and carvacrol have been implicated in the activities of black cumin (Gilani et al., 2004). Improved absorption of nutrients in the intestine of the birds is one of the probable mechanism through which black cumin enhances growth (Kumar et al., 2017). The higher feed consumed by the birds of SB and AA in the present study is reflected in the higher body weights of the birds. There are inconsistencies in literature regarding the effect of black cumin on feed intake of chickens. Some studies have reported higher feed intake (Osman and El-Barody, 1999; Erener et al., 2010; Yatoo et al., 2012), some have reported no effect on the feed intake of birds fed black cumin (Guler et al., 2006; Al-Mufarrej, 2014; Ghasemi et al., 2014), while others reported depressed feed intake (Çetin et al., 2008; Shewita and Taha, 2011; Szczerbińska et al., 2012). Nevertheless, comparison between in ovo and ordinary feeding trials should be made with caution because the former is a totally different way of nutrient supplementation to the animal at a different stage of development. The improved FCR of the birds of SB treatment in the present study is in agreement with the observation made in dietary treatments (Nofal et al., 2006; Durrani et al., 2007; Toghyani et al., 2010; Ghasemi et al., 2014; Jahan et al., 2015). This may be attributed to the bioactive compounds in black cumin. However, a disparate effect was reported by Nasir and Grashorn (2010). Improved performance of birds by dietary black cumin has also been attributed to its antibiotic effect (El-Kamali et al., 1998; Guler et al., 2006). The bioactive substances of black cumin such as melatonin and nigellone have been reported to interact synergistically to enhance utilization of nutrients while also having antibiotic effects (El-Dakhakhny et al., 2000; Abbas et al., 2012). Analgesic, anti-inflammatory, cleaning, and antioxidant functions have been reported because of presence of thymoquinone (Meral et al., 2001; Paarakh, 2010). The resemblance in the performance of the birds of TB and FB groups compared with that of SA and WA in this study suggests that the level of the extract was not high enough to upregulate the growth performance of the birds. Our observation on the similarity in the performance of the birds of SA and WA groups is consistent with a previous study (Mcgruder et al. (2011)).

At hatch, the similarity of the level of triiodothyronine of the birds of AA and SB groups in the present study suggests that black cumin exerts its effects through the thyroid axis. In agreement with our findings, a previous study indicated that black cumin enhanced the concentration of thyroxin, thereby positively influencing the rate of metabolism. Similar to the findings in the present study, an earlier study showed that low doses of black cumin increased thyroxin concentration, thereby increasing the metabolic rate (Mandour et al., 1998). The concentration of plasma T_3 of the chicks of TB,

Table 8. Effect of *in ovo* injection of black cumin extract on haematological parameters of broiler chickens at market age.

Parameters	TB	FB	SB	EB	AA	SA	WA	SEM	*P* value
RBC ($\times 10^6/\mu L$)	1.93	2.89	3.25	2.88	2.08	2.47	2.42	0.167	0.3614
WBC ($\times 10^9/\mu L$)	2.38	2.35	5.33	2.48	2.83	2.35	3.56	0.361	0.2229
PCV (%)	26.00	32.00	39.00	31.00	38.50	35.50	36.00	2.046	0.7289
Hemoglobin (g/dL)	8.30	9.80	12.75	10.00	12.65	11.65	11.95	0.785	0.7918
Basophil (%)	0.50	0.50	0.00	0.50	0.00	0.50	0.50	0.133	0.8991
Eosinophil (%)	2.00	1.50	1.00	1.50	1.00	2.50	1.50	0.228	0.6813
Heterophil (%)	30.00	34.00	25.50	25.50	31.00	34.50	37.50	1.95	0.6861
Lymphocyte (%)	65.00	60.00	68.50	66.50	63.00	59.00	55.50	2.002	0.7144
Monocyte (%)	5.00	4.00	2.50	5.00	3.50	5.00	6.00	0.429	0.4335

Abbreviations: AA, 3 mg ascorbic acid; BC, black cumin; EB, 8 mg BC extracts; FB, 4 mg BC; PCV, packed cell volume; RBC, red blood cells; SA, eggs injected with 0.9% saline solution; SB, 6 mg BC; TB, 2 mg BC; WA, eggs without in ovo injection; WBC, white blood cells.

Table 9. Effect of *in ovo* injection of black cumin extract on serum biochemical parameters of broiler chickens at market age.

Parameters	TB	FB	SB	EB	AA	SA	WA	SEM	*P* value
Creatinine[1]	0.40	0.35	0.30	0.35	0.30	0.45	0.45	0.019	0.0996
ALT (U/L)	63.00^{a}	51.50^{b,c}	45.00^{c}	48.00^{b,c}	45.00^{c}	58.50^{a,b}	58.00^{a,b}	1.920	0.0013
AST (U/L)	128.50^{a}	116.00^{a}	78.50^{b}	118.00^{a}	84.50^{b}	141.00^{a}	136.50^{a}	6.472	0.0005
Total protein[1]	2.60b^{c}	2.40^{c}	3.45^{a,b}	2.45^{c}	4.05^{a}	1.85^{c}	1.75^{c}	0.218	0.0001
Albumin[1]	1.05^{b,c}	0.90^{c}	1.50^{a,b}	0.95^{b,c}	2.00^{a}	0.85^{c}	0.90^{c}	0.114	0.0005
Globulin[1]	1.55^{a,b}	1.50^{a,b}	1.95^{a}	1.50^{a,b}	2.05^{a}	1.00^{a,b}	0.85	0.16	0.0230
Glucose[1]	117.00	98.00	76.50	103.00	68.00	144.50	141.00	9.856	0.2571
Triglycerides[1]	64.00^{b}	54.50^{b,c}	49.50^{b,c}	57.50^{b,c}	35.00^{c}	96.00^{a}	96.50^{a}	6.147	0.0004

Means within a row with different superscript differ significantly at $P < 0.05$.

Abbreviations: AA, 3 mg ascorbic acid; ALT, alanine transaminase; AST, aspartate aminotransferase; BC, black cumin; EB, 8 mg BC extracts; FB, 4 mg BC; SA, eggs injected with 0.9% saline solution; SB, 6 mg BC; TB, 2 mg BC; WA, eggs without in ovo injection.

[1]mg/dL.

FB, WA, and SA was statistically similar, indicating the levels of black cumin in TB and FB did not upregulate this hormone differently.

MDA is a biomarker of lipid peroxidation, and it is used to assess oxidative damage (Jensen et al., 1997). Similar to the birds of AA in this study, the different doses of black cumin extract resulted in improved plasma MDA in the day-old chicks. Earlier studies have shown that black cumin could be used as a natural antioxidant for avians (Guler et al., 2007). Studies have shown black cumin to possess antioxidant properties under *in vitro* and *in vivo* conditions (Ozdemir et al., 2018). In humans, the use of black cumin also reduced MDA alongside glutathione peroxidase (Mostafa et al., 2013). The higher plasma SOD of the chicks of SB than that of the control groups in the present study corroborates the findings of Mostafa et al. (2013) who reported that the use of black cumin resulted in higher SOD in human. The improvement in the oxidative parameters in the chicks at hatch in the present study affirms the observation of Tollba and Hassan (2003) that the use of black cumin relieved thermal stress effect. Rahman and Kim (2016) reported an improvement in the MDA and SOD of broiler chickens fed black cumin. The improvement in the trend of the oxidative parameters of the birds at hatch and after thermal challenge at the market age in this study suggests that there was a carryover effect of the *in ovo* injection of the extract on the birds. Indeed, previous studies have shown that black cumin possess antioxidant activities which could enhance various enzyme activities including SOD, catalase, and glutathione-S-transferase which are involved in oxidative stress modulation in broiler chickens.

Hematochemical parameters may show the health status of an animal (Toghyani et al., 2010). *In ovo* injection of black cumin extract did not influence the hematological parameters of broiler chickens subjected to thermal stress. In contrast to our findings, Nofal et al. (2006) reported there was an improvement in the hematological parameters of birds offered black cumin additives. The similarity in the packed cell volume of the birds is similar to the findings of Hermes et al. (2009). In accordance to our observation, however, the author reported an improvement in the serum biochemical parameters. The similarity in the blood glucose level of the birds of *in ovo* black cumin in this study is partially in agreement with previous studies (Khalaji et al., 2011; Ghasemi et al., 2014; Kumar et al., 2017) who reported there was no difference in the blood glucose. In contrast, Yatoo et al. (2012) reported that the use of 1% black cumin in the diets of broiler chickens reduced blood glucose. The total protein of the birds was closely related, with a slight increase in the total protein of the birds of SB and AA. This observation is similar to the findings of Kumar et al. (2017) who reported the total protein of broiler chickens fed black cumin was higher than that of the control group. The improvement in the total protein may be attributed to the immune-modulating effects of black cumin (Al-Beitawi et al., 2009). The higher reduction in the triglycerides of the birds of TB, FB, SB, EB, and AA groups than that of SA and WA is in accordance with earlier studies (Akhtar et al., 2003; Ghasemi et al., 2014). Conversely, a comparable triglyceride was observed by Kumar et al. (2017). The improved liver and kidney function parameters in this study are in agreement with the findings of Hermes et al. (2009) and Attia and Harthi (2015). Our findings corroborate the hepatoprotective effect of black cumin (Mahmoud et al., 2002). In accordance to our findings, Soliman et al. (2017) reported a reduced serum creatinine of biologically challenged broiler chickens.

CONCLUSION

The results of the present study clearly demonstrate the beneficial effect of *in ovo* supplementation with black cumin on the hatchability, antioxidant status, and growth performance of thermally challenged broiler chickens during incubation. However, *in ovo* injection of 6 mg of black cumin extract improved the posthatch performance and antioxidant status of broiler chickens.

DISCLOSURES

The authors declare no conflict of interest.

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