Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v46i1.62653

The influence of i*n ovo* feeding of black cumin extract on the physiological responses of broilers under hot tropical environments

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ABSTRACT. An experiment was conducted to investigate the influence of *in ovo* feeding of black cumin extract (BC) on the intestinal morphology and physiological responses of broilers thermally challenged during incubation. The study involved the use of seven hundred Marshall broiler hatching eggs, which were assigned to 7 in ovo feeding treatments at embryonic day 17.5, as follows: eggs injected with 8 mg (EN), 6 mg (SN), 4 mg (FN), 2 mg (TN)BC, no *in ovo* feeding (NI), 0.9 % saline solution (SS), and 3 mg vitamin C (VC). Juvenile growth performance, plasma malondialdehyde (MDA), triiodothyronine (T_3), superoxide dismutase (SOD), and haematological and serum biochemical indices were evaluated. The results revealed that the juvenile growth performance of SN birds was better than those of the NI, SS, FN and TN groups. The plasma SOD and T_3 of the birds of SN and VC were significantly higher ($p < 0.05$) than the chickens of NI and SS treatments. The ileal crypt depth recorded in SN birds was lower ($p < 0.05$) compared to SS and NI values. To conclude, *in ovo* black cumin extract enhanced the gut health and lymphoid organs of broiler chickens with no pronounced effect on the thermotolerance of the birds at market age.
Keywords: heat stress; gut; incubation; environment; phytogenic.

Received on February 23 2022.
Accepted on June 15 2023.

Introduction

There has been a growing interest in the thermal resistance of broiler chickens, particularly in tropical environments. Efforts have been made to improve the thermotolerance of broiler chickens through various means, including embryonic and early-age thermal manipulation (Willemsen et al., 2010; Meteyake, Bilalissi, Oke, Voemesse, & Tona, 2020; Oke et al., 2020) by manipulating temperature during incubation (Collin et al., 2011), as well as a few days after hatching (Yahav, 2000; Oke et al., 2020) when the thermal regulation mechanism of the birds is immature, thereby enhancing their ability to cope with thermal challenges later in life. Some interesting results have been documented on the post-hatch growth and the anti-oxidative status of the birds (Yahav, Collin, Shinder, & Picard, 2004; Al-Zghoul & Saleh, 2020).

Oxidative stress can result from the metabolic production of reactive oxygen species and free radicals during the fast development of chick embryos (Deeming & Pike, 2013). Excessive free radicals can cause various pathological conditions, biological damage, and poor growth performance in chickens (Uyanga et al., 2022; Uyanga et al., 2023; Akosile, Kehinde, Oni, & Oke, 2023a; Tokofai, Idoh, Oke, & Agbonon, 2021; Tokofai Orounladji, Idoh, Oke & Agbonon, 2023). In order to preserve the integrity of the organs and systems of chick embryos, there is a need to ensure their optimal antioxidant defense system, as their tissues are high in polyunsaturated fatty acids (Surai, Fisinin, & Karadas, 2016). The antioxidant defense system is crucial in the overall health of chick embryos, particularly during the last phase of incubation (Goel, Bhanja, Mehra, Mandal, & Pande, 2016). The production of birds that can tolerate different environmental stressors is one of the indices of the success of poultry enterprises. One of the viable means of enhancing antioxidant defense during embryonic development and post-hatch growth is *in ovo* feeding. The use of *in ovo* injection is an efficient means of ensuring nutrients are directly delivered to the embryos at a lower cost compared to the dietary route (Kadam, Barekatain, Bhanja, & Iji, 2013).

There has been a growing awareness of the use of phytonutrients as alternatives to growth promoters in the poultry industry (Oke et al., 2017; Oke, 2018; Tokofai, Idoh, Oke, & Agbonon, 2020; Oke et al., 2021a;

Oke et al., 2021b; Ajayi, Smith, Oso, & Oke, 2022; Akosile, Sogunle, Majekodunmi, & Oke, 2023b, Akosile et al., 2023c; Adejuyigbe, Sogunle, Onagbesan, & Oke, 2023) due to their antioxidant and antimicrobial properties (Bhatti et al., 2022). Supplementation of black cumin (*Nigella sativa*) (BC) in poultry diets has received tremendous attention due to its beneficial bioactive compounds, including carvacol, dithymoquinone, thymolthymoquinone (Oke et al., 2021; Bhatti et al., 2022) having anti-inflammatory, antioxidant, immune stimulator, anti-bacterial, anti-parasitic, and anti-histamine properties (Arslan et al., 2005; Kumar, Patra, Mandal, Samanta, & Pradhan, 2017). It has been shown that the antioxidant capacities of black cumin were more potent than the synthetic antioxidants in different regions (Yakup, 2007). Indeed, Guler, Ertas, Kizil, Dalkýlýc, and Ciftci (2007) indicated that the antioxidant properties of black cumin might be beneficial in poultry production. Additionally, zinc, calcium, iron, folic acid, pyridoxine, niacin, thiamin, copper, phosphorus, etc. have been reported in significant quantities in black cumin. Although our earlier findings showed a positive influence of *in ovo* feeding of the extract of BC on broiler chickens (Oke et al., 2021), the mechanism of action of the extract needs to be further elucidated. Natural antioxidants in black cumin may enhance the adaptability of broiler chickens in tropical environments. Moreover, as the positive role of in ovo vitamin C has been established in chickens (Elibol, Türkoglu, Akan, & Erol, 2001), the present study aimed to evaluate the influence of *in ovo* feeding of BC extract on the physiological responses and intestinal morphology of broiler chickens.

Material and methods

Care and use of animals

This study was conducted according to the Institutional Animal Ethics Committee guidelines, Federal University of Agriculture, Abeokuta, Nigeria. The birds used were given appropriate management and laboratory techniques and unnecessary inconvenience was avoided.

Experimental birds and management

Eggs management

A total of 700 hatching eggs of broiler (Marshall) chickens were procured and assigned to 7 treatments having 100 eggs each. At 17.5th d of incubation, *in ovo* injection was carried out.

Black cumin (BC) seed extract

Dry seeds of black cumin were procured from a well-known farmer. The extract was prepared as described in our earlier study (Oke et al., 2021).

In ovo injection procedure

At embryonic day 17.5, the hatching eggs were assigned to seven *in ovo* treatments as follows: intact eggs (NI), eggs with *in ovo* feeding of 8 (EN), 6 (SN), 4 (FN) and 2 (TN) mg BC, saline solution (0.9% saline) (SS), and vitamin C (3 mg egg-1) (VC). The *in ovo* procedure was done as described in our earlier study (Oke et al., 2021)

Post-hatch management

Birds from each *in ovo* treatment group were allocated to five replicates, each on a deep litter system, having 4-cm-deep wood shavings. NRC (1994) nutrient recommendations were adopted for feeding the birds at different ages. Fresh water and feed were provided for the birds with no restriction during the 56 days of the post-hatch phase of the trial.

Data collection

Growth performance

Data on the chickens' feed intake were obtained by deducting feed remnants from the feed supplied to the birds. Also, weekly weights of the chickens were taken and the weight gain was determined as the difference between two consecutive weeks.

Plasma antioxidants and thyroid hormone

During the post-hatch growth, blood samples were collected from two birds per replicate into heparinized bottles and centrifuged for 15 minutes. The plasma samples were analyzed for malondialdehyde (MDA) and

superoxide dismutase (SOD) with the use of commercial assay kits, following the guidelines of the manufacturer. Plasma triiodothyronine (T_3) concentrations were measured following the method of Indu, Sejian, and Naqvi (2014).

Biochemical Indices

Serum was obtained from the blood samples by centrifuging (3,000× g) the samples for fifteen minutes. The blood samples were later analysed for serum metabolites (total protein, aspartate aminotransferase, alanine transferase, triglyceride, glucose, albumin, globulin, and creatinine) using conventional methods (Jain, 1986). Total antibody titers to SRBC were determined by haemagglutination assay to analyze for humoral immunity (Pourhossein et al., 2015).

Acute heat stress at market age

On d 56, two chickens were subjected to thermal challenge in a previously heated poultry house (35°C)

Rectal temperature (RT) and respiratory rate were determined before and after one hour of acute heat stress. A digital thermometer was gently inserted into the cloaca while the birds were restrained calmly and the readings were taken when the thermometer beeped.

Organ relative weights

On day 56, two birds in each replicate of average weights were slaughtered and dissected and different organs were carefully removed and weighed.

Gastrointestinal morphology

At 8th week post-hatch, immediately after slaughter, gastrointestinal samples were taken from two birds per replicate and were fixed in buffered formalin (10%). Jejunal, duodenal and ileal tissue samples in formalin were dehydrated, inserted in paraffin, cut (10 µm) and stained with haematoxylin and eosine. In each segment, morphometric parameters were determined using a light microscopic image analyzer. Crypt depth, villus height, basal width, apical width, and the ratio of villus height to crypt depth were determined.

Statistical analysis

The data collected were subjected to a one-way analysis of variance using SAS (2008) statistical package. When there were differences among *in ovo* treatment, significant ($p < 0.05$) means were separated using Tukey's HSD test.

Results and discussion

The plasma MDA of chickens of NI and SS treatment groups was significantly higher ($p < 0.05$) than the other treatment groups at week 4 (Table 1). The plasma MDA of birds of TN and FN was similar to that of EN but significantly higher than those of birds of SN and VC. The plasma SOD of NI and SS birds was significantly lower than the others. The plasma triiodothyronine of the birds in SN and VC were similar but higher ($p < 0.05$) than those of NI and SS. Plasma SOD of NI and SS was lower ($p < 0.05$) than the values obtained in VC and SN chickens. The lower MDA of the birds injected with BC extract, similar to that of vitamin C *in ovo* in this study, suggests an enhanced activity of antioxidant enzymes in the birds (Faix, Faixova, Placha, & Koppel, 2009). This result aligns with the reports of Guler et al. (2007), which indicated that dietary BC enhanced lipid peroxidation in birds. The presence of thymoquinone, an antioxidant constituent, has been reported to be involved in the scavenging of free radicals (Mahmoud & Mansour, 2000). Consistent with the findings in the present study, Ilhan, Gurel, Armutcu, Kamisli, and Iraz (2005) showed that BC oil enhanced the superoxide dismutase in mice. The authors opined that the antioxidant constituents of BC may prevent cellular ATP degradation through the reduction of the main source of oxygen free radicals. Thyroid hormones are involved in the metabolic processes in animals. The improved plasma T_3 of chickens injected with BC extract *in ovo* at week 4 post-hatch in this trial suggests that the extract enhanced the systemic responses in the birds. Our observation partially agrees with the report of Mandour, Ashry, and Hedaya (1998), who showed that dietary BC enhanced plasma T_3 of broiler chickens.

Table 1. Evaluation of *in ovo* feeding of BC on plasma malondialdehyde, superoxide dismutase (SOD) and T_3 of broilers at starter phase (week 4).

Parameters	TN	FN	SN	EN	VC	SS	NI	SEM	p-Value
MDA (mg mL^{-1})	0.7^{b}	0.7^{b}	0.6^{d}	0.7^{bc}	0.6^{cd}	0.8^{a}	0.9^{a}	0.030	<.0001
SOD (Unit mL^{-1})	2.1^{b}	1.9^{b}	3.1^{a}	2.2^{b}	2.5^{ab}	0.7^{c}	0.7^{c}	0.237	<.0001
T_3 (ng mL^{-1})	2.4^{ab}	2.2^{ab}	2.9^{a}	2.5^{ab}	2.7^{a}	1.7^{b}	1.7^{b}	0.124	0.0056

[a,b,c:] Means within a row with different superscript are different ($p < 0.05$). VC, 3 mg vitamin C; BC, black cumin; EN, 8mg BC extracts; FN, 4 mg BC; SS, 0.9% saline solution treated eggs; SN, 6 mg BC; TN, 2 mg BC; NI, intact eggs. MDA, Malondialdehyde; SOD, Superoxide dimutase; T_3, triiodothyronine.

As shown in Table 2, the albumin of birds of VC was similar to SN and FN but higher ($p < 0.05$) compared to TN, EN, NI and SS. The serum globulin of SN birds was similar to VC birds but higher ($p < 0.05$) compared with NI, SS, TN, FN and EN birds. However, the total protein of birds of SN and VC was higher ($p < 0.05$) compared to the other groups. The creatinine of birds of NI was similar to SS and TN but higher ($p < 0.05$) compared to SN and VC chickens. The ALT and AST were higher ($p < 0.05$) in birds of NI than in the other treatment groups. The serum ALT and AST of VC chickens were lower ($p < 0.05$) than those of TN, FN, SS, and NI. Triglycerides were highest ($p < 0.05$) in birds of NI but lowest in birds of VC, FN, EN, and SN. Aspartate aminotransferase and Alanine transaminase activities have been considered biomarkers that may be used to evaluate hepatotoxicity (Króliczewska et al., 2017). The levels of these enzymes tended to be lower in the birds administered in ovo extracts in the present study. This observation suggests a hepatoprotective activity of the bioactive compounds of BC. Also, serum protein was slightly higher in SN and VC birds in this study. It is well known that protein is primarily synthesized in the liver and can be a biomarker of the status of the hepatocytes (Khan et al., 2020). The comparable values in the blood glucose of chickens in this study are consistent with the observation of Abudabos, Alyemni, Dafalla, and Khan (2018), who reported that phytogenic feed additives did not influence the serum glucose of broiler chickens. Kidney impairment may be reflected by higher creatinine as a result of a reduction in glomerular filtration (Rhiouani, El-Hilaly, Israili, Lyoussi, 2008). The marginal reduction in the serum creatinine of the birds of EN, VC and SN in our study indicates that the constituents of BC enhanced kidney functions. Serum triglyceride is considered to be an indicator of lipid metabolism (He et al., 2015). The lower serum triglyceride of the birds administered *in ovo* BC in the present study is consistent with findings obtained on dietary BC (Ghasemi, Kasani, & Taherpour, 2014). This is, however, in contrast with the observation of Kumar et al. (2017), who reported that BC did not influence the triglycerides of chickens. Reduction in the serum triglycerides by BC has been attributed to its thymoquinone and monounsaturated fatty acids on the cholesterol synthesis of the hepatocytes (Brunton, 1999).

Due to the presence of some active pharmacological constituents such as thymohydroquinone, thymoquinone, nigellimine, etc., BC has been reported to possess immunopotentiating functions (Osman & El-Barody 1999; Al-Beitawi, El-Ghousein, & Nofal, 2009). However, *in ovo* feeding of BC did not elicit a difference in the immunity of the birds in the present study; this may be due to the difference in the route of administration, as most of the earlier studies were dietary applications.

Table 2. Evaluation of *in ovo* feeding of BC on serum biochemical indices and humoral immunity of chickens at starter phase (week 4).

Parameters	TN	FN	SN	EN	VC	SS	NI	SEM	P value
Creatinine (mg dL^{-1})	0.40^{abc}	0.35^{bc}	0.30^{c}	0.35^{bc}	0.30^{c}	0.50^{ab}	0.55^{a}	0.027	0.0055
ALT (U L^{-1})	86.50^{bc}	66.50^{cd}	29.00^{e}	47.00^{de}	35.00^{e}	107.50^{b}	161.00^{a}	12.090	<.0001
AST (U L^{-1})	119.50^{c}	109.00^{cd}	73.50^{de}	95.50^{cde}	65.50^{e}	337.00^{b}	396.50^{a}	35.026	<.0001
Total protein (mg dL^{-1})	$3.05b^{c}$	$3.15b^{c}$	4.20^{a}	3.25^{b}	4.15^{a}	2.45^{d}	2.60^{cd}	0.178	<.0001
Albumin (mg dL^{-1})	1.50^{b}	1.60^{ab}	1.80^{ab}	1.55^{b}	1.90^{a}	1.05^{c}	1.10^{c}	0.085	0.0001
Globulin (mg dL^{-1})	1.55^{c}	1.55^{c}	2.40^{a}	1.70^{bc}	2.25^{ab}	1.40^{c}	1.50^{c}	0.105	0.0009
Glucose (mg dL^{-1})	124.00^{b}	116.00^{bc}	93.00^{c}	114.50^{bc}	105.50^{bc}	170.50^{a}	179.00^{a}	9.543	0.0418
Triglycerides (mg dL^{-1})	97.00^{b}	62.50^{c}	56.50^{c}	71.00^{c}	51.50^{c}	113.00^{ab}	118.50^{a}	7.183	<.0001
Anti-SRBC	3.32	3.32	3.46	3.39	3.46	2.73	2.59	0.12112	0.2441

[a,b,c,d,e:] Means within a row with different superscript are different ($p < 0.05$). VC, 3 mg vitamin C; BC, black cumin; EN, 8mg BC extracts; FN, 4 mg BC; SS, 0.9% saline solution treated eggs; SN, 6 mg BC; TN, 2 mg BC; NI, intact eggs; SN, 6 mg BC; TN, 2 mg BC; NI, eggs without *in ovo* injection, SRBC: sheep red blood cell.

There was no difference in the rectal temperature of the birds before and after heat challenge (Table 3). Before the heat challenge, the respiratory rate of the birds in NI was similar to those of SS and TN but significantly higher than those of the other treatments. There was a similarity in the respiratory rate of FN, SN, EN and VC birds. A similar trend was observed after the thermal challenge. Similar to our findings, Tollba and Hassan (2003) indicated

that the addition of 1% Black seed as a natural feed additive to diets of heat-stressed broilers significantly decreased their respiratory rate and body temperature compared with the control.

Table 3. Evaluation of *in ovo* feeding of BC on rectal temperature and respiratory rate of broilers at 8th weeks post-hatch (before and after heat challenge).

Parameters	TN	FN	SN	EN	VC	SS	NI	SEM	p-Value
Before Heat Challenge									
Rectal Temp (ºC)	41.75	41.60	41.10	41.15	41.20	42.00	42.15	0.148	0.3327
Respiratory Rate (60 sec)	72.50^{abc}	65.00^{c}	65.00^{c}	65.00^{c}	67.50^{bc}	80.00^{ab}	82.50^{a}	2.077	0.0135
After Heat Challenge									
Rectal Temp (ºC)	41.90	41.78	41.25	41.35	41.40	42.40	42.45	0.153	0.1228
Respiratory Rate (60 sec)	102.50^{b}	92.50^{c}	92.50^{c}	95.00^{bc}	90.00^{c}	112.50^{a}	117.50^{a}	2.380	0.0001

VC, 3 mg vitamin C; BC, black cumin; EN, 8 mg BC extracts; FN, 4 mg BC; SS, 0.9% saline solution treated eggs; SN, 6 mg BC; TN, 2 mg BC; NI, intact eggs.

The duodenal villus height of the birds of EN and VC was similar and higher compared to the FN, SS, and NI treatment groups (Table 4). The duodenal villus height to crypt ratio of broiler chickens of VC was comparable to SN, EN, and TN but higher ($p < 0.05$) compared to NI and SS. The ileal basal width of the birds of EN was higher ($p < 0.05$) compared to FN birds. There was a similarity in the ileal villus heights of the birds of SN, EN and VC, which were higher than those of TN, FN, SS and NI. The ileal crypt depth of the birds of SN was lower ($p < 0.05$) compared to NI and SS birds. The ileal villus height to crypt ratio of birds of SN was higher ($p < 0.05$) than birds of SS but comparable to the chickens of TN, EN, FN, VC, and NI. The jejunal villus height to crypt ratio and villus height of EN broilers were higher ($p < 0.05$) compared to the birds of NI and SS. The morphology of the small intestine is crucial to the proper functioning and gut health of avians. Due to the crucial roles of the small intestine in the digestion and absorption of nutrients from the ingested diets, any alteration in its structure or function will influence the growth and development of an animal (Toman, Hajkova, & Hluchy, 2015). The morphological status of the jejunum can be used to assess nutrient absorption efficiency (Varel, Robinson, & Pond, 1987) as it is the principal intestinal area of absorption in chickens (Leeson & Summers, 2001). The higher jejunal villus height of chickens of EN and SN in the present study is an indication that the bioactive compounds of BC positively modulated this region of the small intestine. As it is known that higher villus height has been associated with higher epithelial turnover (Boka, Mahdavi, Samie, & Jahanian, 2014), our observation suggests that there was increased utilization of nutrients from the diets fed to the birds of EN and SN, thereby resulting in their improved growth performance.

Table 4. Evaluation of *in ovo* feeding of BC on intestinal morphology indices of broilers at eighth week post-hatch.

Parameters	TN	FN	SN	EN	VC	SS	NI	SEM	p value
Duodenum									
Villus height (µm)	776.50^{ab}	623.00^{bc}	894.00^{ab}	993.00^{a}	1066.50^{a}	405.00^{c}	391.50^{c}	71.484	0.0003
Apical width (µm)	50.00	55.00	65.00	55.00	50.00	40.00	40.25	3.693	0.6590
Basal width (µm)	76.50	76.50	94.50	99.00	90.00	54.00	54.00	6.086	0.2443
Crypt depth (µm)	157.50	174.50	148.50	173.50	158.00	179.50	178.50	4.276	0.3403
VCR	5.08^{abc}	3.58^{bc}	6.01^{ab}	5.75^{ab}	6.76^{a}	2.27^{c}	2.20^{c}	0.497	0.0025
Ileum									
Villus height (µm)	585.0^{b}	715.0^{b}	1255.0^{a}	1280.0^{a}	1160.0^{a}	420.0^{b}	475.0^{b}	84.138	0.0001
Apical width (µm)	30.00	25.00	35.00	40.00	25.00	30.00	30.00	1.951	0.4270
Basal width (µm)	80.00^{ab}	45.00^{b}	110.00^{ab}	130.00^{a}	100.00a^{b}	55.00^{ab}	55.00^{ab}	9.089	0.0188
Crypt depth (µm)	173.00^{ab}	175.50^{ab}	129.00^{b}	148.50^{ab}	160.00^{ab}	180.00^{a}	180.00^{a}	5.530	0.0353
VCR	3.25^{ab}	4.04^{ab}	9.77^{a}	8.89^{ab}	7.30^{ab}	2.41^{b}	2.64^{ab}	0.875	0.0188
Jejunum									
Villus height (µm)	675.0^{abc}	750.0^{abc}	925.0^{ab}	1050.0^{a}	850.0^{ab}	400.0^{bc}	260.0^{c}	77.784	0.0068
Apical width (µm)	35.00	40.00	35.00	35.00	35.00	25.00	30.00	1.991	0.6577
Basal width (µm)	100.00	110.00	200.00	150.00	125.00	65.00	75.00	16.811	0.4183
Crypt depth (µm)	172.50	175.00	172.50	155.00	165.00	180.00	220.00	6.745	0.2125
VCR	4.11^{abc}	4.29^{abc}	5.36^{ab}	6.83^{a}	5.04^{abc}	2.22^{bc}	1.23^{c}	0.529	0.0111

$^{a,b,c;}$ Means within a row with different superscript are different ($p < 0.05$). VC, 3 mg vitamin C; BC, black cumin; EN, 8 mg BC extracts; FN, 4 mg BC; SS, 0.9% saline solution treated eggs; SN, 6 mg BC; TN, 2mg BC; NI, intact eggs. VCR: Villus height to crypt ratio

At week 4, body weights and body weight gain of chickens of EN and SN were similar, whereas that of SN was significantly higher ($p < 0.05$) than the other treatment groups (Table 5). The FCR of chicken of NI, SS, TN and FN were similar but higher ($p < 0.05$) than that of SN. The feed intake of birds of EN was significantly higher ($p < 0.05$) than VC and NI birds. The FCR of chickens of SN was similar to those of EN and VC but

significantly lower than those of the other treatment groups. The improved performance of the chickens of *in ovo* BC extract at the juvenile age in the present trial partially agrees with the observation of Sogut, Inci, and Ozdemir (2012), who reported that dietary BC improved broiler chickens' growth performance. Earlier studies have established the involvement of flavonoids in the performance of broiler birds (Saeed et al., 2017). Also, the improved weight gain of the birds of SN in this study is in accordance with the findings of Sohail, Jahanzeb, Ahsan, and Ghulam (2012). The enhanced juvenile performance can be attributed to the bioactive compounds (thymoquinone) of BC, which act as digestive enzyme stimulants in the pancreas and intestinal mucosa, thereby aiding digestion and absorption of nutrients, culminating in improved growth performance (Guler et al., 2007).

Table 5. Evaluation of *in ovo* feeding of BC on juvenile age performance of broilers (week 4).

Parameters	TN	FN	SN	EN	VC	SS	NI	SEM	P Value
Body weight gain, g	1062.08^{bc}	1075.88^{bc}	1286.84^{a}	1218.92^{ab}	1087.52^{bc}	1014.57^{c}	990.24^{c}	22.972	0.0001
Feed intake, g	2453.5^{ab}	2524.20^{ab}	2530.1^{ab}	2751.1^{a}	2409.6^{b}	2437.9^{ab}	2395.5^{b}	32.582	0.0353
Feed conversion ratio	2.32^{a}	2.35^{a}	1.97^{b}	2.26^{ab}	2.22^{ab}	2.41^{a}	2.42^{a}	0.036	0.0028

$^{a,b,c;}$ Means within a row with different superscript are different ($p < 0.05$). VC, 3 mg vitamin C; BC, black cumin; EN, 8 mg BC extracts; FN, 4 mg BC; SS, 0.9% saline solution treated eggs; SN, 6 mg BC; TN, 2 mg BC; NI, intact eggs.

Improved growth performance is associated with shallow crypts, longer villus, and larger villus height/crypt ratios (VCR) (Boka et al., 2014). Since VCR is a biomarker of the digestive capacity of the gut (Mahdavi et al., 2010), the enhancement in the VCR of the birds administered higher *in ovo* injection of the extract in the present study indicates that the use of BC had a positive impact on the gut health. Our observation conforms to the earlier study, which indicated that dietary BC could preserve the intestinal mucosa from an oxidative challenge (Bagchi et al., 1999). Improved VCR has also been attributed to the antimicrobial function of BC in reducing microbial activities and aiding the proliferation of new cells in the gut, leading to smaller crypts and higher villus heights (Xu, Hu, Xia, Zhan, & Wang, 2003).

The higher duodenal villus height of the birds TN, EN, SN and VC than those of NI and SS in this study suggests that the nutrients in the diets of the birds were better absorbed during their post-hatch growth. This corresponds to the improved growth performance of the chickens. Higher villi heights have been linked with improved cell mitosis, leading to a better absorptive capacity (Onderci et al., 2006). The findings of Awad, Bohm, Razzazi-Fazeli, Ghareeband, and Zentek (2006) indicated that longer villi of the small intestine are associated with improved nutrient absorption in the intestine.

There was a similarity ($p > 0.05$) in most of the organs except the bursa weight. The weights of the bursa of Fabricius of VC and SN chickens were similar but higher than the others whose values were similar (Table 6). The ultimate pH parameters of broiler chickens were similar ($p > 0.05$) across the treatment groups. The improvement in the body weight gain achieved in this present study could be attributed to better nutrient absorption, which resulted in improved performance of the chickens and this partially agrees with the study of Guler et al. (2007), who indicated that broilers fed the dietary BC seed had higher body weights than the control. The relative weights of organs may reflect the responses of animals to substances or conditions that influence a decrease or increase in their internal organs (Ayodele, Oloruntola, & Agbede, 2016). The results in the present study revealed that the relative weight of the bursa of Fabricius was higher in the chickens of VC and SN. This suggests that the pharmacologically active substance of BC extract up-regulated the lymphoid organs of the birds. The stimulatory role of BC on the T-cell-mediated immune responses has been established (Islam, Begum, Ahsan, Huque, & Ahsan, 2004). Our findings are in concurrence with the observation of Sohail et al. (2012), who indicated that dietary BC increased lymphoid organ weights.

Table 6. Effect of *in ovo* feeding of BC on organ relative weights broiler chickens at 8th weeks post-hatch

Parameters	TN	FN	SN	EN	VC	SS	NI	SEM	p-Value
Intestine (%)	4.60	4.11	3.53	5.00	4.80	4.74	5.38	0.208	0.3055
Spleen (%)	0.12	0.16	0.15	0.12	0.12	0.13	0.09	0.008	0.4253
Bursa (%)	0.03^{b}	0.03^{b}	0.14^{a}	0.04^{b}	0.10^{a}	0.03^{b}	0.02^{b}	0.013	0.0007
Liver (%)	1.87	1.96	2.31	2.60	2.37	2.11	2.01	0.105	0.5976
Kidney (%)	0.73	0.51	0.54	0.58	0.56	0.48	0.54	0.029	0.4164
Heart (%)	0.58	0.51	0.57	0.45	0.62	0.42	0.49	0.024	0.3536
Gizzard (%)	3.25	2.81	2.87	2.54	3.22	2.78	2.78	0.113	0.7268
Proventriculus (%)	0.16	0.09	0.15	0.06	0.10	0.11	0.25	0.018	0.0877

^{a,b,}Means within a row with different superscript are different ($p < 0.05$). VC, 3 mg vitamin C; BC, black cumin; EN, 8mg BC extracts; FN, 4 mg BC; SS, 0.9% saline solution treated eggs; SN, 6 mg BC; TN, 2 mg BC; NI, intact eggs.

Conclusion

Overall, it can be concluded that *in ovo* feeding of BC extracts improved the intestinal morphology of broiler chickens. 6 and 8mg of the extract elicited similar responses to vitamin C. Moreover, the metabolic and antioxidant status of the birds were significantly improved from the eggs injected with 6mg of the extract, and acute heat stress did not show a pronounced effect on the birds and there was no remarkable impact on the thermotolerance of the birds at the slaughter age.

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