Response of lymphatic tissues to natural feed additives, curcumin (*Curcuma longa*) and black cumin seeds (*Nigella sativa*), in broilers against *Pasteurella multocida*

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ABSTRACT The antibiotic residues and pathogenic resistance against the drug are very common in poultry because of antibiotics used in their feed. It is necessary to use natural feed additives as effective alternatives instead of a synthetic antibiotic. This study aimed to investigate the immune response of *Nigella sativa* and *Curcuma longa* in broilers under biological stress against *Pasteurella multocida*. The total 100, one-day-old chicks were divided into 5 groups. Groups 1 and 2 served as control negative and control positive. Both control groups were receiving simple diet without any natural feed additives, but the infection was given in group 2 at day 28 with the dose of 5.14×10^7 CFU by IV. Groups 3A and 3B were offered 2% seed powder of *Nigella sativ*a, groups 4A and 4B were offered *C. longa* 1% in powdered form, and group 5A and 5B were offered both *C. longa* 1% and *N. sativa* 2% in the feed from day 1 and groups 3B, 4B, and 5B were challenged with *P. multocida*. The haemagglutination inhibition titter against Newcastle Disease virus (NDV), feed conversion ratio, mortality, gross, and histopathology were studied. The results of this study revealed that hemagglutination inhibition titers against NDV were highly significant ($P < 0.05$) in treated groups, highest titers (3A, 6.8; 3B, 6.4; and 5A, 7.2) were obtained from treated Groups. The feed conversion ratio of *N. sativa* + *C. longa* treated groups (5A, 1.57, and 3A, 1.76) were higher than that of other nontreated groups. The gross and histopathological changes were much severe in control positive, but fewer changes were seen in treated groups. Therefore, we recommend that natural feed additives, black cumin *(N. sativa)* and turmeric (*C. longa*), act as an immune enhancer in broilers against *P. multocida*.

Key words: *Curcuma longa*, *Nigella sativa*, feed conversion ratio, gross pathological change, histopathological change

2021 Poultry Science 100:101005
https://doi.org/10.1016/j.psj.2021.01.028

INTRODUCTION

The poultry industry is one of the most imperative sectors of agriculture in the world. It is growing rapidly as a dynamic industry in South Asian countries. The wonderful role of commercial broilers and layers is to fulfil the growing demand for protein in the form of eggs and meats (Raheem et al., 2020). The *Pasteurella multocida* is a gram-negative bacterium that causes various

Received November 13, 2020.
Accepted January 2, 2021.

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diseases in different animals such as fowl cholera in poultry (Peng et al., 2019). Fowl cholera is a usually infectious avian bacterial disease causing high morbidity and mortality, resulting in huge financial losses in the poultry industry (Animal Health, 2008; Baksi et al., 2018; Poolperm et al., 2018).

Since ancient times, different strategies have been applied to improve animal health, productivity, and profitability (Yadav and Jha, 2019). Antibiotics used as an antibiotic growth promoter (**AGP**) in the feed of livestock and also used as subtherapeutic (Sivagami et al., 2020). However, the use of antibiotics as feed additives is dangerous because of cross-resistance and also deposits into the tissues which affect the meat quality (Dutta et al., 2019). From 2006, the European Union has banned AGP in animal feed because of its potential risks (Bennani et al., 2020). Now the demand for alternative substances (antibiotic-free) is growing rapidly (Fisher, 2019).

Phytogenic feed additives (**PFA**) are products which are obtained from plants and used in human and animal feed to improve their health status and also to increase digestive enzymes secretion (Pandey et al., 2019). The PFA can be available in different forms such as herbs, spices, essential oils, or oleoresin (Singh and Gaikwad, 2020). The pure forms PAF and their different combinations have been used in many in vitro and in vivo experiments to reveal the immune-potential, anti-inflammatory, antioxidant, anthelmintic, coccidiostats, antiviral, and antibacterial properties (Oladeji et al., 2019). The useful effects of PFA on poultry may come from feed intake and activation of digestive secretions (Windisch et al., 2008; Pirgozliev et al., 2019).

The black cumin (*Nigella sativa*) seeds are the most valuable medicinal seeds in history widely. It is grown in different parts of the world, especially in the Middle East and South Asia countries, to support the health (Koç et al., 2019; Yimer et al., 2019). The seeds of *Nigella. sativa* (*N. sativa*) have different types of pharmacologically active substances, such as thymol, dithymoquinone, thymoquinone, carvacrol, alpha-hedrin, nigellicine-N-oxide, and nigellidine, which help in strengthening of paraimmunity (Ghaly et al., 2017; Saleh et al., 2018). The seeds of *N. sativa* have been used to treat coughs, dropsy, vomiting, diarrhoea, obesity, abdominal pain, stomachic, hepatic disorders, intermittent fevers, asthma, rheumatism, skin diseases, diuretic, joint pain, diaphoretic, dyspepsia, carminative, migraine, and chronic headache (Hussain and Hussain, 2016; Srinivasan, 2018; Arif, 2019; Akbar, 2020). The *N. sativa* and its various extracts have antibacterial activity and also inhibit the production of aflatoxin (Abdallah, 2017). The importance of *N. sativa* is highly increased in the treatment of several diseases, such as antitumor activity (Majdalawieh and Fayyad, 2016; Majdalawieh et al., 2017), antioxidant activity (Ahmed et al., 2018), anti-inflammatory activity (Ikhsan et al., 2018), antibacterial activity (Georgescu et al., 2018), and stimulating effects on the immune system (Bektaş et al., 2019). Most of the researches show that *N. sativa* seeds have antiparasitic, anthelmintic, antidiabetic, analgesic, a bronchodilator, renal protective spasmolytic, and antihypertensive properties (Koç et al., 2019; Selvaraju and Dhanraj, 2019). The grinded seeds of *N. sativa* have beneficial effects on metabolism, growth, and meat quality in broilers and also improve the performance layers and its egg quality (Kumar and Patra, 2017; Abou-Elkhair et al., 2018; Kumar et al., 2018; Seidavi et al., 2020). The *N. sativa* and its ingredients enhanced cell-mediated immunity but suppressed humoral immunity. Therefore, scientists all over the world have great interest in this seed, and *N. sativa* has been extensively studied (Dajani et al. 2016; Tavakkoli et al., 2017).

Curcuma longa (*C. longa*) is generally known as turmeric. It is mainly used in powdered form. It also has therapeutic properties (Omosa et al., 2017; Soleimani et al., 2018). The volatile oils and curcuminoid pigments, oleoresins, and extracts are the main secondary metabolites of turmeric that are responsible for the pharmacological actions of turmeric powder (Nelson et al., 2017; Rathore et al., 2020). Turmeric is used as a blood purifier and used in the prevention and treatment of skin diseases (Ahmed et al., 2020). It was found that the main functions of *Curcuma rhizome* were hepatoprotective (Sadashiva et al., 2019; Ibrahim et al., 2020), anti-inflammatory (Tejada et al., 2016; Xiang et al., 2017), antimicrobial (Singh et al., 2017; Ilangovan et al., 2018), antiviral (Alsamydai and Jaber, 2018), antifungal (Hu et al., 2017), wound healing (Tejada et al., 2016), antitumour (Shakeri et al., 2019), antivenom (Sani et al., 2019), and immunomodulator (Mehala and Moorthy, 2008). The results revealed the therapeutic potential of *C. longa* crude extracts, including polysaccharides and curcuminoids as a supportive supplement for cancer patients whose immune systems were crushed during chemotherapy. The effect of *C. longa* was investigated by the performance of broilers. The *C. longa* increases the total number of leukocytes and erythrocytes count. Turmeric has no adverse effects on health and food, so it has been concluded that turmeric can be used as a feed additive to improve the overall performance of broiler (Ahlawat, 2017).

MATERIALS AND METHODS

Animals and Ethics Statement

We used the one-day-old Arbor Acre broiler as experimental birds purchased from Anhui Anqin Poultry Company Ltd. (Anhui, PR China). The one-day-old Arbor Acre broiler was kept under a healthy and controlled environment, and the temperature was also maintained according to the age and behavior of birds (28°C–33°C). We tried our best to provide them with a clean and comfortable environment. All birds were taken care according to the Institutional Animal Care and Use Committee guidelines by the school of animal science and technology, Anhui Agricultural University,

Hefei, PR China (AHAU 2019-010). We are very sorry to do the experiments at the cost of their lives, but these chicks made a great contribution to scientific research to save more chickens in future and improve the health of the human being.

Bacterial Strain and Media

In this study, we used the clinical strain of *P. multocida* obtained from Key Laboratory of Veterinary Pathobiology and Disease Control, College of Animal Science and Technology, Anhui Agricultural University, Hefei, 230036, Anhui, PR China. The bacteria were grown in lysogeny broth medium at 37°C and 150 RPM. This study was conducted at the experimental shed of the pathology department, laboratory of veterinary pathobiology and diseases control, Anhui Agricultural University.

Experimental Design

One hundred one-day-old chicks were purchased from the local market and divided into 5 groups of 20 viz. 1, 2, 3, 4, and 5. The feed and water were given ad libitum. Group 1 served as a noninfected nontreated control group in which no infection with bacteria and no treatment with *N. sativa* and *C. longa* was given. Group 2 was challenged with *P. multocida* with a dose rate of 5.14×10^7 CFU by IV at day 28 (Zhang et al., 2013). Group 3 was divided into 2 subgroups 3A and 3B of 10 birds each. Group 3A was offered *N. sativa* 2% in powder form in the feed from day 1, not challenged, while group 3B was offered *N. sativa* 2% in powder form in the feed from day 1 and was challenged with *P. multocida* at day 28. Group 4 was be divided into 2 subgroups 4A and 4B of 10 birds each. Group 4A was given *C. longa* 1% in powder form in the feed from day 1 and not challenged, while group 4B was offered *C. longa* 1% in powder form in the feed from day 1 and was challenged with *P. multocida* at day 28. Group 5 was divided into 2 subgroups 5A and 5B of 10 birds each. Group 5A was offered both *N. sativa* 2% and *C. longa* 1% in powder form in the feed from day 1, and group 5B was offered both *N. sativa* 2% and *C. longa* 1% in powder form in the feed from day 1 and was challenged with *P. multocida* at day 28.

Vaccination

All routine vaccines were administered to experimental birds according to standard vaccination schedule and protocol.

Mortality

The mortality and postmortem examination for each dead bird was carried out to determine the cause of death. The dead birds were weighed to adjust in feed efficiency and weight gain.

Feed Conversion Ratio

The FCR was calculated on day 42. The average weight of all day-old chicks was 45.11 g on day 1. The feed consumption and average weight gain were calculated on the 28th day of the experiment to evaluate the feed conversion rate (**FCR**) according to the following formula (Marcu et al., 2013):

$$\text{FCR:} \frac{\text{Feed consumed}}{\text{Body weight gain}}$$

Gross Pathology

At the end of the trail (after 7 wk), all chicks were slaughtered to observe the gross pathological changes.

Histopathology

After postmortem examination, tissue samples from the spleen, liver, and lungs were quickly excised and preserved in 10% buffered formalin; the preserved tissues were quickly processed for histopathological examination. All standard protocols for histopathological examination, including fixation, washing, dehydration, clearing, embedding, block preparation, sectioning, and mounting, were adopted (Abdallah et al., 2020).

Staining

Eosin and hematoxylin were used for the staining of tissue sections. Then stained slides were examined under different magnifications for prominent lesions (Awad et al., 2009).

Hemagglutination Inhibition

On the 14th, 21st, and 28th day, the ordinary plain vacutainers (red top) were used to collect the blood sample from the wing veins of 5 randomly selected birds in each group. Allow blood samples to coagulate to separate the serum and for Hemagglutination Inhibition (**HI**) test (Kokate et al., 2017). The HI tests were performed to determine the antibody titer against NDV (Wambura and Mzula, 2017; Baksi et al., 2018). Reference NDV (Velogenic) antigen was obtained from the laboratory of veterinary pathobiology and diseases control, Anhui Agricultural University. The HI positive results shows prominent button formation in the bottom of microtitration plate wells. Whereas, HI negative results shows clumped RBCs form a uniform thin layer in the wells of microtitration plate. HI titer was equal to the highest serum dilution level that inhibits hemagglutination activity (Wambura and Mzula, 2017; Baksi et al., 2018; Choudhury et al., 2017). The geometric mean titers (GMT) of different groups were calculated.

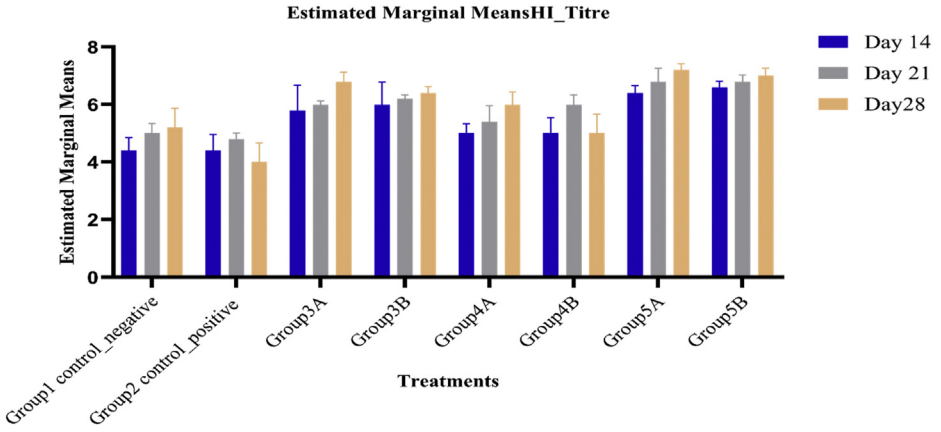

Figure 1. Estimated marginal mean titer of each group, which are highly significant ($P < 0.05$) from each other.

Statistical Analysis

The statistical analysis was performed by SPSS 16.0 (SPSS Inc., Chicago, IL). The results of all experiments were analyzed by one-way analysis of variance, followed by Tukey's honestly significant differences for post-hoc testing to compare the significance (P) between the means of different groups. $P < 0.05$ was considered to indicate a significant difference between the values compared.

RESULTS

Antibody Titer Against NDV in Different Bird's Groups

HI test was performed against NDV at day 14, 21, and 28 of the age of flock to evaluate the immunomodulatory effects of *N. sativa* and *C. longa* in trail poultry broiler birds. The GMT and cumulative geometric mean titers (**CGMT**) values of HI test (Figure 1) are described in the following paragraphs.

Group 1 served as noninfected, nontreated control group. The GMT values of HI test against NDV at day 14, 21, and 28 were $\log_2^{4.4}$, $\log_2^{5}$, and $\log_2^{5.2}$, respectively, calculated, and the CGMT value was 4.86 ± 0.41 (Table 1). Group 2 was challenged with *P. multocida* at day 28 and served as a positive control in which no treatment with *N. sativa* and *C. longa*. The GMT values of HI test against NDV at day 14, 21, and 28 were $\log_2^{4.4}$, $\log_2^{4.8}$, and $\log_2^{4}$, respectively, calculated, and the CGMT value was 4.40 ± 0.40 (Table 1). Group 3A was offered 2% *N. sativa* powder in the feed from day 1 to the end of the trail and no challenge with *P. multocida*. The GMT values of HI test against NDV at day 14, 21, and 28 were $\log_2^{5.8}$, $\log_2^{6}$, and $\log_2^{6.8}$, respectively, calculated, and the CGMT value was 6.20 ± 0.52 (Table 1). Group 3B was also offered 2% *N. sativa* powder in the feed from day 1 to the end of the trail and challenged with *P. multocida* at day 28. The GMT values of HI test against NDV at day 14, 21, and 28 were $\log_2^{6}$, $\log_2^{6.2}$, and $\log_2^{6.4}$, respectively, calculated, and the CGMT value was 6.20 ± 0.20 (Table 1). Group 4A was offered 1% *C. longa* powder in the feed from day 1 to the end of the trail and not challenged with *P. multocida*. The GMT values of HI test against NDV at day 14, 21, and 28 were $\log_2^{5}$, $\log_2^{5.4}$, and $\log_2^{6}$, respectively, calculated, and the CGMT value was 5.46 ± 0.50 (Table 1). Group 4B was offered 1% *C. longa* powder in the feed from day 1 to the end of the trail and challenged with *P. multocida* at day 28. The GMT values of HI test against NDV at day 14, 21, and 28 were $\log_2 5$, $\log_2 6$, and $\log_2 5$, respectively, calculated, and the CGMT was 5.13 ± 0.1 (Table 1). Group 5A was offered 2% *N. sativa* and 1% *C. longa* in powder form in the feed from day 1 to the end of the trail and not challenged with *P. multocida*. The GMT values of HI test against NDV at day 14, 21, and 28 were $\log_2^{6.4}$, $\log_2^{6.8}$, and $\log_2^{7.2}$, respectively, calculated, and the CGMT value was 6.80 ± 0.40 (Table 1). Group 5B was offered 2% *N. sativa* and 1% *C. longa* in powder form in the feed from day 1 to the end of the trail and challenged with *P. multocida* at day 28. The GMT values of HI test against NDV at day 14, 21, and 28 were $\log_2^{6.6}$, $\log_2^{6.8}$, and $\log_2^{7}$, respectively, calculated, and the CGMT value was 6.80 ± 0.20 (Table 1).

Table 1. Antibody titer against NDV in different bird groups (mean ± standard error; n = 3).

Groups	Days (HI titer) GMT			CGMT ± S.D
	14 d	21 d	28 d	
Groups 1	4.4	5.0	5.2	4.86 ± 0.41
Groups 2	4.4	4.8	4.0	4.40 ± 0.40
Groups 3A	5.8	6.0	6.8	6.20 ± 0.52
Groups 3B	6.0	6.2	6.4	6.20 ± 0.20
Groups 4A	5.0	5.4	6.0	5.46 ± 0.50
Groups 4B	5.0	6.0	5.0	5.13 ± 0.11
Groups 5A	6.4	6.8	7.2	6.80 ± 0.40
Groups 5B	6.6	6.8	7.0	6.80 ± 0.20

Feed Conversion Ratio of Each Group

Group 1 served as noninfected, nontreated control group. The average feed intake and average body weight gain was 1,140 g ± 460.88 g and 523 g ± 189.14 g, respectively (Figure 2). The average feed conversion ratio was 2.17 ± 0.49 (Table 2). Group 2 was challenged with *P. multocida* by IV at day 28 and served as a positive control in which no treatment was given. The average feed intake and average body weight gain were

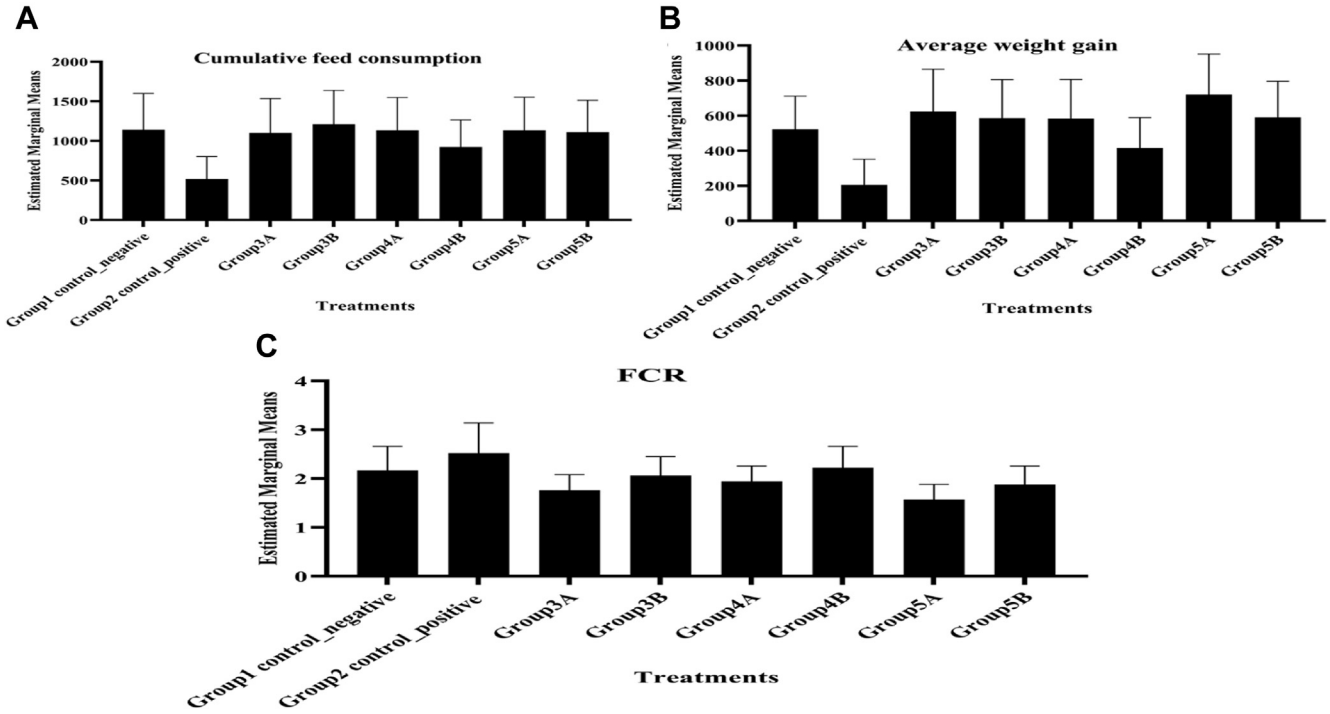

Figure 2. Cumulative feed consumption, average weight gain, and FCR each group were highly significant ($P < 0.05$) from each other.

518 g ± 285.28 g and 205 g ± 146.79 g, respectively (Figure 2). The average feed conversion ratio was 2.52 ± 0.62 (Figure 2). Group 3A was offered 2% *N. sativa* powder in the feed from day 1 to the end of the trail and not challenged with *P. multocida*. The average feed intake and average body weight gain were 1,100 g ± 435.56 g and 624 g ± 241.20 g, respectively. The average feed conversion ratio was 1.76 ± 0.32 (Table 2). The Group 3B was also offered 2% *N. sativa* powder in the feed from day 1 to the end of the trail and challenged with *P. multocida* at day 28. The average feed intake and average body weight gain were 1,210 g ± 429.46 g and 586 g ± 219.96 g, respectively. The average feed conversion ratio was 2.06 ± 0.39 (Table 2). The Group 4A was offered 1% *C. longa* powder in the feed from day 1 to the end of the trail and not challenged with *P. multocida.* The average feed intake and average body weight gain were 1,134 g ± 414.57 g and 584 g ± 222.82 g, respectively. The average feed conversion ratio was 1.94 ± 0.32 (Table 2). Group 4B was offered 1% *C. longa* powder in the feed from day 1 to the end of the trail and challenged with *P. multocida* at day 28. The average feed intake and average body weight gain were 923 g ± 343.93 g and 415 g ± 174.94 g, respectively. The average feed conversion ratio was 2.22 ± 0.44 (Table 2). Group 5A was offered 2% *N. sativa* and 1% *C. longa* in powder form in the feed from day 1 to the end of the trail and not challenged with *P. multocida*. The average feed intake and average body weight gain were 1,134 g ± 418.99 g and 720 g ± 232.04 g, respectively. The average feed conversion ratio was 1.57 ± 0.31 (Table 2). Group 5B was offered 2% *N. sativa* and 1% *C. longa* in powder form in the feed from day 1 to the end of the trail and challenged with *P. multocida* at day 28. The average feed intake and average body weight gain were 1,110 g ± 404.14 g and 590 g ± 207.10 g, respectively, calculated. The average feed conversion ratio was 1.88 ± 0.38 (Table 2).

Mortality of Birds before Challenged

A complete record of mortality was noted daily from each group of experimental birds. Before challenging *P. multocida*, some mortality occurs in the following experimental groups. One bird out of twenty was found to have died in group 2 (Table 3).

Table 2. Feed conversion ratio of each group (mean ± standard error; n = 3).

Groups	Feed consumption (g)	Average weight gain (g)	Feed conversion ratio
Groups 1	1,140 ± 460.88	523 ± 189.14	2.17 ± 0.49
Groups 2	518 ± 285.28	205 ± 146.79	2.52 ± 0.62
Groups 3A	1,100 ± 435.56	624 ± 241.20	1.76 ± 0.32
Groups 3B	1,210 ± 429.46	586 ± 219.96	2.06 ± 0.39
Groups 4A	1,134 ± 414.57	584 ± 222.82	1.94 ± 0.32
Groups 4B	923 ± 343.93	415 ± 174.94	2.22 ± 0.44
Groups 5A	1,134 ± 418.99	720 ± 232.04	1.57 ± 0.31
Groups 5B	1,110 ± 404.14	590 ± 207.10	1.88 ± 0.38

Table 3. Before and after infection mortality during experiment (mean ± standard error; n = 3). Mean values followed by the same letter in the same column do not vary significantly ($P < 0.05$).

	Days						
	Mortality before challenged	Mortality after challenged					
Groups	2	31	34	37	39	40	Total mortality percentage
Groups 1	1	0	0	0	0	0	5
Groups 2	0	2	3	3	2	3	65
Groups 3A	0	0	0	0	0	0	0
Groups 3B	0	0	1	0	0	0	10
Groups 4A	0	0	0	0	0	0	0
Groups 4B	0	1	1	0	0	0	20
Groups 5A	0	0	0	0	0	0	0
Groups 5B	0	0	0	0	0	0	0

Clinical Signs and Necropsy Findings after Challenged

The *N. sativa, C. longa,* and combination of both *N. sativa* and *C. longa* in powder form were given in the feed from day 1 to all groups. All groups were closely watched for any clinical sign of disease after challenging with *P. multocida* at day 28 in group 2, 3B, 4B, and 5B. All experimental birds were taking their feed and water properly till 48 h, but after that time, most of the birds from group 2 (positive control) show depression and poor feed consumption, and within the first week after infection, the birds of group 2 showed sever type of clinical signs such as fever, anorexia, ruffled feathers, mucous discharge from mouth, white diarrhoea arthritis, and reduced feed consumption. Other groups also showed clinical signs and symptoms such as reduced feed and low weight gain. At day 31, two birds from group 2, and one bird from group 4B were died, whereas, at day 34, three birds from group 2, one from group 3B, and one from group 4B were died. The number of birds died from group 2 was 3, 2, and 3 at day 37, 39, and 40, respectively, but most birds of groups 3B, 4B, and 5B were showing activeness, started feeding, and getting toward recovery.

Necropsy Findings

The group 2 and group 4B necropsy findings were hemorrhagic carcass and fibrino necrotic dermatitis on abdomen and breast muscle (Figure 3). Petechial hemorrhages were seen on the heart and liver, the liver was enlarged, swollen, and cooked appearance (Figure 3). Numerous small, irregular-shaped yellow necrotic foci were present on the liver. Large size spleen in some birds and hemorrhagic and odematic lungs were also found (Figure 3). Dark, friable lungs in some birds were also obvious—petechial hemorrhages on the intestine, duodenal enteritis, and caseous material in hock joint.

Histopathology

Group 1 served as a negative control group, in which no natural feed additives and no challenge were given. Figure 4A shows normal liver section; histologically it is divided into 2 lobules, the central and peripheral lobule. The central lobule is the central vein, and periphery lobule is portal triads. Under 40× magnification, the liver cells look polygonal or cuboidal in shape with large, single, and spherically shaped nuclei in the center. Sinusoids are capillaries that have wide sinusoidal lumens, and they are lined by a discontinuous endothelium and large intercellular gaps. These sinusoids provide a maximum exchange of materials between blood and hepatocytes. Figure 4B shows the normal histologic appearance of the spleen. The lymphocytes gathered around the splenic arteriole and formed a white pulp. Red pulp comprises of many splenic sinusoids. Seventy-five percent of the splenic volume is red pulp, while the white pulp is separated by marginal zone. Figure 4C represents the normal histologic appearance of lungs tissue sections. The tissues of the lungs are composed of thin-walled alveoli, and alveoli are composed of a single layer of squamous epithelium. This slide contains sections of many bronchioles of various sizes. Bronchioles can be recognized because they are lined by cuboidal epithelium (smaller bronchioles leading to alveoli) or ciliated columnar epithelium (larger bronchioles).

The group 2 served as a positive control. After giving infection at day 28, 13 birds died out of 20 till the end of experiments. On histopathology, the liver, spleen, and lungs showed specific microscopic lesions associated with *P. multocida* infection (Figure 5). However, Group 3B (Infection + Nigella sativa), the morphology of liver section is normal, spleen section of morphology of all cells is normal, lungs section morphology of all cell is normal (Figure 6). Moreover, Group 4B (Infection + Curcuma longa), the liver section representing a mild congestion and mild fatty change, spleen section representing a mild congestion, and lungs section representing a mild congestion (Figure 7). Furthermore, Group 5B (Infection + Curcuma longa + Nigella sativa), the normal liver section, spleen section representing a normal section, and lungs section representing a normal section (Figure 8).

DISCUSSION

This study was carried out to check the response of lymphatic tissue of broiler chicken that has been

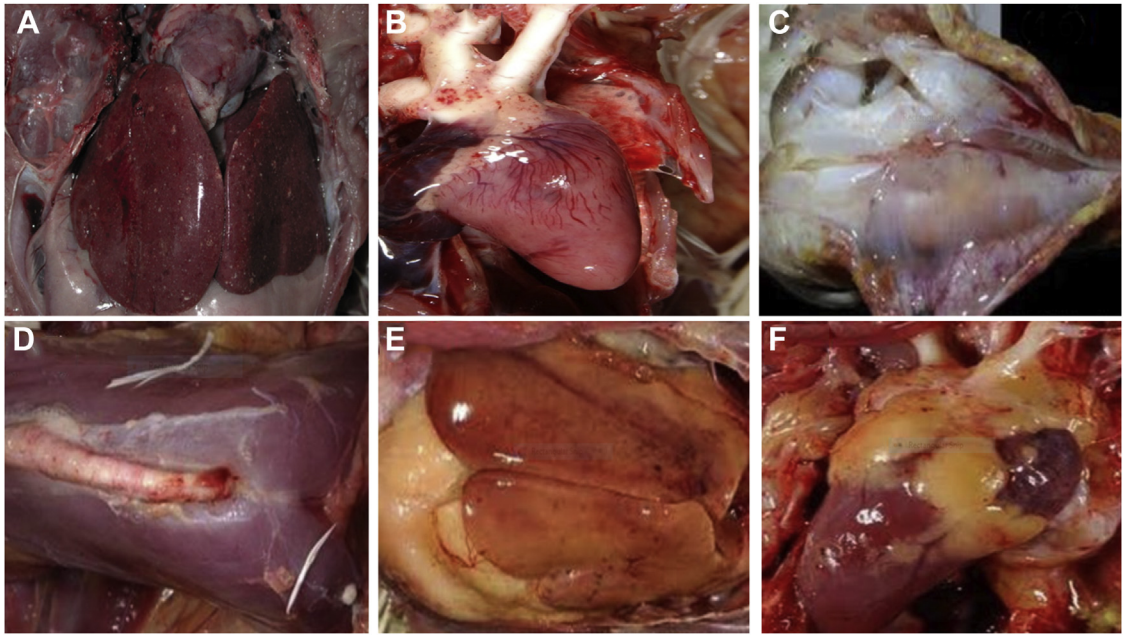

Figure 3. Gross pathological changes. (A) Group 2 showing numerous, small, irregular-shaped yellow necrotic foci on liver; (B) group 2 showing hemorrhages on heart; (C) group 2 representing caseous material in joints; (D) group 2 representing the swelling of sternal bursa; (E) group 4B representing enlarged, swollen, cooked appearance, and petechial hemorrhages on the liver; (F) group 4B showing hemorrhages on heart.

experimentally challenged with *P. multocida* and supplemented with *N. sativa* and *C. longa*. The experiment was conducted to evaluate the protective and immunomodulatory properties of *N. sativa* and *C. longa* in broilers birds. The number of dead birds in group 1 was one, whereas in group 2, 3A, 3B, 4A, 4B, 5A, and 5B, it was 13, 0, 1, 0, 2, 0, and 0, respectively. All these groups were highly significant ($P < 0.05$) from each other. But in the study of Hermes et al. (2011), the results were that the average percentage of mortality rate was relatively high (23.6%) in the positive control group.

The most typical acute case symptom was green diarrhoea, but in chronic case, it is inflammation of hemorrhagic carcass, hock joints, sternal bursa and footpads—petechial hemorrhages on the heart and liver. The liver was enlarged, swollen, and cooked in appearance. Numerous small, irregular-shaped yellow necrotic foci were present on the liver. Large-size spleen in some birds and hemorrhagic and odematic lungs were also found. Dark, friable lungs in some birds were also obvious. The petechial hemorrhages on the intestine, duodenal enteritis were observed, and caseous material in the hock joint was also obvious (Ghaly et al., 2017; Tang et al., 2017). Mostly all other birds remained alive because of the immunomodulatory and antibacterial effects of *N. sativa* and *C. longa*; our results are lined with those of Arslan et al. (2017), and according to their study, black seed (*Nigella sative*) increased the total WBCs, and the difference was significant for the white hens ($P < 0.02$).

Black seed generally enhanced cell-mediated immune response and antibody titer. In addition to its antiviral and antihelminthic effects, *N. sativa* also showed antibacterial activity such as gram-positive and gram-negative bacterial strains (Shamim Molla et al., 2019). The birds receiving *C. longa* (group 4A and 4B) in their

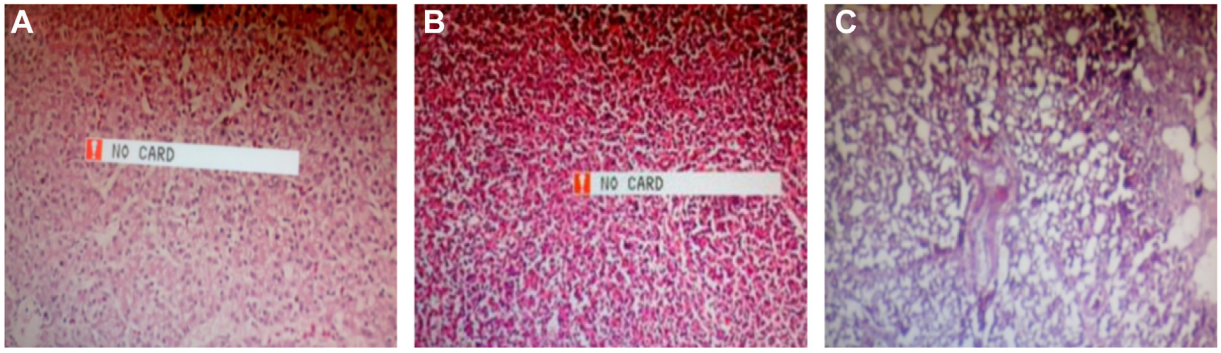

Figure 4. Group 1, control negative group (no feed additives + no infection). (A) Liver section normal; (B) spleen section normal; (C) lungs section normal.

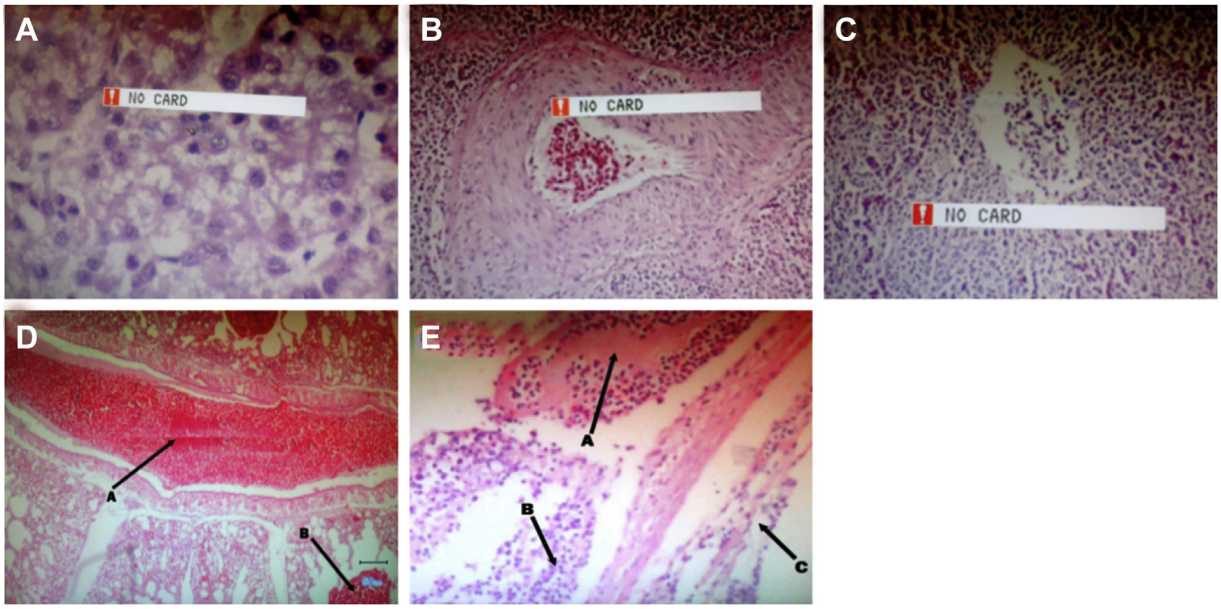

Figure 5. Group 2, control positive (no feed additives + infection); (A) liver section showing congestion, infiltration of inflammatory cell, fatty changes and coagulative necrosis of hepatocyte can also be seen; (B) spleen section showing hyperemia and congestion; (C) spleen section showing congestion, hyperemia, atrophy of cell, dead tissue accumulation, blast cell accumulation, degeneration and necrosis of parenchyma; (D) lungs section showing (A) engorgement of blood vessel with RBCs, (B) congestion of RBCs in mesobronchus; (E) lungs section showing (A) pulmonary odema, (B) infiltration of heterophils, (C) sloughing of epithelium.

feed were also immune against *P. multocida*. That is why they remain normal after infection, and our results are lined with those of Al-Mufarrej (2014).

In our study, the HI titer of all experimental groups was measured at day 14, 21, and 28. The CGMT of group 1, 2, 3A, 3B, 4A, 4B, 5A, and 5B was 4.86 ± 0.41, 4.40 ± 0.40, 6.20 ± 0.52, 6.20 ± 0.20, 5.46 ± 0.50, 5.13 ± 0.11, 6.80 ± 0.40, and 6.80 ± 0.20, respectively. These CGMT titers were significantly different from each other, but according to the study of Fallah and Mirzaei, 2016, it was revealed that the inclusion of *C. longa* in poultry diets improved antibody titer and that there was a significant difference in feed consumption. At the same time, there was no significant difference in packed cell volume, RBC, and Hb. In WBC there was a significant difference in H/L ratio, albumin and globulin. Significantly ($P < 0.05$) higher level of globulin suggested that birds of the treated group had better humoral immune status than the control group. Results indicate that powder of *N. sativa* seed increases antibody titer in broiler birds (Dair and Ali, 2016). But the birds supplemented with both *N. sativa* and *C. longa* have a greater effect on blood serum and increase antibody titer.

The FCR of all experimental groups was measured at day 1, 7, 14, 21, 28, 35, and 42. The FCR of groups 1, 2, 3A, 3B, 4A, 4B, 5A, and 5B was 2.17 ± 0.49, 2.52 ± 0.62, 1.76 ± 0.32, 2.06 ± 0.39, 1.94 ± 0.32, 2.22 ± 0.44, 1.57 ± 0.31, and 1.88 ± 0.38, respectively. The *C. longa* effect on the FCR of birds is in line with the findings of Choudhury et al. (2017). These results are also similar to those of Ürüşan and Bölükbaşı (2017). Supplementation of *N. sativa* in the broiler ration significantly ($P < 0.05$) improved the weight of the birds of various groups compared with those of the control group (Tehseen et al., 2016); this finding was also supported by Kumar

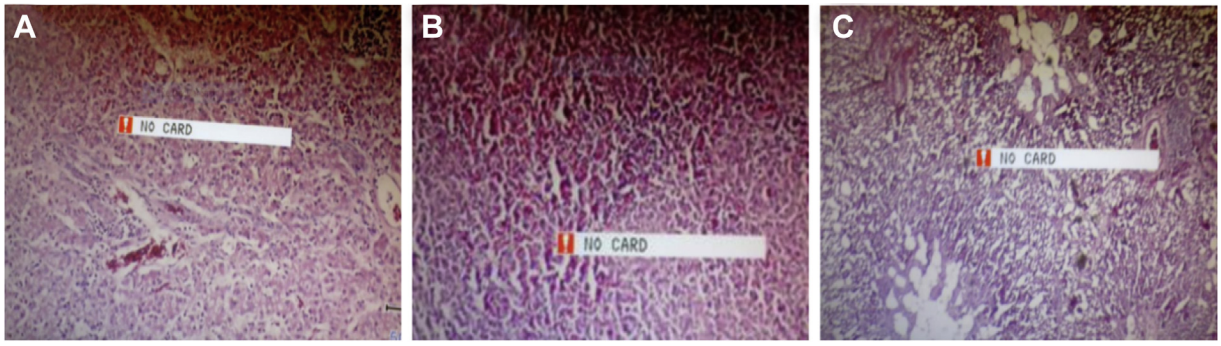

Figure 6. Group 3B (Infection + *Nigella sativa*), (A) morphology of liver section is normal; (B) spleen section of morphology of all cells is normal; (C) Lungs section morphology of all cell is normal.

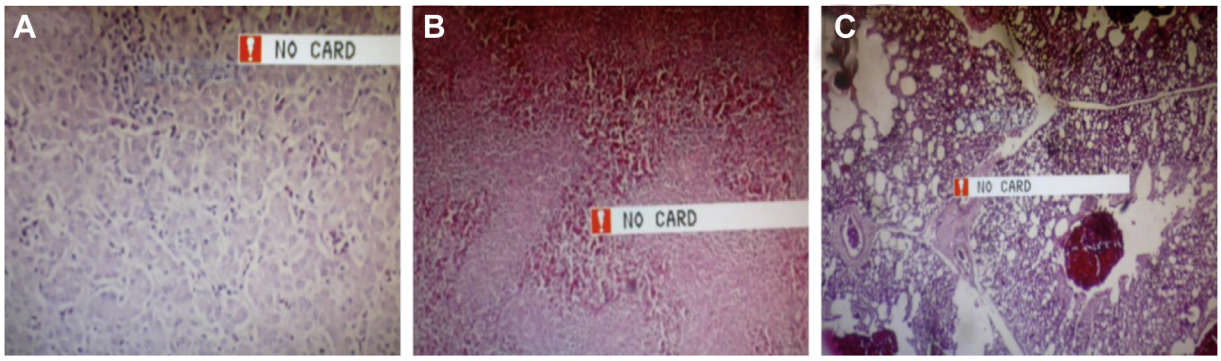

Figure 7. Group 4B (Infection + *Curcuma longa*); (A) liver section representing a mild congestion and mild fatty change; (B) spleen section representing a mild congestion; (C) lungs section representing a mild congestion.

et al. (2017). The cumulative effect of *N. sativa* and *C. longa* is greater on FCR of broiler than when these products are individually used.

Histopathology of *N. sativa*–taking birds was normal because *N. sativa* has different types of pharmacologically active substances such as thymol, dithymoquinone, thymoquinone, carvacrol, alpha-hedrin, nigellicine-N-oxide, and nigellidine. These substances are effective in treating various ailments and proved to be beneficial in improving immunity (Islam et al., 2019). The *N. sativa* is one of the most valued medicinal seeds in history (Dubey et al., 2016). *N. sativa* is a phytogenic immunostimulants that help in establishment and strengthening of para immunity (Yimer et al., 2019). The histology of *C. longa*–supplemented birds was also normal because of curcuminoid pigments (diarylheptanoids; 30–45%) and volatile oil (15–20%), which contains zingiberene (25%), turmerone (60%), and minor amounts of sesquiterpenes, phellandrene, eucalyptol, sabinene, and borneol (Lim, 2016; Hosseini et al., 2017). In current years, the assessment of the antioxidant power of foods has got abundant attention. Most work has been performed on the anticancer and antioxidant activities of compounds obtained from the rhizomes of *C. longa*. *C. longa* has been widely used in both dried and fresh forms as a blood purifier, tonic, and stomachache relief and externally in the treatment and prevention of skin diseases (Vaughn et al., 2016). The foremost actions of *Curcuma rhizome* are hepatoprotective, anti-inflammatory, antimicrobial, antiviral, antifungal, wound healing, antitumor, and antivenom agents (Alsamydai and Jaber, 2018; Rahmani et al., 2018). The recent studies have also revealed the immunomodulatory properties of hot water extracts of *C. longa* and also examined using human peripheral blood mononuclear cells. To separate the active components liable for the activities, more partition with ethanol, ethyl acetate, and N-butanol was carried out gradually. The productions of cytokines (tumor necrosis factor, transforming growth factor, granulocyte macrophage colony-stimulating factor, and interleukins) have been moderated by a polysaccharide-enriched portion as shown in enzyme-linked immunosorbent assay and cytokine protein array (Abdollahi et al., 2018). This study clearly indicates that these 2 herbal products of *N. sativa* and *C. longa* have high immunomodulatory and antibacterial effects. So, these products are useful against *P. multocida*.

CONCLUSION

The use of natural feed additives to treat common medical diseases is a novel idea but still a challenging step in medicine. These traditional medicinal plants have received much attention because of many reasons such as low adverse effect, ease of access, and low cost

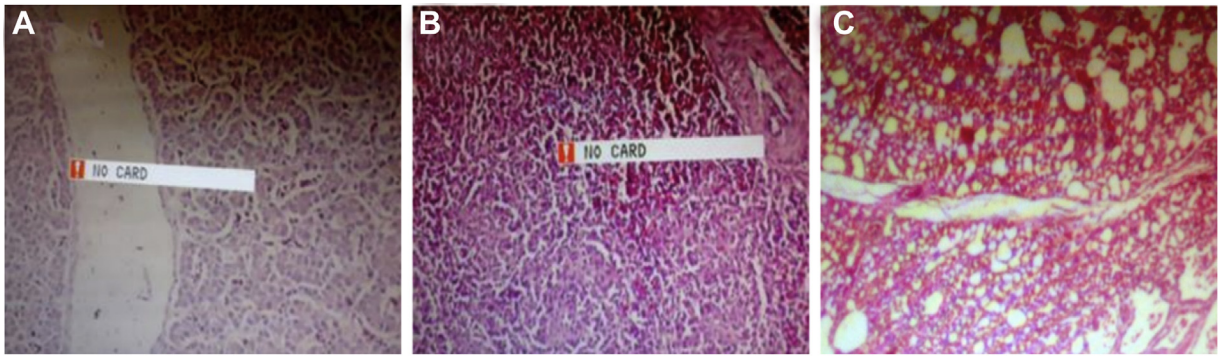

Figure 8. Group 5B (Infection + *Curcuma longa* + *Nigella sativa*); (A) normal liver section; (B) spleen section representing a normal section; (C) lungs section representing a normal section.

as compared to synthetic medicines. The inclusion of *C. longa* and *N. sativa* as an alternative to synthetic antibiotics in poultry feed has several benefits because of its antimicrobial and antioxidant properties. The results of this study indicated that the supplementation of natural feed additives *C. longa* and *N. sativa* improves the immune status, FCR, digestibility, and overall growth performance of broiler and also proves their effectiveness against *P. multocida* compared with the control group. The *C. longa* and *N. sativa* decreased abdominal fat and blood cholesterol level in blood diseases and that they may serve as catalysts for future research to demonstrate the efficacy and safety of both *C. longa* and *N. sativa* for the treatment of many human diseases. Moreover, the dietary supplementation of *C. longa* and *N. sativa* may lead to the development of low-cholesterol chicken meat as demanded by health-conscious consumers. Further researches are needed to get a better understanding of the effect of natural feed additives in poultry production and their beneficial impact on human health.

ACKNOWLEDGMENTS

Funding: This work was supported by the grant from National Natural Science Foundation of China, 31772707, and the University Synergy Innovation Program of Anhui Province, GXXT-2019-035.

DISCLOSURE

The authors have no conflicts of interest associated with this publication. They confirmed that the manuscript has been read and approved for submission by all the named authors.

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