Comparative Immunomodulatory Efficacy of Rosemary and Fenugreek against *Escherichia Coli*-Infection via Suppression of Inflammation and Oxidative Stress in Broilers

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Abstract

Broiler chickens are frequently infected with *Escherichia coli* (*E.Coli*) bacteria, which often leads to the emergence of many diseases and huge economic losses. Hence, the current study was conducted to evaluate the relative efficacy of dietary rosemary and fenugreek, under *E.Coli*-infection in broilers, via evaluation of growth performance, biochemical indices, immune response and histo-morphological changes. Eighty Cobb broilers were allotted to four equal groups (*n = 20* chicks/group); control non-infected (CN), control infected (CI), rosemary infected (RI) and fenugreek infected (FI) groups. The RI and FI groups revealed a significant elevation in their body weight and body weight gain compared with the CI group. However, both groups showed a significant decline in serum aspartate and alanine aminotransferase activities, as well as uric acid and creatinine levels. A significant decrease in total antioxidant capacity, catalase and superoxide dismutase activities were noted among CI chicks. Moreover, distinctly higher activities were evident in both RI and FI groups. Assessment of immunomodulatory markers showed a significant increase in immunoglobulin G along with a significant decline in interleukin-6 level in both RI and FI groups, with the lowest IL-6 value within FI group. Histopathological evaluations focused on the deleterious effect associated with *E-Coli* infection of broilers' liver, kidney, intestine, spleen, bursa of fabricius, and thymus. Partial histological improvement was noticed among RI group, and nearly normal tissues were recorded in FI group.

Overall, our findings suggest the ability of fenugreek to mitigate the adverse effects of *E.Coli*-infection on broiler performance and tissue profiles, by improving the general health status of the broiler chickens.

Introduction

In the global poultry industry, Escherichia coli (*E.Coli*) lead to significant economic losses each year (Lau et al., 2010). *Ecoli* infection is usually controlled with antibiotics. However, the emergence and continued use of the antibiotics in poultry feed has raised cross-resistance that poses substantial risks for human health (Asai et al., 2011; Ghozlan et al., 2017). One possibility is the application of probiotics, prebiotics and herbaceous plants or their essential oils (Sarica et al., 2007), to replace antibiotics without negative health impact or any loss of productivity (Demir et al., 2003; Maiorano et al., 2016).

Rosemary (*Rosmarinus officinalis*), a widespread household plant, is used as a natural additive to animal feed with its antibacterial, antifungal and antioxidant activities (Genena et al., 2008; Mohamed et al., 2016a), as well as a flavoring agent for food, beverages, and cosmetics preparations (Ibarra et al., 2010). It possess a number of therapeutic applications in medicine for the treatment or management of various pathological conditions such as inflammatory diseases, respiratory and GIT disorders (Al-Kassie and Abd-Al-Jaleel, 2011; El-Boshy et al., 2015). It is mainly composed of 0.5% volatile oil, flavonoids (diosmetin, genkwanin, diosmin, glucoside and luteolin), phenolic acids (rosmarinic, chlorogenic, neochlorogenic, labiatic and caffeic acids), carnosic acid, rosmaricine and isorosmaricine, triterpenic acids and others (Khan and Abourashed, 2010). Jafarı-sales and Pashazadeh (2020) indicated that 1.8 cineole and α-pinen was the highest essential oils in rosemary, and had a remarkable anti *E.Coli* activity, So it could be

used as a suitable alternative to synthetic antibiotics. Furthermor, **Ojeda et al. (2013)** reported a relationship between the antibacterial activity of different rosemary essential oils against Gram-positive and Gram-negative bacteria, and they related the activities to the changes in membrane permeability and disruption of the Ecoli cell membrane invitro.

Historically, fenugreek (*Trigonella foenum-fraecum*) is considered one of the oldest medicinal herbs (Djeridane et al., 2006). Its seeds are commonly used by people in Asia, Africa and Mediterranean countries as one of the ingredients in daily diets (Basch et al., 2003). In modern food technology, it is used as a food stabilizer, adhesive and emulsifier (Meghwal and Goswami, 2012). It is known to have several pharmacological properties (Benayad et al., 2014), including hypoglycemia (Sharma et al., 1990; Zia et al., 2001), hypocholesterolemia (Stark and Madar, 1993; Srinivasan, 2006), gastro-protective (Sujapandian et al., 2002), chemo-preventive (Amin et al., 2005), antioxidant (Kavirasan et al., 2007), anti-inflammatory **(Ahmadian et al., 2001)** and appetite stimulation (Petit et al., 1993). Previously reported data on the phytochemical composition of fenugreek, highlighted the presence of alkaloids (Petropoulos, 2002), flavonoids and phenolic acids (Kenny et al., 2013), polysaccharides (Petropoulos, 2002), triterpenoids (Shang et al., 1998), steroidal sapogenins (Taylor et al., 1997) and nicotinic acid (Rajalakshmi et al., 1964). The fenugreek seeds contain about 7.5 % of total lipids; neutral lipids, glycolipids, and phospholipids. Additionally, it shows a high content of palmitic, linolenic acids, and former acid (Petropoulos, 2002), a large carbohydrate fraction (mucilaginous fiber and galactomannan), as well as 20–30% proteins high in tryptophan and lysine; pyridine-type alkaloids; flavonoids; free amino acids (4-hydroxyisoleucine, arginine, lysine, and histidine); saponins; glycosides; vitamins, minerals, (28%) mucilage, 5 % of a stronger-smelling, bitter fixed volatile oils (Snehlata and Dande, 2012). The strong antioxidant free radical scavenging activity of fenugreek seeds correlates with the presence of carboxyl group in the seed oil that were more dominated by unsaturated essential fatty acids **(Akbari et al.,2013;** Baba et al., 2018). **Qureshi et al. (2015)** revealed the antibacterial properties of fenugreek seeds in vitro with zone of inhibition as 2.1 mm against *E.coli* on the Mueller Hinton Agar. This high growth inhibitory effect related to the presence of major compounds known to have antibacterial activity such as tannins and flavonoids (Chalghoumi et al., 2016).

Therefore, in this paper, we report on the *in-vivo* ameliorative effects of broiler ration supplementation with two medicinal plants; rosemary leaves (*Rosmarinus officinalis*) and fenugreek seeds (*Trigonella foenum-graecum*), challenging with experimental infection of *E.coli* for six weeks, on growth performance, some selective biochemical, immunological and antioxidant parameters, along with histopathological changes associated with hepatic, renal, intestinal, splenic, thymic and bursal tissues.

Materials And Methods

Experimental broiler chicks, E-coli strain and natural products

Eighty, one-day-old, apparently healthy commercial Cobb broiler were obtained from Alasma Masr Poultry Company, Egypt. *E.coli* strain O78 was obtained from Animal Health Research Institute, Ismailia, Egypt. *E.coli* colonies were grown in nutrient broth for 24 hours at 37^{o}c and viable number was adjusted to 4 x106 colony forming units (CFU). Each chick was inoculated with 0.5 ml of *E.coli* O78 bacterial inoculum, at 7 days old of age as the following: 0.25 ml intranasal and 0.25 ml via eye drop route. Rosemary leaves and fenugreek seeds were purchased from local commercial market of herbs and medicinal plants (Al-kateb Company, Egypt), were grounded by the home blender, and then were added to the balanced ration by 5gm plant powder/kg diet for each.

Chemicals and reagents

All commercial test kits for Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Uric Acid (UA), Creatinine (Cr), Cholesterol (Ch) and Glucose (G) were obtained from BIO-Merieux (Brains/France) and Ticho-Diagnostic (Sees, France). Diagnostic kits for chicken interleukin-6 (IL-6) ELISA kit (Genorise Scientific, INC), Chicken immunoglobulin G (IgG) ELISA kit (Bethyl Laboratories, INC.), Superoxide Dismutase (SOD) ELISA kit (Kamiya Biomedical), Catalase (CAT) catalase assay kit (Cayman Chemical) and Total Antioxidant Capacity (TAC) OxiSelect™ assay kit (Cell Biolabs, Inc) were used in the current study.

Birds grouping and treatment schedule

Upon arrival, birds were weighed and kept under standard sanitary conditions in floor pens covered with unused wood shavings litter, with free access to the balanced commercial basal ration (Table 1) and fresh tap water ad-libitum until the end of the experiment. The temperature was adjusted according to the age, 32^{o}C at the first week and then decreased 2^{o}C per week. All chickens were vaccinated according to the vaccination schedule. Birds were then allotted into four equal groups (*n = 20* chicks/group) as follow; control non-infected (CN) group, fed on balanced commercial ration free from any feed additives. Control infected (CI) group, fed on balanced commercial ration free from any feed additive but experimentally infected with *E.Coli* at one week of age (Majo et al., 1997). Rosemary infected (RI) group, fed on balanced commercial ration supplied with rosemary at the level of 0.5 % (5gm rosemary leaves powder /kg ration) from one day to 6 weeks old (Ghazalah and Ali, 2008) and experimentally infected with *E.Coli* at one week of age. Fenugreek infected (FI) group, fed on balanced commercial ration supplied with fenugreek at the level of 0.5 % (5gm fenugreek seed powder /kg ration) from one day to 6 weeks old (Elbushra, 2012) and experimentally infected with *E.Coli* at one week of age.

Growth performance parameters

Each chick was individually weighed at the beginning and end of the experiment (one day and 6th week old), respectively. Body weight gain (BWG) was calculated at 6th weeks of the experiment by subtracting the body weight between two consecutive weights. Feed consumption (FC) was calculated by subtracting the amount of feed remaining at the end of the 6th week from the amount of feed given at the beginning of the experiment. The amount of feed consumed was then divided by the weight gain for each group to obtain the feed conversion ratio (FCR) (Nobo et al., 2012).

Blood and tissue sampling

At the end of the experiment, five chicks were randomly obtained from each group for blood and tissue specimens' collection. Approximately 3 ml of blood samples were obtained by the puncturing the heart of each bird, collected in a plain centrifuge tube and then used for the preparation of serum for assay of biochemical, immunological and antioxidant parameters (TAC and CAT). After blood sampling, chicks were gently sacrificed, and small specimens from the liver, kidney, intestine, spleen, thymus and bursa were obtained for histopathological examination. Furthermore, parts of liver and kidney were stored at -20^{o}c for the antioxidant assay.

Sera biochemical parameters

Blood sera were then used for assessment of hepatic and renal injury biomarkers. ALT and AST activities was determined colorimetrically according to the method of **Crowley (1967).** UA was determined by uricase –POD enzymatic colorimetric method according to **Fossati et al. (1980)**. Cr was performed by photometric colorimetric test for kinetic measurement, methods without deproteinization, using readymade kits as described by the method of **Owen et al., (1954).** Serum glucose was determined according to **Kinoshita et al. (1979).** Cholesterol was determined by the enzymatic colorimetric method; CHOD-POD, according to the method described by Allain et al. (1974).

Cytokine's estimation

IL-6 was assessed and IgG concentrations were calculated according to the method of Koivunen and Krogsrud (2006**).**

Evaluation of antioxidant indices

SOD concentration was measured according to Koivunen and Krogsrud (2006**).** CAT was assessed according to Wheeler et al., (1990), meanwhile TAC was calculated according to Hariane and Moya (2015**).**

Histopathological examination

Liver, kidney, intestine spleen, bursa, and thymus samples, obtained from sacrificed birds of all groups, were freshly collected and then fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5–7 μm thickness and finally stained with Hematoxylin and Eosin (H&E) for histopathological examination. Routine histological procedures were carried out according to the method of Suvarna et al., (2012).

Statistical analysis

Data collected from biochemical, immunological and antioxidant assays of all treated groups were statically analyzed in compare to control group for the mean and standard error using statistical software program (SPSS for windows, version 16, USA). The difference between means of different experimental

groups were carried out using one-way ANOVA with Duncan multiple comparison tests. Dissimilar superscript letters in the same column show a significance (P > 0.05) (Landau and Everitt, 2004).

Results

The main clinical symptoms, observed among the *E.coli* infected birds, were dullness, depression, raffled feathers, huddling, reduced fed and water consumption, which appeared within 24 hrs. post infection. Respiratory signs were developed 2–3 days post infection; as sneezing, rhinitis and wet eyes. As summarized in **Table 2**, mortality was highest in CI group (25%) followed by that of RI (10%) and then the lowest percentage were recorded within FI (5%).

Growth Performance parameters

Growth performance parameters was summarized within Table 3 (supplementary data). The CI group showed a significant decline in BW and BWG compared to CN. Meanwhile, RI and FI groups revealed a significant increase in BW and BWG compared with CI, with more significance in FI group. Moreover, non-significant change was reported in FI group compared with CN (Fig. 1A, B). Concerning FC and FCR, CI group showed a significant decrease when compared with CN. Additionally, a significant decline of FC and FCR was noted among RI and FI groups in comparison with CI, with the less FC and FCR in RI group (Fig. 1C, D).

Cholesterol and glucose levels

Regarding cholesterol and glucose levels, CI group showed a significant increase and a significant decrease in cholesterol and glucose levels, respectively when compared CN (Fig. 2A, B). RI and FI groups revealed a significant decline in cholesterol level compared with CI (Fig. 2A). RI group showed a significant increase, while FI group revealed a significant decrease in glucose level in comparison with CI (Fig. 2B). RI group showed a non-significant change in glucose level when compared with CN (Fig. 2B).

AST and ALT activities, UA and Cr concentrations

As shown in Fig. 3, the CI group showed a significant increase in AST and ALT activities along with a significant elevation in UA and Cr levels when compared with CN. On the other hand, both RI and FI groups showed a significant decline in their levels compared with CI, with the least recorded value in FI.

Immunological profile

Pointing to IL-6 and IgG levels, the CI group revealed a significant increase in their levels compared with CN (Fig. 4A, B). Meanwhile, both RI and FI groups showed a significant decrease in IL-6 level (Fig. 4A) when comaped with CI, along with a significant elevation in IgG level (Fig. 4B) when comapared with CN and CI groups, with the lowest value of IL-6 and the highest IgG level obtained from FI group.

Hepatic and renal SOD activities

Referring to hepatic and renal SOD activities, the CI group showed a significant decrease in their levels when compared with CN. In contrary, RI and FI groups showed a significant elevation of SOD levels in comparison with CI (Fig. 5). A non-significant change in hepatic and renal SOD levels was noted among CN, RI and FI (Fig. 5).

Sera antioxidant indices

With regard to antioxidant indices, the CI group revealed a non-significant decline in TAC and CAT levels compared with CN group (Fig. 6A, B). RI and FI groups showed a significant increase of TAC and CAT levels compared with CI one (Fig. 6A, B). On the other hand, non-significant changes in TAC and CAT activities were recorded among RI and FI groups (Fig. 6A, B).

Histopathological evaluations

In all four treated-groups; hepatic, renal, intestinal, splenic, thymic and bursal specimens were processed further for histopathological analysis. Architectural changes were successively recorded in all selected organs of experimental broilers. The histological structures of all previously mentioned organs in response to different treatments were illustrated in Figs. 7, 8, 9, 10, 11 and 12, respectively.

The microscopical examination of hepatic tissue sections, obtained from CN group, demonstrated normal hepatic architecture along with normally arranged hepatocytes separated by hepatic sinusoids and radiated from the central vein. The hepatocytes appeared crowded polygonal cells with centrally located spherically basophilic nuclei and acidophilic cytoplasm (Fig. 7A). In contrast, CI group showed thick hepatic capsule with marked degenerative changes among hepatocytes, Kupffer cell hyperplasia, mononuclear leukocytic infiltration around the central vein, evidence of marked congestion and dilatation of central vein and sinusoids as well as marked diffuse necrobiotic changes of hepatic tissue. Such degenerative changes were evidenced by vacuolar degeneration in some pyknotic hepatocytes. Moreover, fibrous connective proliferation was observed around the portal area admixed with mononuclear leukocytic infiltration (Fig. 7B). RI birds' liver revealed very mild degenerative changes in hepatocytes with mononuclear cellular infiltration. The central vein slightly dilated and congested compared to control group (Fig. 7C). Meanwhile, FI birds' liver showed near normal hepatocellular organization and architecture, with very mild degenerative changes of some hepatocytes and less mononuclear cell infiltration, others showed regeneration in the rest of the cells. Additionally, the central vein appears normal (Fig. 7D).

The selected renal sections obtained from CN group revealed normal renal histological structures of the glomeruli and surrounding tubules (Fig. 8A). However, infected birds showed marked degenerative changes of tubular cells and areas of mild interstitial infiltration of mononuclear leukocytic cells were noticed among the renal cortex of this treated group. Additionally, congestion of the renal blood vessels

and inter-tubular capillaries were also observed along with extravasated RBCs among this group (Fig. 8B). The degenerative changes of tubular cells are indicated by vacuolar and hydropic degeneration. Additionally, individual epithelial cells were shrunken with pyknotic nuclei. Concerning RI birds, the kidneys showed mild congestion of the renal blood vessels and inter-tubular capillaries. Additionally, the lining epithelium of the convoluted tubules was mostly appeared degenerated (Fig. 8C). The degenerated changes of renal structures were seen to be disappeared in FI group which exhibited near normal renal features (Fig. 8D).

Control untreated birds revealed normal intestinal architecture with uniform intestinal villi lined by columnar epithelium with goblet cells in between, as well as intestinal glands located between the bases of villi in intestinal mucosal layer (Fig. 9A). Even the intestine of infected birds showed vacuolation, atrophy, sloughing and necrosis of intestinal villi along with leukocytic infiltration (mainly hetrophils, macrophage and lymphocyte) associated with edema and necrosis of the muscularis mucosa (Fig. 9B). The intestinal tissue architecture of RI birds revealed some degenerative changes of the intestinal architecture but less than that picture recorded in an infected group alone (Fig. 9C). Meanwhile, FI birds showed normal villus architecture with mild cellular infiltration in intestinal mucosa and sub-mucosa when compared with the control (Fig. 9D).

Histological examination of splenic sections obtained from CN broilers showed no difference in splenic architecture enclosing normal white and red pulps (Fig. 10A). Additionally, the splenic red pulp formed mainly from cords of reticular and blood cells associated with immunocompetent cells; macrophages and lymphocytes. The white pulp is the splenic lymphatic tissue, composed mainly of lymphoid follicles with periarterial sheath (Fig. 10A). Meanwhile, the infected group showed noticeable pathological changes among splenic parenchyma when compared to control group. These changes include lymphocytic depletion and degeneration (Fig. 10B). Additionally, massively congested areas within the splenic red pulp were noted. Marked increasing of the area red pulp on the expense of the white one was also recorded among this infected group. RI group showed a significant difference from that of infected birds without any treatment including relative improvement of white pulp containing small-sized lymphoid follicles with mild to moderate congestion of splenic blood vessels along red pulp (Fig. 10C). Splenic parenchyma restored its architecture to almost the normal picture and appeared to be regenerated after fenugreek treatment with mild congestion of splenic blood vessels (Fig. 10D).

The present light microscopic study of thymic sections from control untreated birds revealed thin connective tissue capsule surrounded the gland, numerous fine septa of connective tissue originated from the capsule were divided the organ into incompletely separated lobules. Each lobule organized into a peripheral cortex and a central medulla with numerous thymocytes, few macrophages, and diffuse Hassall's corpuscles found (Fig. 11A). On the other hand, the thymus of CI group showed marked lymphocytic depletion when compared with the thymus of the control non-infected group along with blood vessels congestion and extravasated haemo biotic cells (Fig. 11B). Lymphocytic necrotic areas were also noted near the area of thymic cortex concomitant with an irregular arrangement of thymic cells within cortex and medulla. Hence, the boundaries between the cortex and medulla were mingled together.

Both RI and FI revealed thymic architectural improvements but the best pictures were observed in the infected group treated with fenugreek (Fig. 11C, D).

It is clearly noticed that the bursal sections obtained from CN group showed normal longitudinal mucosal folds projected into the lumen covered by follicular epithelium, numerous follicles filled the lamina propria of each fold. Each bursal follicle was composed a peripheral cortex and a central medulla. The cortex composed mainly of many closely packed small lymphocytes meanwhile medulla contained fewer cells of various sizes (Fig. 12A). Meanwhile that of the infected bird's revealed mild to moderate lymphoid depletion with severe diffuse edema of the interfollicular connective tissue in the lamina propria (Fig. 12B). In the medulla of the follicles, some lymphocytes showed karyopy knosis. Regarding RI group, there was still tendency of interfollicular edema and mild lymphoid depletion among the examined sections (Fig. 12C), however, FI group showed an improvement of the degenerative changes when compared with infected group with less edematous area among the interfollicular connective tissue (Fig. 12D).

Discussion

The prohibition of nutritive antibiotic use in Europe, as well as the increased awareness of the consumers, triggered a need for natural and safe feed additives to achieve better poultry production. Herbal plants are used in animal nutrition as appetite, digestion stimulants, physiological functions stimulants, prevention and treatment of certain pathological conditions, and antioxidants **(Mohamed et al., 2016$_a$; Mohamed et al., 2016$_b$**; Ismaiel et al., 2017; Abdellatief et al., 2017; Emam et al., 2018; Farouk et al., 2020; Gad et al., 2021). The current study focused on the comparative efficacy of rosemary and fenugreek as feed additives, growth promoters, immunostimulants and tissue protective agents against *E-Coli* infection in broilers.

The decreasing effect of *E-Coli* infection on B.W., B.W.G, FC, and FCR, noted in the present study, may be attributed to colonizing of *E-coli* in the intestinal wall and secreting toxins so affect intestinal integrity which reflected on feed intake and so on weight gain (Gomis et al., 1997; El-Baky et al., 2014). This assumption is proved by intestinal histopathological examination where *E-Coli* badly affect intestinal tissues with atrophy, sloughing and necrosis of intestinal villi and glands along with leukocytic infiltration, which came in accordance with **Moursi et al. (2008)**.

Moreover, the present findings indicated a decreasing of BW among RI birds compared with CN group, which came in agreement with Hernández et al. (2004); Abd El-Latif et al. (2013) and Soltani et al. (2016), this likely was due to reduction in feed intake that resulting from the strong flavor of rosemary which need an adaptation period for accommodating oral and nasal sensing, preparing the gastrointestinal tract for food reception, and modulating digestive secretions and gut motility. Additionally, it may be also due to the fact that rosemary leaves contain high crude fiber particularly, cellulose which may hampered nutrient utilization by chickens (Barelli, 2013; Soltani et al., 2016). Oppositely to the results of Mathlouthi et al. (2012) who recorded good growth performance effects of rosemary, that may be due to the

difference in the used rosemary form, source, and concentration (Yesilbag et al., 2011). On the other hand, fenugreek cleared an elevating effect on BW and BWG, which come in agreement with Park and Kim (2015). Meanwhile, it not harmony with that results of Saki et al. (2014) **and** Patel et al. (2014). Our finding may be attributed to fenugreek controlling effect on potential pathogens in gut microflora, thus move the animals from immune defense stress to increase absorption of essential nutrients, improving the digestive capacity of the small intestine and thereby helping animals to grow better, as mentioned by Hashemi and Davoodi (2011). Such results clearly confirmed by histopathological evaluation that revealed less degenerative changes in RI intestine and normal villus architecture in fenugreek infected intestinal tissue. This good histological picture came in accordance with **Gurkan et al. (2015).**

In the current study, *E-Coli* infection resulted in an increase in AST, ALT, UA, Cr and cholesterol, with a decline in glucose level. These findings are in accordance with **Zaki et al. (2012)** and Abdel Ziz et al. (2016) who recorded that *E. coli* infection in chickens resulted in significant increase in liver enzymes (AST and ALT) activities. Our results also were in complete harmony with those reported by Joan and Pannel (1981), who stated that the *E-Coli* infection produced alteration in cellular permeability due to changes in cell membrane which allows the escape of these enzymes into serum in abnormal high level. Our findings are magnified by histopathological examination of hepatic and renal tissues which expressed as hepatocytic vacuolar degeneration and marked necrobiotic changes of hepatic tissue, along with renal tubular degenerative changes. This histopathological figures came in agreement with **Moursi et al. (2008).**

On contrary, rosemary succeed to decrease AST, ALT, UA and Cr levels, which was proved by histopathological examination that revealed mild degenerative changes in hepatocytes. Similar data obtained by Albasha and Azab (2014); **Mohamed et al. (2016$_a$)** who recorded the protective effect of rosemary supplementation against cadmium, gentamicin and lead acetate induced hepatorenal toxicity, respectively. Azab et al. (2016) related the hepatoprotective effect of rosemary to its principal antioxidant constituents (rosmarinic acid, diterpenoids such as carnosic acid, carnosol, carotenoid and alpha-tocopherol) which inhibit free radicals' generations. Also **Mohamed et al., (2016$_a$)** related the renal protecting effect of rosemary to synergistic interactions between its individual components with his antioxidant properties. Moreover, rosemary showed hypercholesteremic properties in RI group, which also recorded by Ghazalah and Ali (2008); Polat et al., (2011),who related it to leaves defatted portion rich in fibrous content that preventing intestinal cholesterol absorption. Additionally, fenugreek mediated a decrease of AST, ALT, UA, Cr and glucose levels than CI, which magnified by histopathological examination that revealed near normal hepatocellular and renal architecture. These results also recorded by Mamoun et al. (2014); Park and Kim (2015). Mentreddy (2007) who related hypoglycemic effect of fenugreek to the steroidal saponins, alkaloids and 4-hydroxy-isoleucine soluble dietary fiber fraction, exerting delaying effect on sucrose digestion and inhibition of carbohydrate hydrolyzing enzyme, as well as stimulating insulin secretion from the β pancreatic cells.

Our findings of increased IgG and IL-6 levels in CI group, besides came in accordance with (Eleiwa et al., 2011). The microbial pathogens stimulate the immune responses which produce cytokine IL-6, favor B-

cell maturation and produce neutralizing antibodies IgG that neutralize bacterial toxins (D'Elios et al., 2011). Since, the efficacy of immune system in chickens mainly depends on the bursa of fabricius and thymus for lymphocytic differentiation and initiating humeral and cellular-immune responses. So the marked bursal and thymic lymphocytic depletion, induced by *E.coli* experimental infection, was previously reported by Madian et al. (2008) who reported an immunosuppressive effect of *E.coli*. Meanwhile, Nakamura et al. (1986) related this depletion to a combination of direct effects of *E-coli* toxic components and non-specific stress factors.

Generally, herbs rich of flavonoids, vitamin C, and carotenoids benefits the immune system, and presenting immunostimulant effect through; enhanced phagocytic activity, modulation of cytokine secretion, histamine release, immunoglobulin secretion, plasma myeloperoxidase and lysozyme activity increase **(Mirzaei-Aghsaghali, 2012)**. Rosemary abled to increase IgG, decrease IL-6 in RI birds, these results are agreed with Da Rosa et al., (2013) who related its anti-inflammatory activity to effect on decreasing the proinflammatory cytokines with increasing of the anti-inflammatory cytokine in mice suffered from pleurisy. Additionally, fenugreek succeeded to increase IgG, and decrease IL-6 in FI group, this immunostimulant effects is related to high total phenolic content following both fenugreek gastric and duodenal digestion (Jayawardena et al. 2015).

E.coli endotoxin resulted in elevating the systemic cytokines (TNF and IL-6), which enhance production of superoxide anion, release of lysozyme, H_2O_2 and chemotaxis, as an adaptive mechanism to decrease reactive oxygen formation, besides increasing its uptake, resulting in the production of potent oxidant bactericidal agents (Dutta and Bishayi, 2009). Meanwhile, when the stress is too high, antioxidant activity is decreased and apoptosis is activated (Surai, 2015), which cleared the decrease in the SOD, CAT and TAC levels in our *E-Coli* infected group. Generally, antioxidant supplementation resulted in increased interleukin levels, elevated total lymphocytes, increased killer cell activity and antibody response to antigen stimulation. Moreover, using of antioxidants herbs in broiler feed is not important only for their health, but also for the oxidative stability of their meat products (Fellgenber and Speisky, 2006).

Rosemary can elevate the SOD, CAT and TAC in RI birds. **Soltani et al. (2016)** related the antioxidant properties of rosemary to the high polyphenols containing hydroxyl groups that probably stop free radical formation. Furthermore, Polat et al. (2011) observed the greatest activity of SOD through broiler supplementation of rosemary in comparison to vitamin E. Moreover, fenugreek abled to suppress oxidative stress indicated by the increase in SOD, CAT, and TAC level in FI birds. Maharana and Dadhich (2016) related these findings to the oxidative stress suppression, reduction in cell apoptosis, and fibrosis to trigonelline present in fenugreek. Additionally, Mohammadzadeh et al. (2015) recorded an elevation of catalase enzymes activities after treating rats with acetaminophen-liver toxicity by fenugreek.

Conclusion

Considering the obtained findings, it can be concluded that rosemary or fenugreek supplementation is beneficial in reduce and improve the biochemical and histological alterations induced by *E. coli*-infection

in broilers. However, the present study suggests the protective, anti-inflammatory, antioxidant, and immunomodulatory effects of rosemary or fenugreek on *E. coli*-induced toxicity; the most protective efficacy was recorded in infected chicks treated with fenugreek. Moreover, the using of fenugreek as feed additive may be a good strategy against oxidative stress induced by *E. coli*, knowing that it is prohibited to administer in case of hypoglycemia. To strengthen these findings, further investigations are needed to explore the mechanism action of rosemary and/or fenugreek against *E. coli* toxicity in broilers.

Declarations

Ethics approval and consent to participate

The current scientific research scenario was considered and approved by Committee Research Ethics Board at Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Consent for publication

Not applicable

Competing interests

The authors have no conflict of interest.

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Author's Contribution

Sameh M. Farouk, Haidy G. Abd-El-Rahman, Osama A. Abdallah and Nashwa G. EL-Behidy contributed equally to the design and implementation of the research, to the analysis and discussion of the results.

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Tables

Table (1): Composition of broiler chicken's standard basal ration.

Ingredient	**Starter ration (0-3 weeks)**	**Grower ration (4-6 weeks)**
yellow corn %	55%	51.56%
Soya bean48%	30%	35.6%
Corn gluten62%	8.8%	2%
Soya bean oil	2.2%	6.1%
Mono calcium phosphate	1.6%	1.8%
Lime stone	1.5%	1.7%
Iodized Sodium chloride	0.40%	0.42%
*Vitamins, minerals mix, choline	0.3%	0.3%
D.L methionine	0.1%	0.29%
L-lysine hydrochloride	0.1%	0.03%
Calculated chemical analysis:		
Crude protein (c.p.) %	23%	21%
ME Kcal per Kg	3050	3160
Crude Fat	3.58%	6.24%
Crude fiber	3.63%	3.82%

*Each 3 kg of vitamins and minerals mixture contain Vit. A, 12000000 IU; Vit. D3, 2200000; Vit. E, 10000mg; Vit.K, 2000mg; Vit.B1, 1000mg; Vit.B2, 5000mg; Niacin, 30000mg; Pantothenic, 10000mg; Vit.B6, 1500mg; Vit. B12, 10mg; Folic, 1000mg; Biotin, 50mg; Selenium, 100mg; Copper, 4000mg; Iron, 30000mg; Manganese, 60000mg; Zinc, 50000mg; Iodine, 1000mg; Cobalt,100mg; Choline chloride, 300000mg and Calcium carbonate was the carrier till 3 Kg. The ration formulated to full-fill the requirements according to **NRC (1994).**

Table (2): The mortality percentage (*N= 20*) in chicken administrated Rosemary and Fenugreek for 6weeks and experimentally infected with *E.Coli*.

Groups	Number of dead chicks	Mortality %
CN	0	0
CI	5	25
RI	2	10
FI	1	5

Figures

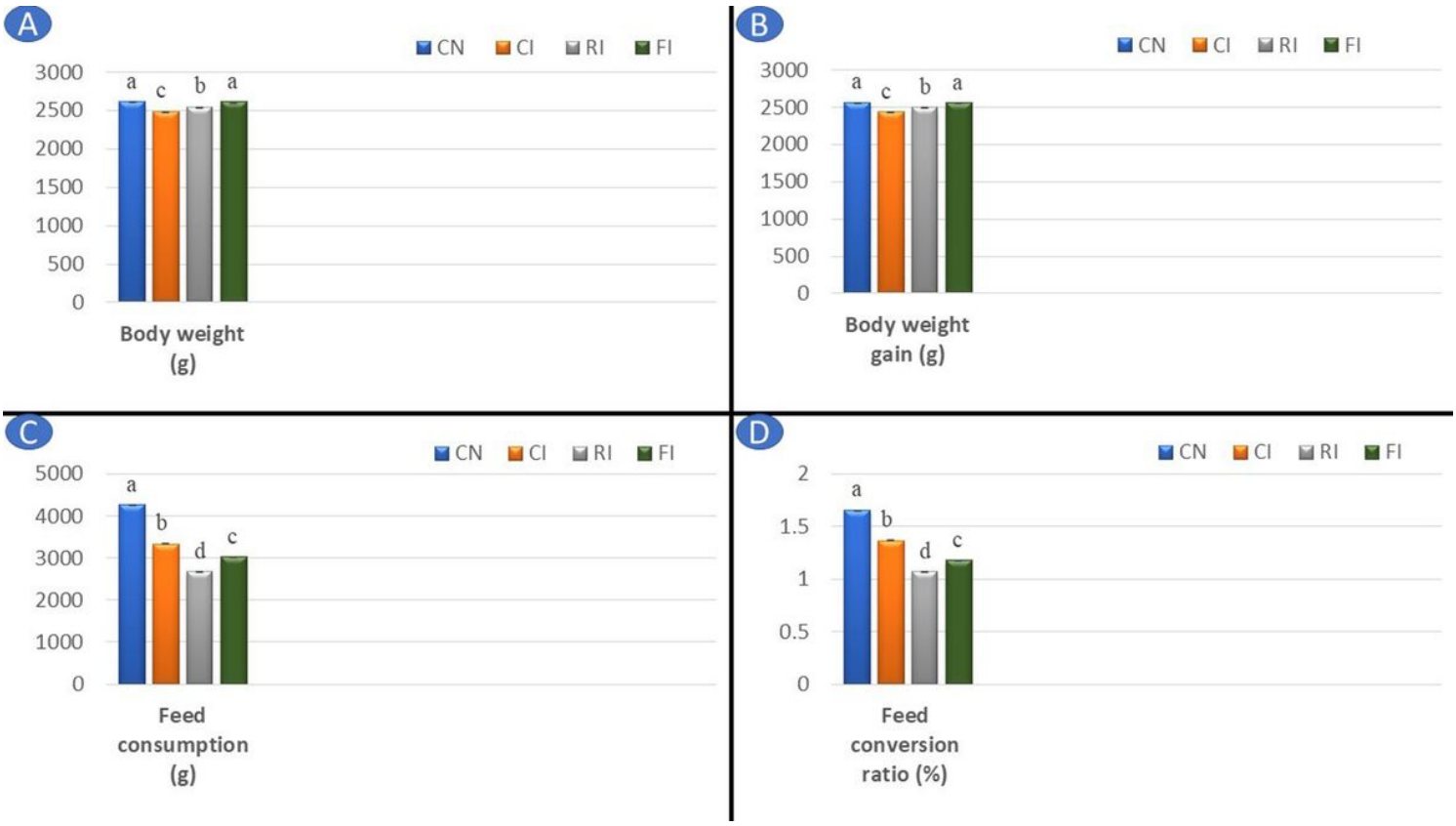

Figure 1

Mean ± SE of body weight (A), body weight gain (B), feed consumption (C) and feed conversion ratio (D) in all experimental groups.

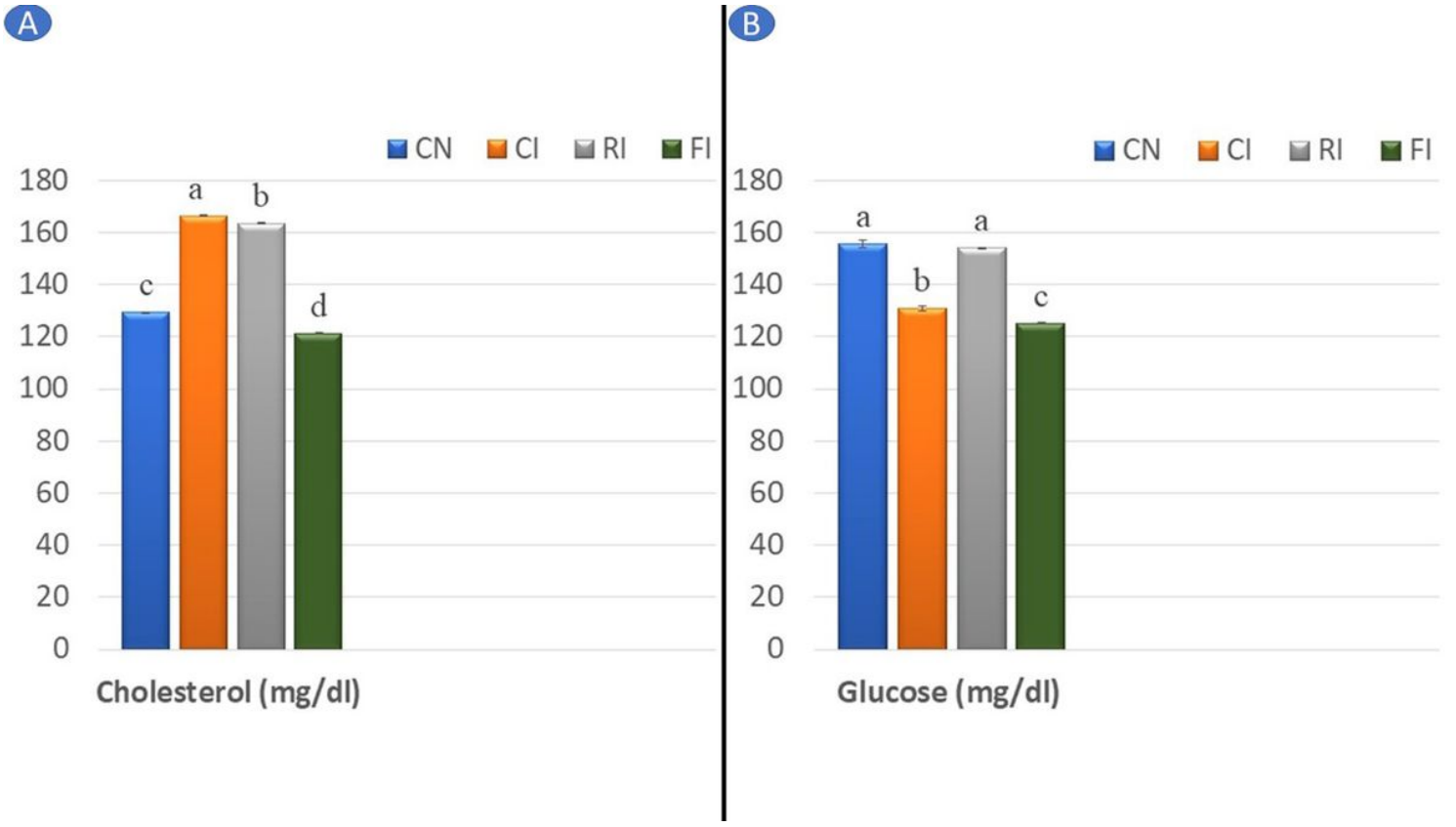

Figure 2

Mean ± SE of cholesterol (A) and glucose (B) levels in all experimental groups.

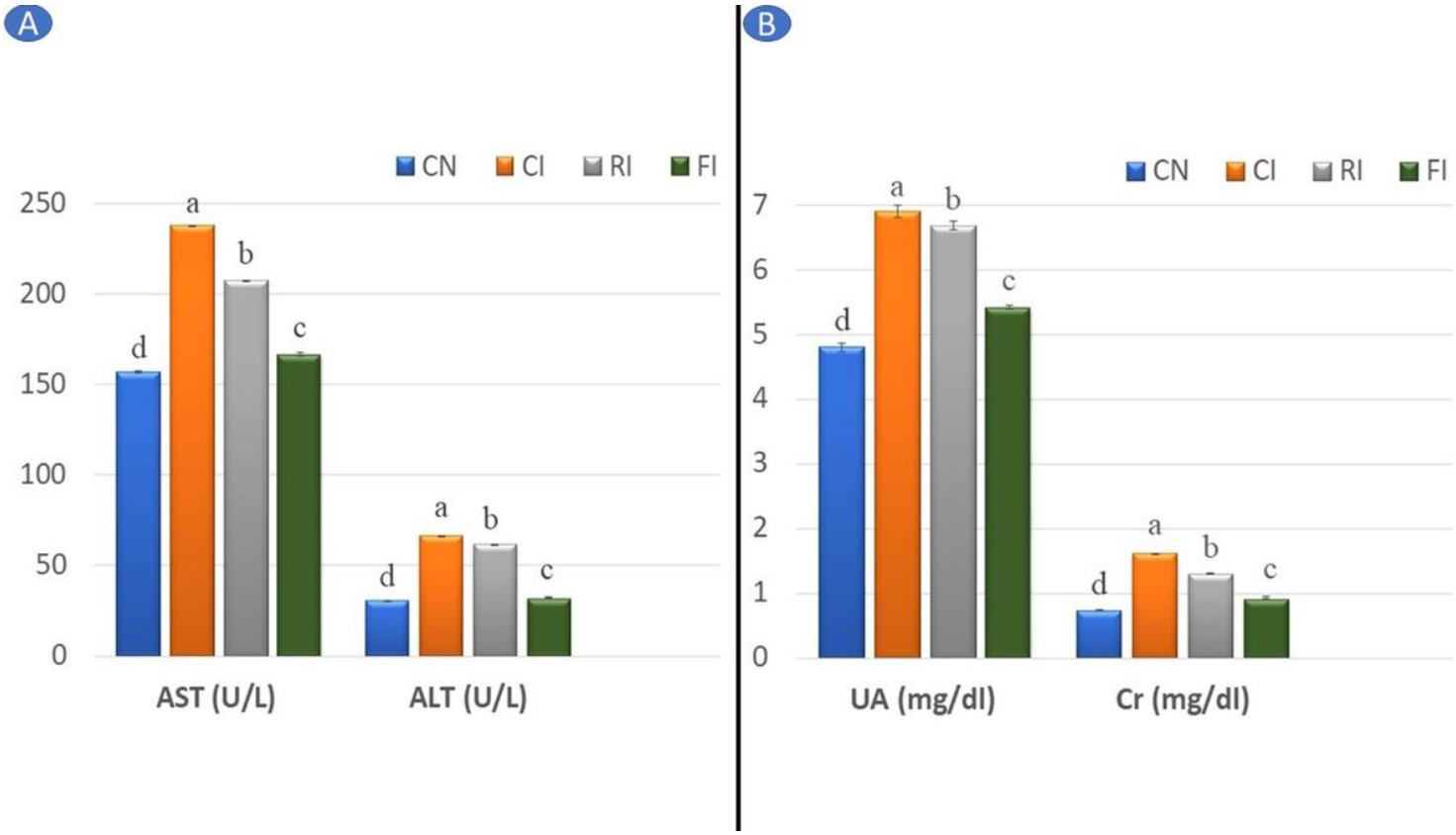

Figure 3

Mean ± SE of AST and ALT activities (A), and UA and Cr levels (B) in all experimental groups.

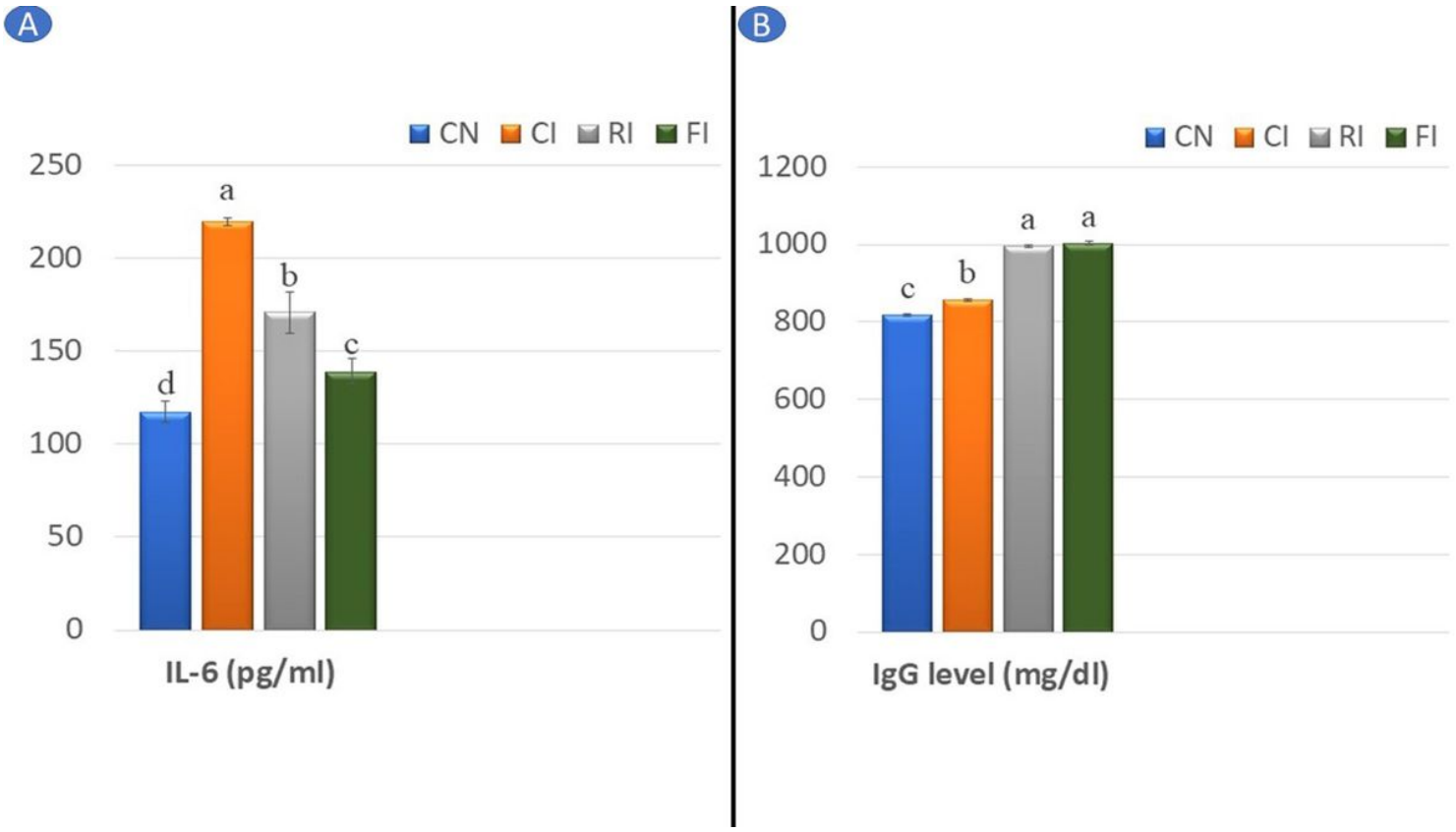

Figure 4

Mean ± SE of IL-6 (A) and IgG (B) levels in all experimental groups.

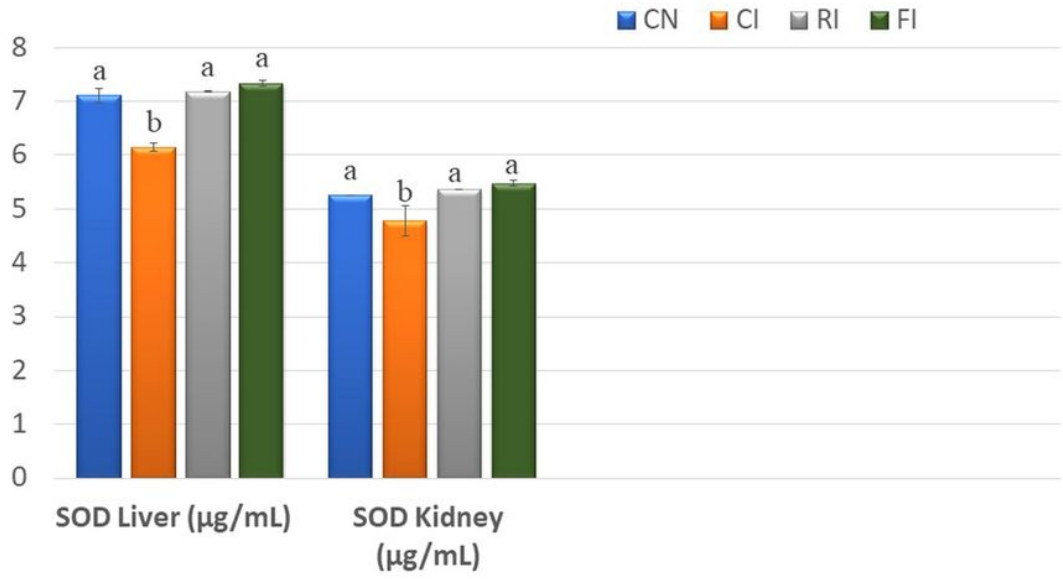

Figure 5

Mean ± SE of SOD in hepatic and renal tissues of all experimental groups.

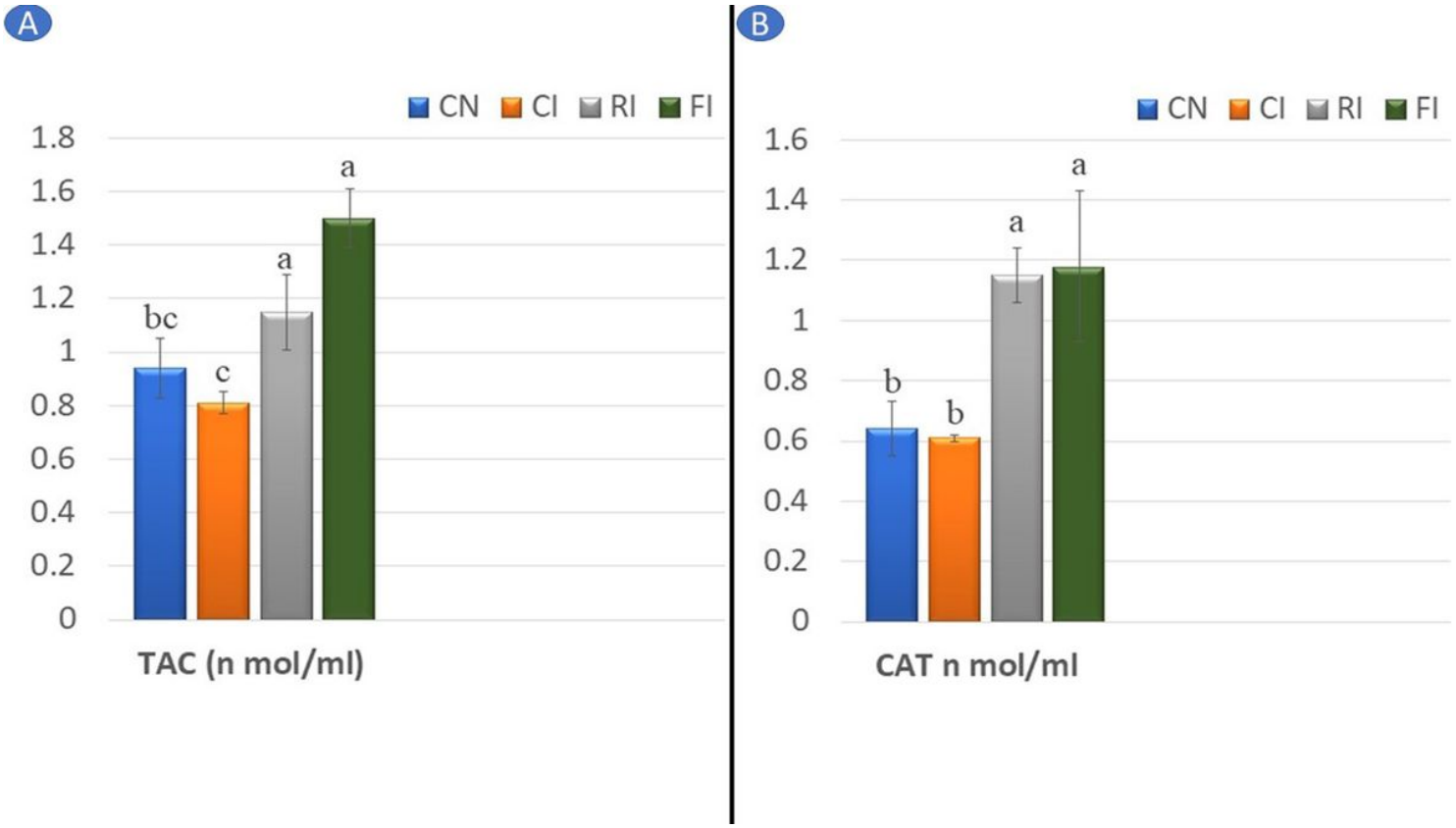

Figure 6

Mean ± SE of TAC (A) and CAT (B) levels in all experimental groups.

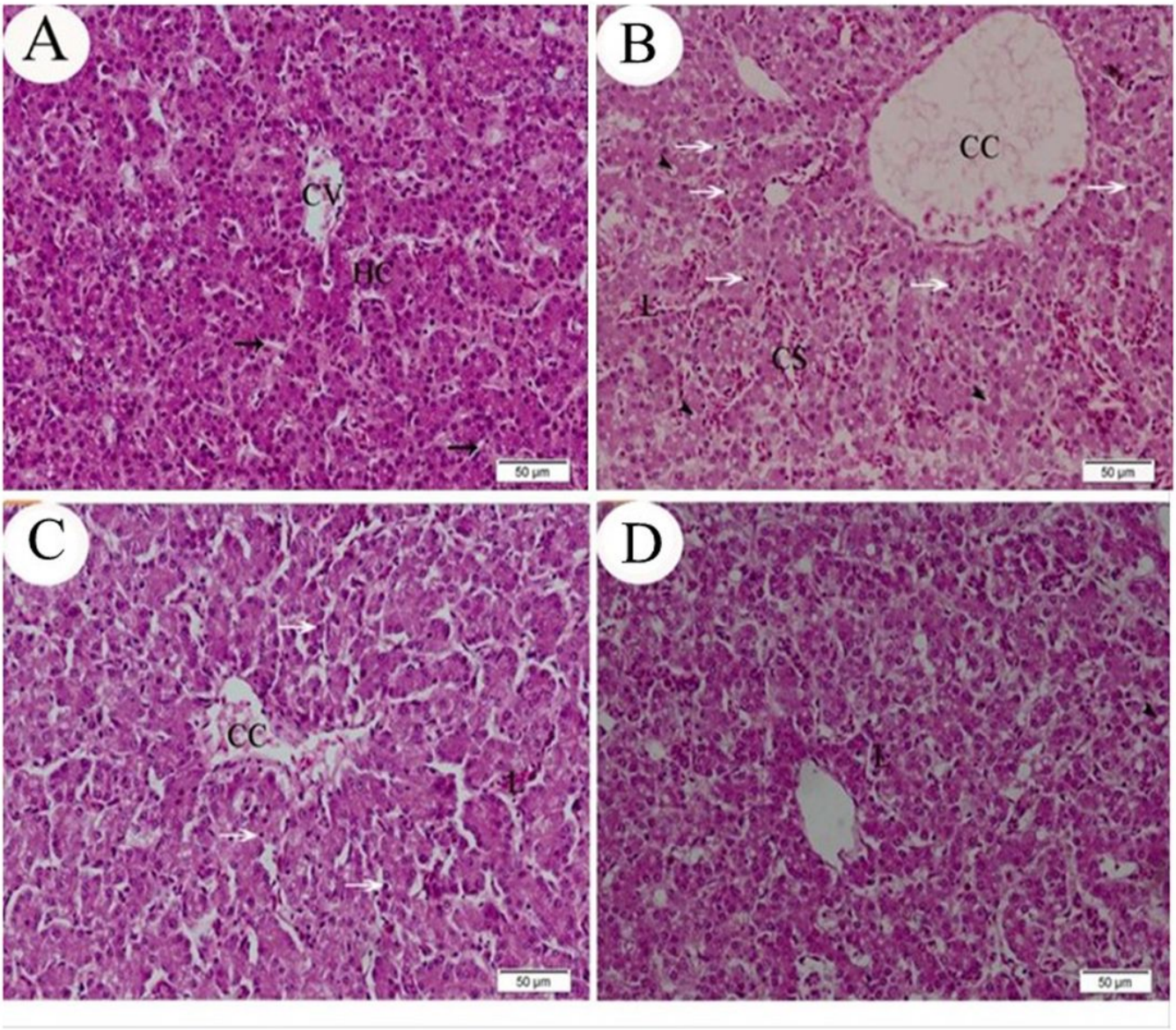

Figure 7

Representative photomicrograph of broiler liver. A; CN group, B; CI group, C; RI group and D; FI group. Here, Hepatocytes (HC) radiated from central vein (CV); Hepatic sinusoids (thin black arrows); vacuolar degeneration in some pyknotic hepatocytes (head arrows); Kupffer cell hyperplasia (thin white arrowheads); Evidence of marked congestion and dilatation of central vein (CC) and hepatic sinusoids (CS) along with Leukocytic infiltration (L). H&E stain.

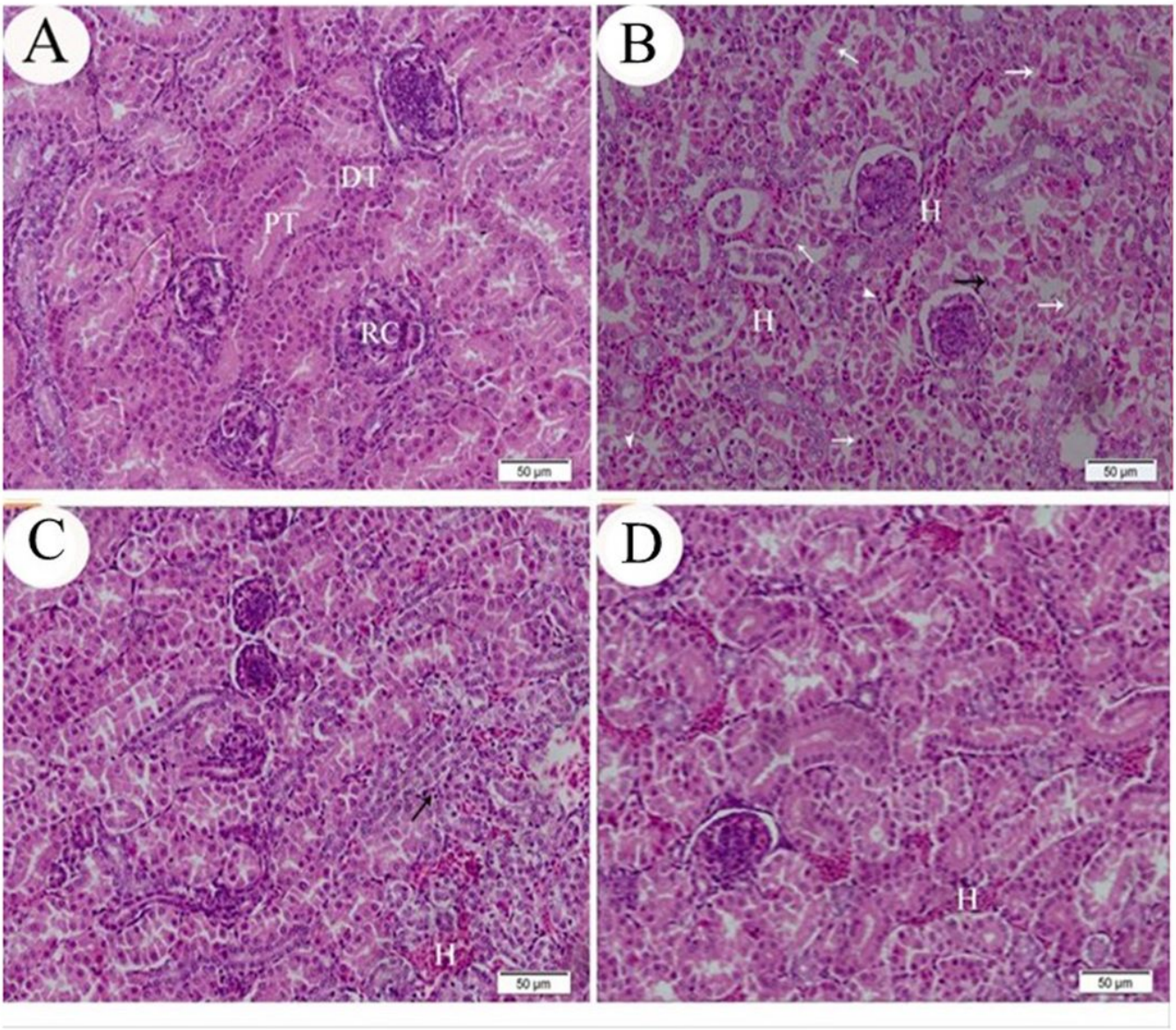

Figure 8

Representative photomicrograph of broiler kidney. A; CN group, B; CI group, C; RI group and D; FI group. Here, Renal corpuscles (RC) and surrounding tubules; proximal tubules (PT) and distal tubules (DT); Sever congestion and hemorrhages in the peritubular capillaries (H); Vacuolization of epithelial lining renal tubules (white arrows); Pyknosis of some tubular nuclei (arrowheads); Leukocytic infiltration (black arrows). H&E stain.

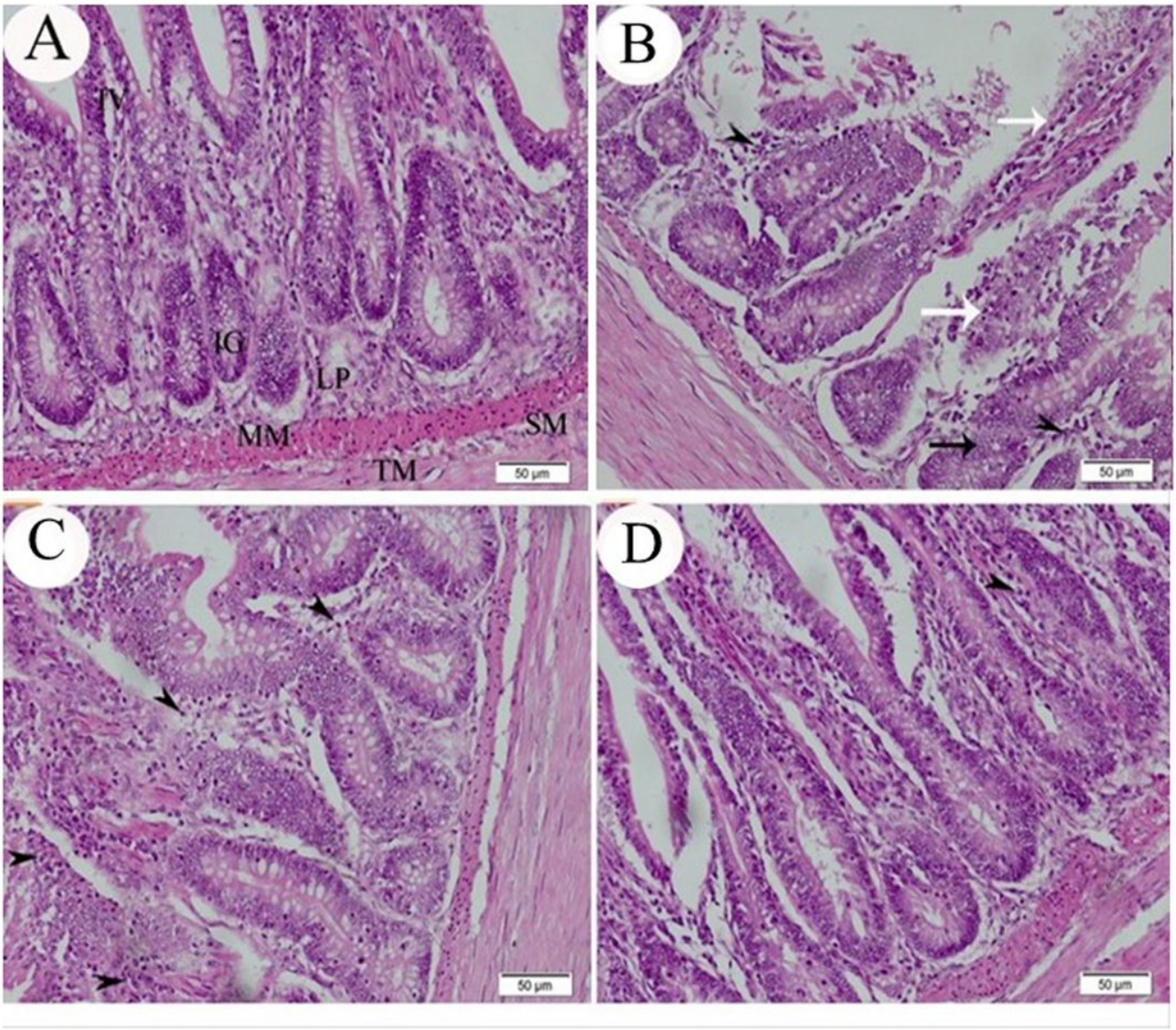

Figure 9

Representative photomicrograph of broiler intestine. A; CN group, B; CI group, C; RI group and D; FI group. Here, Intestinal villi (IV); Intestinal glands (IG); Lamina propria (LP); Muscularis mucosa (MM); Tunica submucosa (SM); Tunica musculosa (TM); destructive intestinal villi (white arrows) and gland (black arrow); Leukocytic infiltration (head arrows). H&E stain.

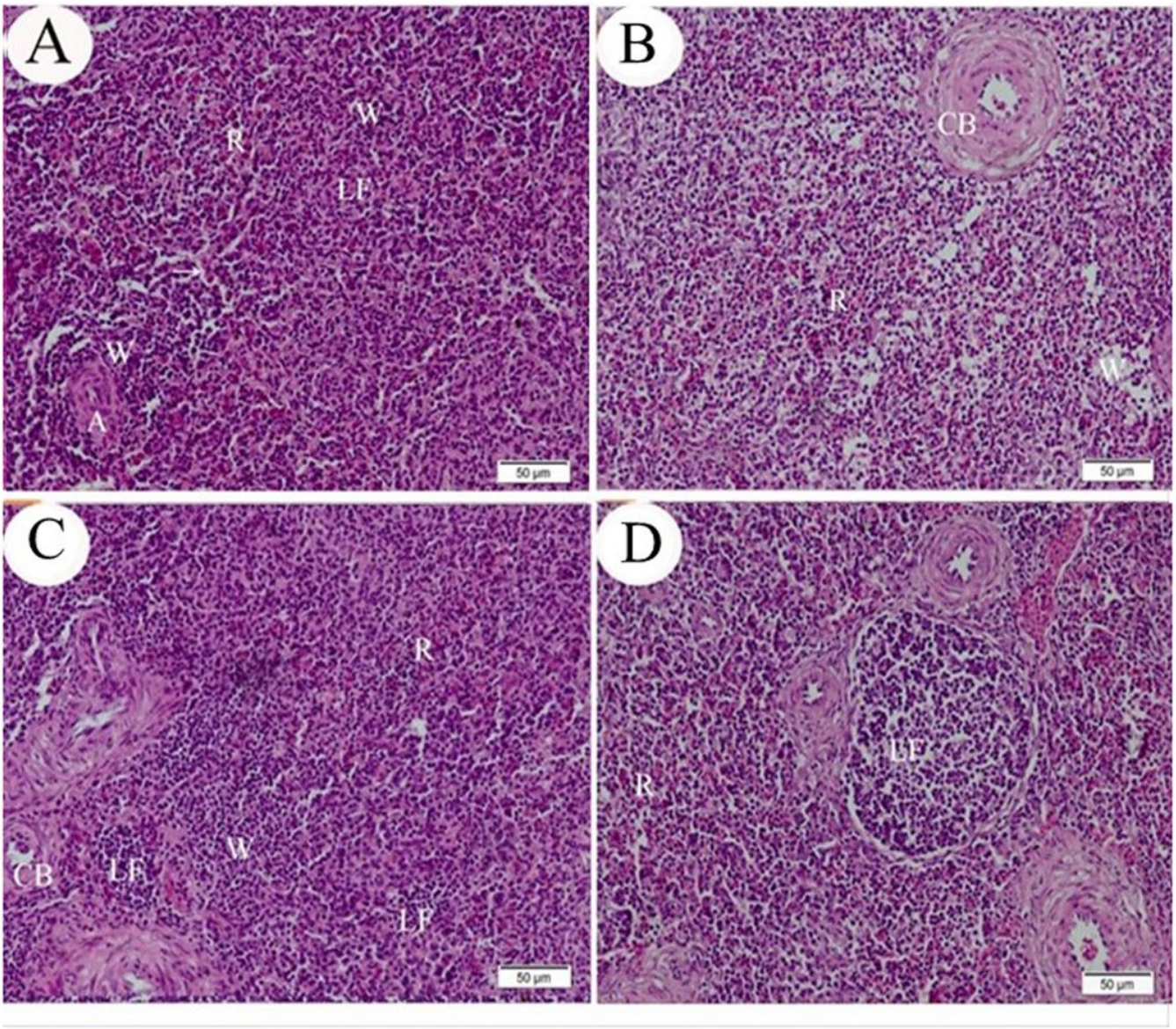

Figure 10

Representative photomicrograph of broiler spleen. A; CN group, B; CI group, C; RI group and D; FI group. Here, Red pulp (R); White pulp (W); Lymphoid follicle (LF); Artery of white pulp (A); Congested blood vessels (CB). H&E stain.

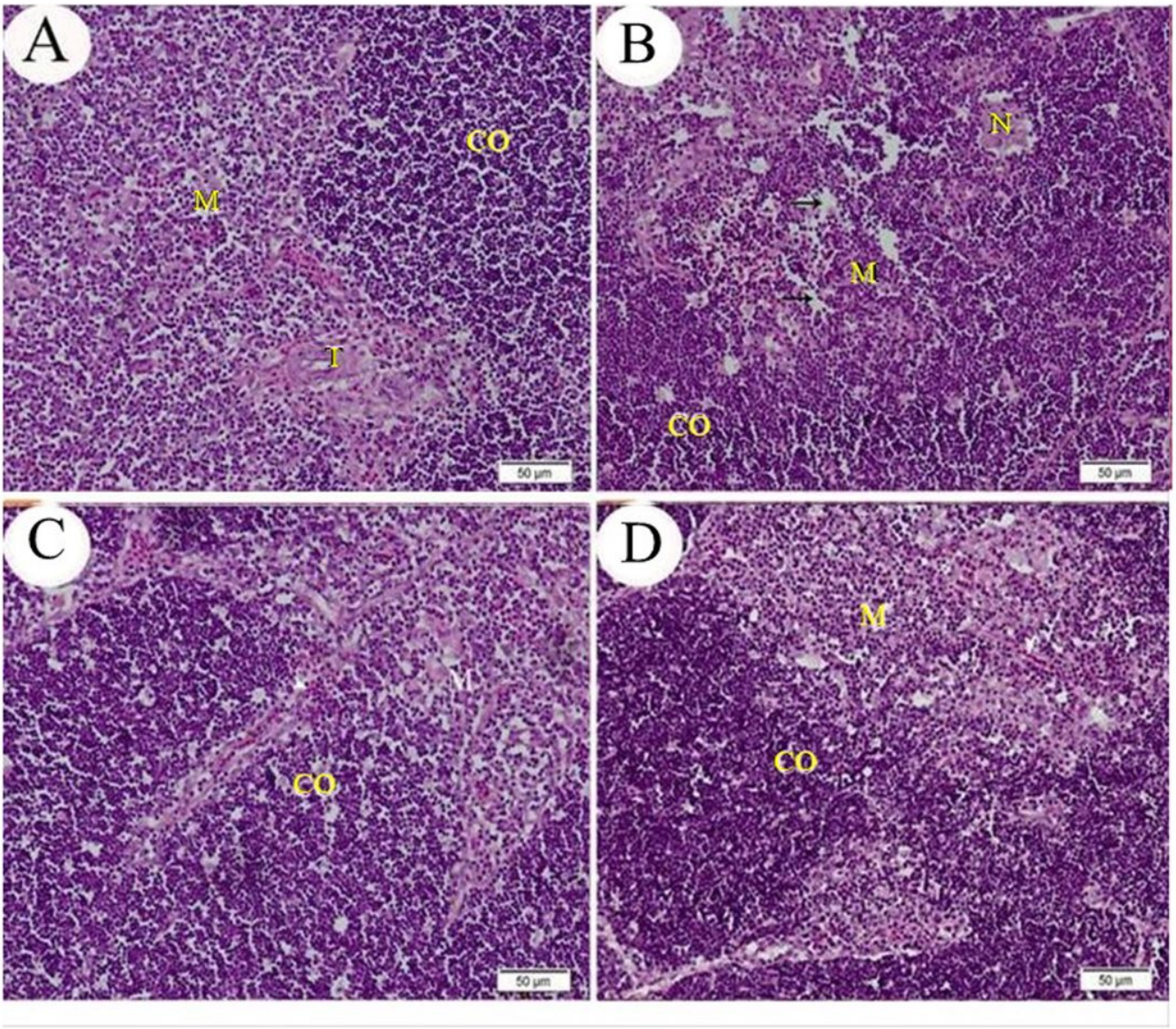

Figure 11

Representative photomicrograph of broiler thymus. A; CN group, B; CI group, C; RI group and D; FI group. Here, Cortex (CO); Medulla (M); Thymic corpuscle (T); Fine connective tissue septa (white arrows); Necrotic area (N); Lymphocytic depletion (black arrows); Heamobiotic cells (head arrows). H&E stain.

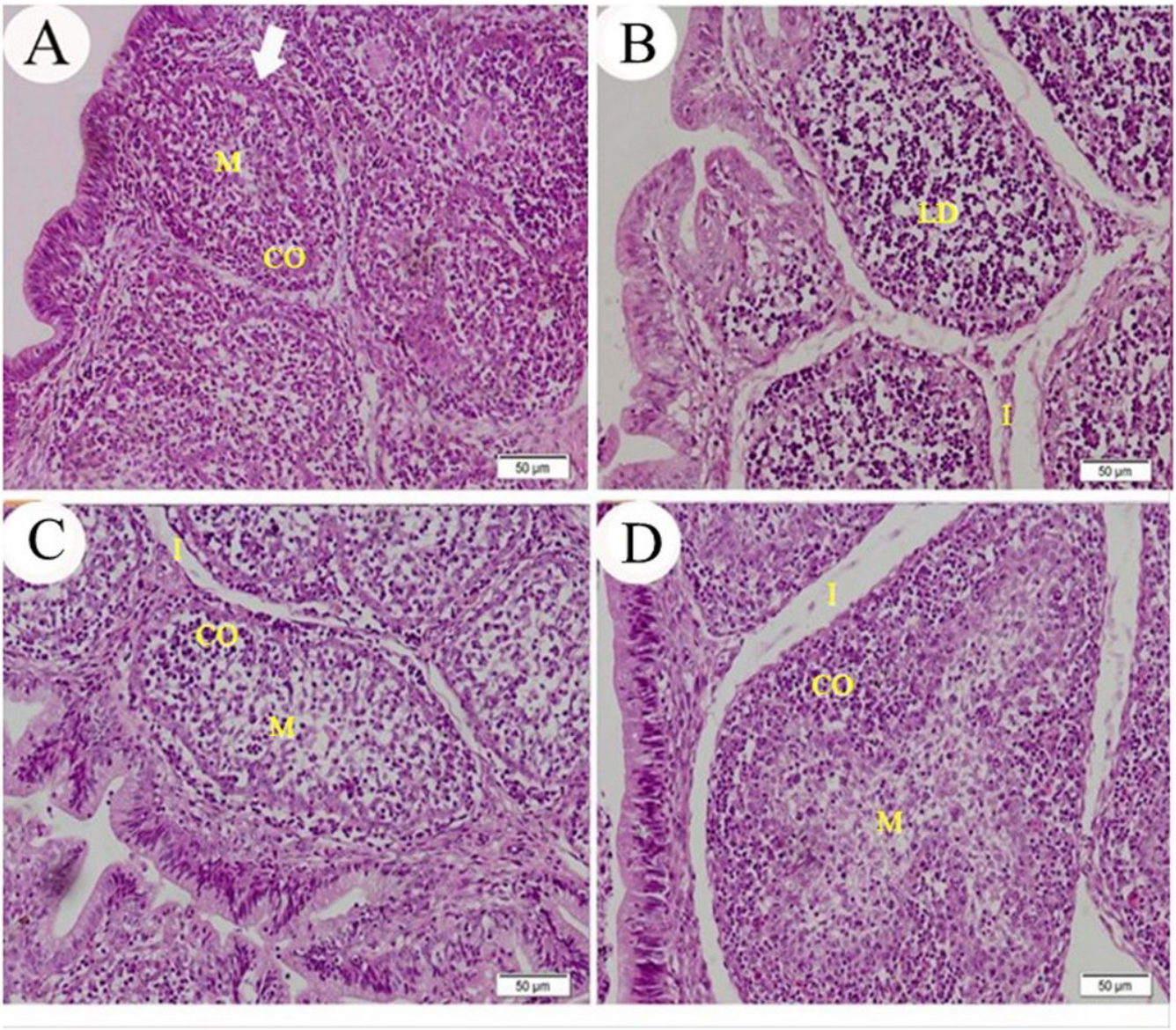

Figure 12

Representative photomicrograph of broiler bursa of Fabricius. A; CN group, B; CI group, C; RI group and D; FI group. Here, Lymphoid follicle (thick arrow); Cortex (CO); Medulla (M); marked lymphocytic depletion (LD); Interfollicular connective tissue (I). H&E stain.

Supplementary Files

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