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Original Research Article

EFFICACY OF AMOXICILLIN (ATCOMOX®) AND/OR ALLICIN ON PERFORMANCE, HAEMATOLOGICAL, BIOCHEMICAL, AND HISTOPATHOLOGICAL CHANGES IN *CLOSTRIDIUM PERFRINGENS* INFECTED CHICKENS

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Abstract: The efficacy of amoxicillin and/or allicinin healthy and experimentally *Clostridium perfringens*-infected broiler chickens was investigated. The chicks were equally divided into six groups, and all medications were orally administered via drinking water for five consecutive days: Group 1: non-infected and non-treated; Group 2: infected and non-treated; Group 3: infected and amoxicillin-treated (20 mg/kg b.wt); Group 4: infected and allicin-treated (25 mg/kg b.wt); Group 5: infected and treated with amoxicillin (20 mg/kg b.wt) and allicin (25 mg/kg b.wt); Group 6: infected and treated with amoxicillin (10 mg/kg b.wt) and allicin (25 mg/kg b.wt). Growth performance,haematological and biochemical parameters were recorded. Significant decreases in total protein, albumin, RBCs, Hb, and PCV and a considerable increase in WBCs, AST, ALT, ALP, creatinine, and uric acid in infected chickens were observed. Administration amoxicillin and/or allicin for treatment of Clostridium perfringens infection resulted in improvement in haematological and biochemical changes following infection. A dose of amoxicillin (10 mg) and allicin (25 mg)/kg bwt for treatment of *Clostridium perfringens* infection in broiler chickens is recommended due to great synergistic effect, reduced mortality, greater safety, and increased economic potential.

Key words: amoxicillin; allicin; efficacy; broilers; biochemical; hematological

Introduction

Necrotic enteritis (NE) is a serious problem in the modern poultry industry (1). It causes reduced growth performance, increased feed costs, decreased absorption and digestion, reduced weight gain, and increased feed conversion ratio due to damage in the intestinal mucosa (2). *Clostridium perfringens* is a gram-positive, anaerobic, spore-forming bacterium found in the gastrointestinal tract of poultry and can be isolated from faeces, dust, feed, and litter (3).

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Antimicrobial therapy for bacterial infection is important for reducing massive losses in the poultry industry (4). NE is prevented by using antimicrobials such as amoxicillin, which is one of the most effective β lactam antibiotic (5). Good absorption, penetration into tissues, and broad-spectrum of antimicrobial activity make amoxicillin very useful in veterinary medicine (6). It inhibits the biosynthesis of cell wall mucopeptides during bacterial multiplication and has bactericidal action (7).

Allicin is an organosulfur compound present in garlic, a species of the family Alliaceae (8). Numerous phytochemicals, including allicin could interfere with the formation of phospholipid layers

of the cell wall (9). Consequently, bacteria cannot grow in the presence of allicin (10). It has been shown that garlic enhances the broiler chicken's growth and feed conversion as a natural feed additive (11).

The use of antibiotics as growth promoters is becoming a serious problem. There are some important factors that restrict the use of antibiotics, such as the drug resistance in bacteria and presence of drug residues in meat. To combat the poor performance and the increased susceptibility to diseases resulting from the removal of the antibiotics from the poultry diets, alternatives are sought. The utilisation of growth promoters of natural origin has therefore attracted much interest in recent years (12).

Allicin has a distinctively pungent smell and exhibits antibacterial, antifungal, anti-inflammatory, and antioxidant properties (13). The mechanism of the antioxidant or anti-stress activity of allicin, such as trapping free radicals, have been reported (14). When allicin decomposes, it forms 2-propene sulfenic acid, and this compound binds to the free-radicals. Allicin was reported to reduce cholesterol in the serum and liver (15), inhibit bacterial growth (16) and reduce oxidative stress (12). Allicin also has immuno-stimulatory effect (17).

The present study was carried out to evaluate the efficacy of amoxicillin and/or allicin treatment in broilers experimentally infected with *Clostridium perfringens*, and to evaluate the advantages and possible side effects of such treatment.

Materials and methods

Drugs

Amoxicillin (Atcomox 40%)® is an antibiotic manufactured by ATCO Pharma Co, Egypt, as oral soluble powder. The recommended dose is20 mg/kg b.wt (18). Allicin is an organosulfur compound extracted from the garlic and obtained from the Technofeed Company, USA. The recommended dose is 25 mg/kg b.wt (19).

Experimental chicks:

One-hundred-and-eighty apparently healthy, one-day-old unsexed Hubbard broiler chicks were obtained from the El-Kahera poultry company, in Egypt. Chicks were divided into six groups (each of 30 chicks). Each group was subdivided into five replicates with six chicks each. Chicks were housed on the floor in separate units following strict hygienic regime. The starting temperature of 32°C was reduced by 2°C each week. Continuous lightning was used; feed and water were provided *ad-libitum* and fed free from any medications balanced commercial ration. Chicks were vaccinated on the 7th day of age against New Castle disease with the HitchnerB_1 vaccine and against Gumboro disease at 14 days of age. The duration of this study was 48 days. The Ethical Committee of the Faculty of Veterinary Medicine, Benha University, approved the study protocol (approval number 10518).

Experimental infection

Clostridium perfringens type A was obtained from the Animal Health Research Institute in Dokki, Giza, Egypt. Five groups of broilers were infected with *Clostridium perfringens* at 19 days of age; the birds were challenged via oral gavages with a toxigenic strain of *Clostridium perfringens*t type A by inoculation of 1ml of 6×10^8 cfu daily, for three consecutive days (at the 19th, 20th and 21st days of age). The treatments occurred from the 23rd to 27th days of age as described by Botlhoko TD (20).

Experimental design

The first group was left uninfected while the other five groups were infected. Group 1: non-infected and non-treated; Group 2: infected and non-treated; Group 3: infected and amoxicillin-treated (20 mg/kg b.wt); Group 4: infected and allicin-treated (25 mg/kg b.wt); Group 5: infected and treated with amoxicillin (20 mg/kg b.wt) and allicin (25 mg/kg b.wt); Group 6: infected and treated with amoxicillin (10 mg/kg b.wt) and allicin (25 mg/kg b.wt). All treatments were administered orally in drinking water for five consecutive days.

Blood samples

Blood samples were collected at the end of 1st, 10th, and 20th day post-drug administration from chicks of each group (which corresponds to 28, 38, and 48 days of age). Six birds from each group were used for the collection of blood samples via wing vein in clean dry tubes. Each blood sample was

divided into two equal parts, and first blood part was collected on heparin and used for haematological studies. The second part was collected in centrifuge tubes; left in a slope position to clot at the room temperature. Clear serum samples were obtained by centrifugation at 2000 g for 10 minutes and transferred carefully in clean dry vials and kept frozen at-20°Cuntil used for biochemical analysis.

Efficacy of the drugs on growth performance

Chicks were individually marked and weighed just prior to infection and weighed on 28th, 38th, and 48th day of age. By subtracting the body weight between two successive weightings for each group, body weight gain was recorded.

Feed consumption and feed conversion were calculated for all groups. The feed conversion was calculated as grams of consumed feed per grams of body weight gain (21). Feed conversion ratio (FCR) was determined as Feed consumption (FC; gm) period/ Weight gain (gm) period.

Effect on haematological parameters

The blood's haematological characteristics, such as red blood cell count (RBCs) (22), white blood cell count (WBCs) (23), haemoglobin (Hb) concentration, and packed cell volume (PCV) (24) were determined.

Effect on biochemical parameters

The serum AST and ALT were measured as previously described (25). Alkaline phosphatase (ALP) (26), serum total proteins (27), serum albumin (28), creatinine and urea were assessed in the serum based on the methods from (29, 30), respectively.

Histopathology

Samples of intestine, liver, and kidney were collected from slaughtered chickens at 28th, 38th, and 48th days of age and fixed in 10% formalin solution for at least 24 hrs. Histopathology was performed according to the methods described in histopathology textbook by Bancroft JD and Gamble M (31).The formalin preserved intestine, liver and kidney tissue were processed in an automated tissue processor. The processing consisted of an initial 2 step fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70%, 90% and 100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, embedded and blocked out. Paraffin sections (4–5 μm) were stained with hematoxylin and eosin (HE). Stained sections were examined for inflammatory reactions, degenerative and necrotic changes or any other pathological changes in the intestine, liver and kidney of the experimental chickens.

Statistical Analysis

The results were expressed as mean ± SE using the analysis of variance test (one-way ANOVA) followed by Duncan's multiple range test to determine the differences between the averages. All analyses were performed by Statistical Package for Social Science software (SPSS (20) software (SPSS Inc., Chicago, USA).

Results

Clostridium perfringens experimentally infected broiler chickens displayed clinical signs when left untreated. Mild clinical signs appeared 24 to 36 h post-infection. These signs were loss of appetite, drooping wings, diarrhoea, depression, polydipsia, emaciation, dehydration, and ruffled feathers. These clinical signs disappeared under the influence of amoxicillin and/or allicin either alone or in combination.

The effects of amoxicillin and/or allicin on the body weight, body weight gain, FC and FCR of control and infected chickens are shown in Table 1. The effect on total RBCs, WBCs count, Hb content and PCV% in healthy and infected chickens are shown in Table 2. Changes in serum AST, ALT, ALP, total protein, albumin, creatinine and uric acid of control and infected chickens are shown in Tables 3 and Table 4, respectively.

Table 1: The effect of amoxicillin and/or allicin given in drinking water for 5 successive days on growth performance parameters in healthy and experimentally infected broiler chickens with *Clostridium perfringens* at 28th, 38th and 48th days of age (n= 6)

Parameters	Groups	Days post-treatment		
		1st day	10th day	20th day
Body weight (gm)	1	1328±36.11^{a}	1880±9.48^{a}	2332±14.96^{a}
	2	978±60.03^{c}	1350±58.83^{d}	1710±44.27^{d}
	3	1108±55.35^{b}	1568±56.85^{c}	2022±65.86^{c}
	4	1144±24^{b}	1632±28.70^{bc}	2104±26.38^{bc}
	5	1188±33.82^{b}	1714±42.14^{b}	2206±32.80^{b}
	6	1132±39.29^{b}	1670±47.32^{bc}	2146±36.82^{b}
Body weight gain (gm)	1	770.4± 29.29^{a}	552±27.09^{a}	452±24.37^{a}
	2	414.2±65.42^{c}	372±22.89^{c}	360±14.83^{b}
	3	561.8±53.67^{b}	460±20.97^{ab}	454±18.42^{a}
	4	594.4±20.86^{b}	488±20.59^{ab}	472±27.27^{a}
	5	635.8±35.61^{b}	526±14.00^{ab}	492±31.20^{a}
	6	557.2±38.38^{b}	538±28.01^{a}	476±22.49^{a}
Feed consumption (gm)	1	526.2±7.76^{a}	940±21.98^{a}	754±8.98^{a}
	2	423±4.48^{c}	820.4±18.92^{b}	725±23.06^{ab}
	3	506.4±2.71^{b}	810.2±21.64^{b}	684.2±14.32^{b}
	4	515.8±5.37^{ab}	807.6±11.21^{b}	699.2±11.19^{b}
	5	526±5.18^{a}	822.4±12.13^{b}	704.4±13.09^{b}
	6	518.2±4.91^{ab}	811.2±9.61^{b}	695±12.27^{b}
Feed conversion rate (%)	1	0.68±0.26^{b}	1.72±0.18^{b}	1.66±0.09^{b}
	2	1.12±0.06^{a}	2.22±0.28^{a}	2.01±0.15^{a}
	3	0.93±0.05^{ab}	1.77±0.18^{b}	1.50±0.07^{b}
	4	0.87±0.25^{ab}	1.65±0.11^{b}	1.48±0.04^{b}
	5	0.82±0.14^{b}	1.56±0.12^{b}	1.43±0.04^{b}
	6	0.91±0.12^{ab}	1.50±0.12^{b}	1.46±0.05^{b}

Mean values having different letters in the same column for each parameter differ significantly ($p \leq 0.05$)

Table 2: The effect of amoxicillin and/or allicin given in drinking water for 5 successive days on RBCs, WBCs, Hb and PCV in healthy and experimentally infected broiler chickens with *Clostridium perfringens* at 28th, 38th and 48th days of age (n= 6)

Parameters	Groups	Days post-treatment		
		1st day	10th day	20th day
RBCs ($x10^6/\mu l$)	1	3.87±0.09^a	3.52±0.21^a	3.88±0.10^a
	2	1.94±0.05^c	1.68±0.05^c	1.93±0.13^b
	3	2.89±0.06^b	2.90±0.03^b	2.31±0.15^b
	4	2.85±0.10^b	3.03±0.09^b	2.16±0.21^b
	5	2.72±0.03^b	3.01±0.03^b	2.22±0.12^b
	6	2.82±0.11^b	2.85±0.05^b	2.12±0.06^b
WBCs ($x10^3/\mu l$)	1	22.8±0.78^b	25.1±0.39^c	27.5±1.91^b
	2	39.2±1.49^a	35.2±1.49^a	37±2.07^a
	3	32.6±2.98^b	29.1±1.31^{bc}	28.5±1.94^b
	4	29.6±0.18^b	29±2.19^{bc}	29±0.52^b
	5	28.8±0.39^b	30.6±2.37^{abc}	28.88±0.39^b
	6	28.3±2.57^b	30.2±2.52^{abc}	28.32±2.57^b
Haemoglobin (g/dl)	1	10.62±1.04^a	10.65±0.36^a	10.17±1.02^a
	2	7.74±0.24^b	7.48±0.20^c	7.16±0.46^b
	3	8.37±0.11^b	8.17±0.56^{bc}	9.06±0.67^a
	4	8.65±0.20^b	8.18±0.32^{bc}	10.15±0.40^a
	5	8.48±0.15^b	8.62±0.31^{b}c	9.76±0.42^a
	6	8.82±0.36^b	9.02±0.33^b	9.38±0.29^a
PCV (%)	1	36.05±2.16^a	34.40±3.15^a	33.60±2.42^a
	2	26.40±1.53^b	26.10±2.36^b	26.12±1.40^b
	3	33.10±2.42^a	23.10±2.42^a	29.81±1.75^{ab}
	4	33.75±1.35^a	33.75±1.35^a	30.14±2.69^{ab}
	5	33.06±1.47^a	33.06±1.47^a	30.92±1.49^{ab}
	6	31.41±0.27^a	32.11±1.85^a	30.53±1.64^{ab}

Mean values having different letters in the same column for each parameter differ significantly ($p \leq 0.05$)

Table 3: The effect of amoxicillin and/or allicin given in drinking water for 5 successive days on AST, ALT, ALP and total protein in healthy and experimentally infected broiler chickens with *Clostridium perfringens* at 28th, 38th and 48th days of age (n= 6)

Parameters	Groups	Days post-treatment		
		1st day	10th day	20th day
AST (U/L)	1	184.8±6.76^{c}	174.4±12.1^{b}	196.2±17.7^{b}
	2	317.6±9.81^{a}	284.4±21.4^{a}	383.2±17.1^{a}
	3	238.6±19.9^{b}	207.6±5.70^{b}	211.4±16.1^{b}
	4	226.6±6.53^{b}	209.4±18.4^{b}	214.2±11.5^{b}
	5	224.2±8.48^{b}	196.8±4.93^{b}	225.2±18.4^{b}
	6	232.2±17.8^{b}	211.4±2.01^{b}	223.2±15.7^{b}
ALT (U/L)	1	21.8±1.39^{c}	21.2±0.66^{b}	20.6±0.92^{b}
	2	36.4±2.55^{a}	35.2±2.26^{a}	32.4±1.96^{a}
	3	27.8±1.82^{b}	32.2±1.39^{b}	22.8±1.80^{b}
	4	28.8±2.17^{b}	22.4±1.69^{b}	21.1±0.24^{b}
	5	29.6±2.51^{ab}	23.4±2.06^{b}	21.2±1.45^{b}
	6	26.2±2.05^{b}	24.8±2.03^{b}	23.4±2.11^{b}
ALP (U/L)	1	322.6±13.6^{c}	321.2±27.46^{b}	317.8±18.7^{b}
	2	448.2±15.87^{a}	421.2±5.07^{a}	408.6±10.85^{a}
	3	378.6±14.72^{ab}	339.4±22.03^{b}	324.2±10.5a^{b}
	4	395.2±25.17^{b}	334.4±18.10^{b}	329.8±17.84^{ab}
	5	362.6±17.92^{b}	340.8±24.66^{b}	334.6±13.61^{ab}
	6	369.2±23.32^{b}	342.6±16.79^{b}	328.2±26.26^{ab}
Total protein (mg/dl)	1	5.66±0.12^{a}	5.52±0.11^{a}	5.43±0.09^{a}
	2	4.16±0.05^{c}	4.22±0.05^{b}	4.38±0.10^{b}
	3	4.82±0.09^{b}	4.92±0.21^{a}	5.18±0.30^{a}
	4	4.92±0.37^{b}	5.06±0.22^{a}	5.13±0.37^{a}
	5	4.74±0.10^{bc}	4.98±0.30^{a}	5.27±0.21^{a}
	6	5.02±0.31^{b}	5.02±0.29^{a}	5.03±0.19^{ab}

Mean values having different letters in the same column for each parameter differ significantly ($p \leq 0.05$)

Table 4: The effect of amoxicillin and/or allicin given in drinking water for 5 successive days on albumin, creatinine and total uric acid in healthy and experimentally infected broiler chickens with *Clostridium perfringens* at 28th, 38th and 48th days of age (n= 6)

Parameters	Groups	Days post-treatment		
		1st day	10th day	20th day
Albumin (g/dl)	1	3.88±0.15^{a}	3.69±0.09^{a}	3.56±0.05^{a}
	2	2.19±0.04^{c}	2.28±0.10^{c}	2.36±0.10^{c}
	3	2.86±0.27^{b}	2.92±0.26^{b}	3.44±0.09^{ab}
	4	2.90±0.27^{b}	3.06±0.21^{b}	3.37±0.07^{ab}
	5	3.02±0.22^{b}	2.98±0.20^{b}	3.28±0.08^{b}
	6	3.16±0.21^{b}	3.04±0.19^{b}	3.22±0.02^{b}
Creatinine (mg/dl)	1	1.26±0.18^{c}	1.52±0.10^{c}	1.32±0.12^{b}
	2	2.72±0.09^{a}	2.68±0.09^{a}	2.52±0.14^{a}
	3	1.90±0.05^{b}	2.08±0.08^{b}	1.64±0.15^{b}
	4	2.06±0.12^{b}	1.90±0.05^{b}	1.56±0.11^{b}
	5	2.12±0.08^{b}	1.94±0.12^{b}	1.44±0.10^{b}
	6	1.88±0.06^{b}	2.02±0.19^{b}	1.42±0.09^{b}
Uric acid (mg/dl)	1	5.42±0.12^{b}	5.18±0.32^{b}	5.16±0.24^{b}
	2	7.27±0.10^{a}	6.56±0.09^{a}	6.62±0.10^{a}
	3	6.36±0.20^{c}	5.88±0.26^{ab}	5.46±0.18^{b}
	4	6.18±0.23^{c}	6.10±0.47^{ab}	5.34±0.13^{b}
	5	6.14±0.28^{c}	5.58±0.31^{ab}	5.23±0.10^{b}
	6	6.11±0.16^{c}	6.04±0.40^{ab}	5.30±0.36^{b}

Mean values having different letters in column for each parameter differ significantly (p≤0.05)

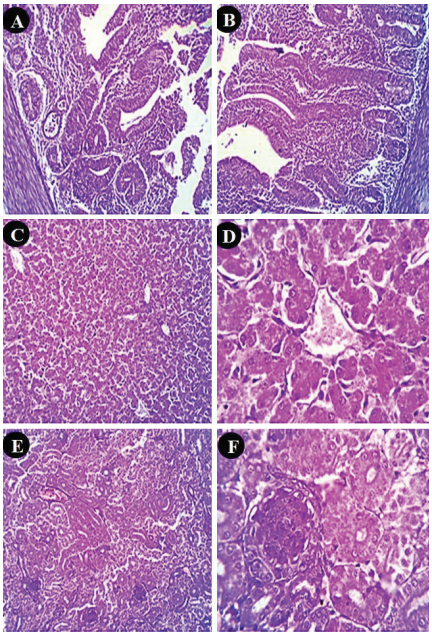

Figure 1: Photomicrograph of chicken's small intestine (A&B), liver (C&D) and kidney (E&F) showing normal histomorphological structure. H&E,(X 200, 400)

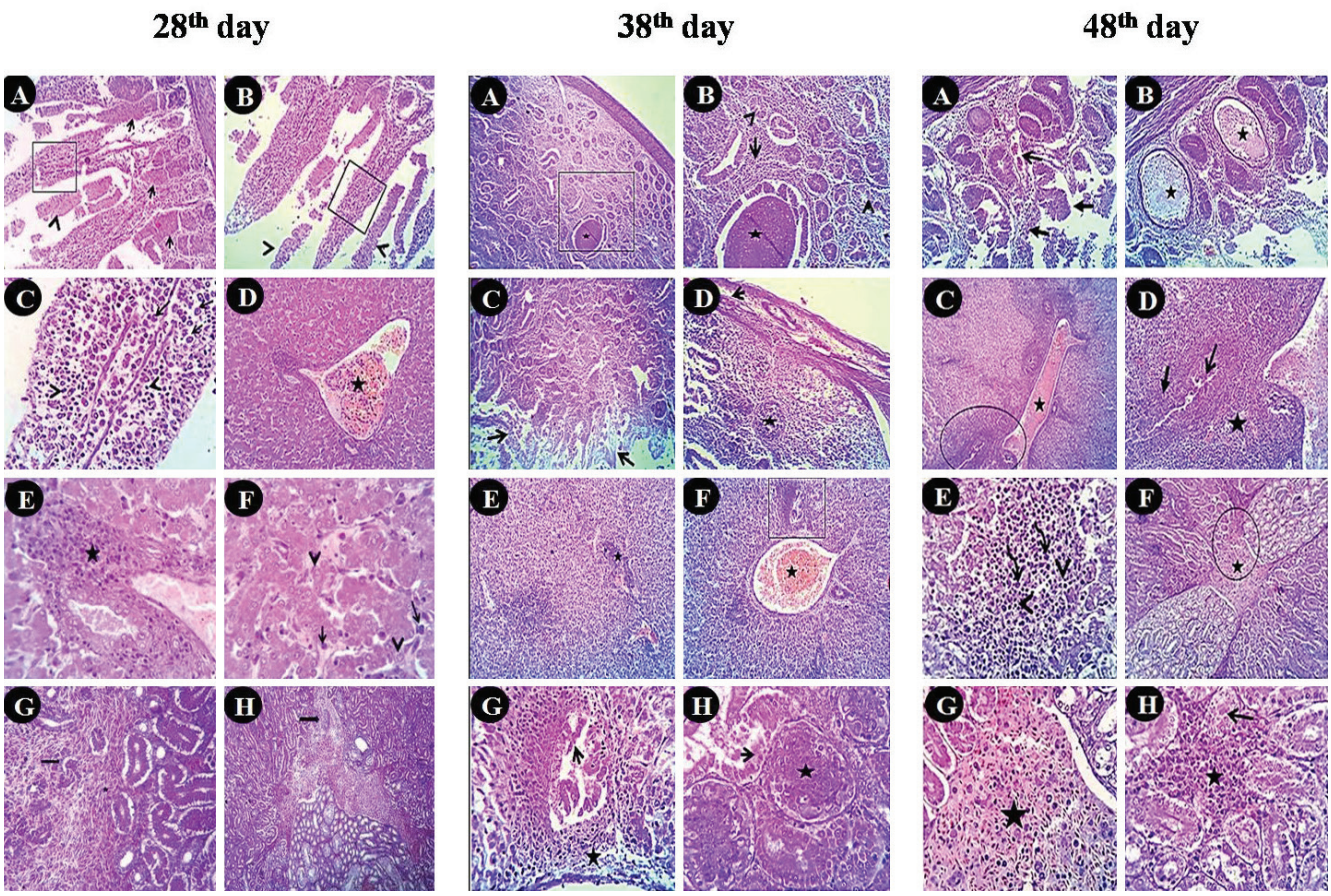

Figure 2: Photomicrograph of chicken's small intestine, liver and kidney of infected non-treated group: H&E

At 28th day (X 100, 200, 400);_Intestine: (A&B) showing villous necrosis (square) and sloughing (arrowheads), distorted crypts and glands (arrows), (C) mild infiltration of lymphocytes (arrowheads) and macrophages (arrowheads) in the lamina propria of the villi. Liver: (D) showing congestion of hepatic blood vessels (star), (E) portal aggregation of round cells (lymphocytes, macrophages) (star), (F) mild degenerative changes in most hepatocytes (arrowheads) and hypertrophied Kupffer cells (arrows). Kidney: (G) showing diffuse haemorrhage areas (star) containing scattered necrotic tubules replacing the renal parenchyma (arrow). (H) showing interstitial extravasated erythrocytes containing lymphocytic aggregations (arrow).

At 38th day (X 100, 200, 400);_Intestines: (A&B) massive round cell infiltration in the mucosa and submucosa (square & open arrow) with necrotic glands (arrowheads), some glands containing necrotic materials in their centres with cystic dilatation (star). (C) Showing villous necrosis (open arrows), (D) showing lymphoid follicles with necrotic changes (star), congested capillaries and oedema (arrow) in the serosa. Liver: (E) showing portal and interstitial round cell aggregations (star). (F) Moderate congestion of hepatic blood vessels (star). (G) Mildly hyperplastic bile ducts (arrow), surrounded by large number of round cells (star). Kidney: (H) showing necrotic changes in the tubular epithelium (arrow) with hypertrophic and hyperplastic mesangial and endothelial cells in some glomeruli (star).

At 48th day (X 100, 200); Intestine: (A&B) showing villous necrosis (closed arrows), congested mucosal and submucosal blood vessels (open arrow). Some of the intestinal glands were cystic and filled by secretory material and degenerated cells (stars). Liver: (C) showing massive portal and perivascular round cell infiltration (circle) and congested hepatic blood vessels (star). (D) Hyperplastic bile ducts (open arrow) surrounded by fibrosis (closed arrow) and large number of round cells (star) mainly lymphocytes (arrowheads) and macrophages (curved arrow). Kidney: (F&G) Showing a large mass of hepatoid like structure (star). (H) Showing focal degenerative and necrotic changes in some tubular epithelium (open arrow) and focal interstitial aggregation of round cells (star).

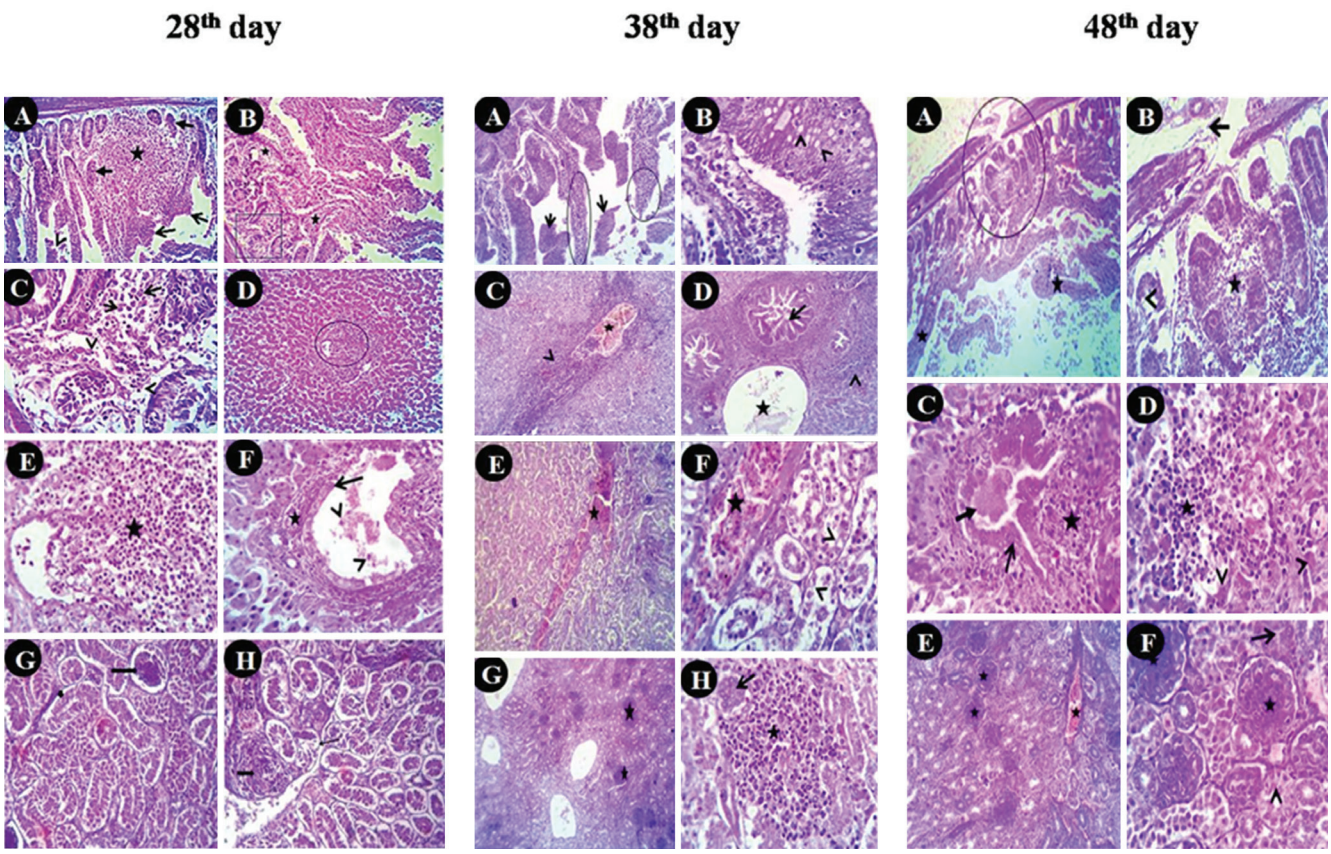

Figure 3: Photomicrograph of chicken's small intestine, liver and kidney of infected and amoxicillin treated group: H&E

At 28th day (X 200, 400); Intestine: (A&B) showing villous necrosis (open arrows), desquamation of the epithelial lining (arrowhead), Focal distortion and degeneration of the intestinal crypts and gland (closed arrows) and (C) moderate infiltration of round cells in the lamina propria and sub-mucosa (stars). (C) Lymphocytes (arrowheads) and macrophages (open arrows). Liver: (D&E) showing focal hepatic necrosis especially periportal (circle) which replaced by moderate aggregation of round cells (star). (F) Showing hyperplasia of bile ducts (open arrow) with partial destruction of the epithelial lining (arrowhead) and periductal fibrosis (star). Kidney: (G) showing dissociated tubular epithelium (arrowhead) and contracted glomeruli (arrow). (H) Showing regenerative attempts (thick arrow) and thickened tubular basement membrane (thin arrow).

At 38th day (X 100, 200, 400); Intestine: (A&B) showing villous necrosis (circle), sloughed epithelium (arrows) and focal goblet cell metaplasia (arrowheads). Liver: (C&D) showing biliary hyperplastic changes (open arrow), massive round cells infiltration in the portal area (arrowheads) and congestion of hepatic blood vessels (stars). Kidney: (E&F) showing dilatation of the renal blood vessels (star), Focal necrotic changes in some tubular epithelium (arrowheads), (G) Most of the glomeruli showing mild to moderate proliferative reactions in the mesangial and endothelial cells(stars). (H) Focal regenerative tubules (arrow) beside focal aggregation of round cells (star).

At 48th day (X 100, 200, 400); Intestine: (A) showing widespread villous necroses (star), (B) moderate round cells infiltration in the mucosa and sub-mucosa (star), glandular and crypt distortion (arrowhead), edematous in the muscular and sub-serosa (open arrow). Liver: (C) showing mild to moderate round cell aggregation in the portal area (star) with mild biliary hyperplasia (open arrow) and presence of static secretory materials in their lumina (closed arrow). (D) Multifocal interstitial round cells infiltration (star) with necrotic changes in some hepatocytes (arrowheads). Kidney: (E) showing mild to moderate congestion of renal blood vessels and capillaries (stars). (F) Focal degenerative (open arrow) and necrotic changes (arrowhead) in some tubular epithelium. Some glomeruli show mesangial and endothelial hyperplastic and hypertrophied changes (stars)

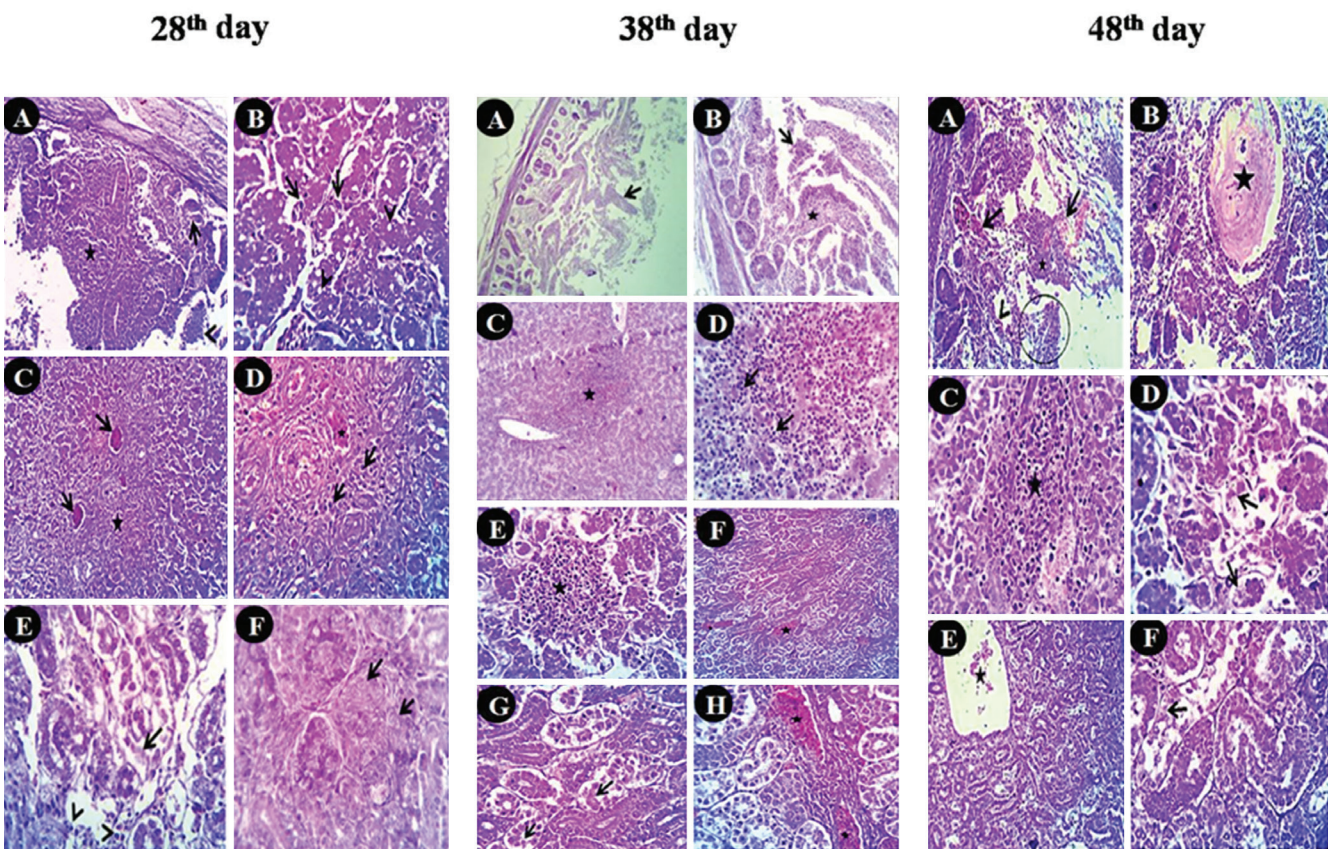

Figure 4: Photomicrograph of chicken's small intestine, liver, and kidney of infected and allicin treated group: H&E

At 28th day (X 200, 400); Intestine: (A) showing villous necrosis (star), desquamation (arrowhead) and crypt distortion (open arrow). Liver: (B) showing hypertrophied Kupffer cells (open arrows), fatty change I na number of the hepatocytes (arrowheads). (C) Congested hepatic blood vessels (arrows) with portal fibroblasts proliferation (star) and (D) round cells infiltration, mainly lymphocytes (open arrow). Kidney: (E) showing focal degenerative and necrotic changes in some tubular epithelium (open arrow), mild interstitial round cell infiltration (arrowheads). (F) Focal regeneration in some tubular epithelium (open arrow)

At 38th day (X 100, 200, 400); Intestine: (A&B) showing villous necrosis (open arrows) with moderate round cell infiltration in the mucosa and submucosa (star). Liver: (C&D) showing portal and interstitial round cells aggregation (star) mainly lymphocytes (open arrows). Kidney: (F&G&H) showing focal necrotic changes in some renal tubular epithelium (arrows) with congested renal blood vessels (stars)

At 48th day (X 100, 200); Intestine: (A&B) Intestine: (A) showing massive villous necroses (circle), dilated capillaries (open arrows) in the mucosa and submucosa with moderated round cells infiltration (star). (B) Some of the intestinal crypts are cystically dilated and filled with mucinous secretion (star)

Liver: (C&D) showing interstitial aggregation of round cells (star), necrotic changes in some hepatocytes (open arrow). Kidney: (E&F) showing moderately congested renal blood vessels (star) with focal necrotic changes in some tubular epithelium (open arrow)

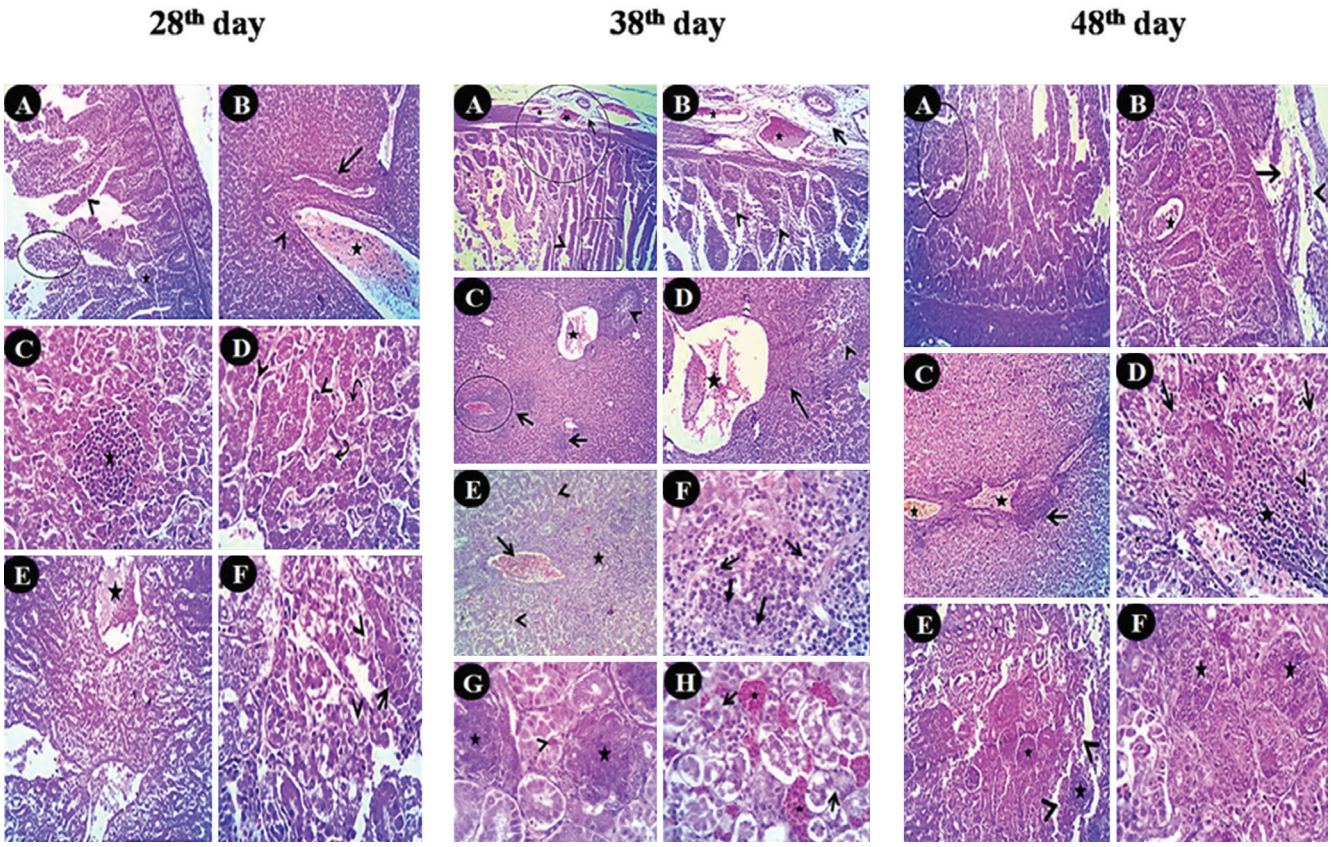

Figure 5: Photomicrograph of chicken's small intestine, liver and kidney of infected and treated with 20 mg amoxicillin and 25 mg allicin/kg b.wt. H&E

At 28th day (X 100, 200, 400); Intestine: (A) showing villous necrosis (circle), desquamation (arrowhead) and round cell infiltration in the mucosa (star). Liver: (B) showing congested hepatic blood vessels (star), portal biliary (open arrow) and fibroblast proliferation (arrowhead). (C&D) showing focal interstitial aggregation of round cells (star) and dilated sinusoids with hypertrophied kupffer cells (arrowheads) ,degenerative changes in some hepatocytes (curved arrows). Kidney: (E&F) showing moderately congested renal blood vessels (star). Focal degenerative and necrotic changes in tubular epithelium (arrowheads) and focal regenerative processes in some tubules (open arrow).

At 38th day (X 100, 200, 400); Intestine: (A&B) showing villous necrosis (square), crypt and gland destruction (arrowheads), the muscular coat and the subserosal tissue showing congested blood vessels (stars) and exudative oedema (open arrows). Liver: (C&D) showing moderate to severe vascular congestion (star) with prominent lymphocytosis in portal areas (open arrows) and bile duct hyperplasia (arrowheads). (E&F) portal and interstitial round cells infiltration (star) with partial replacement of the hepatocytes by lymphocytes (open arrow) and macrophages (closed arrow), necrotic changes in most parenchyma (arrowheads). Kidney: (H) showing moderate congestion of intertubular capillaries (star) with focal degenerative and necrotic changes in some tubular epithelium (open arrows), (G) some glomeruli showed hypertrophic and hyperplastic mesangial and endothelial cells (stars).

At 48th day (X 100, 200, 400); Intestine: (A&B) showing characteristic villous necrosis (circle). The crypts and the glands are cystically dilated and filled by necrotic debris (star), muscular coat showing focal vascular dilatation (arrowhead) and exudative edematous reaction (open arrow). Liver: (C) showing mild to moderate congestion of hepatic blood vessels (stars) with round cells infiltration in the portal area (open arrow). (D) Higher magnification of the previous figure to show hydropic degeneration (open arrows) and infiltration of the portal area by mononuclear cells (star) mainly lymphocytes (arrowhead).
Kidney: (E) showing necrotic changes in some tubules (arrowheads) and glomerular hyperplastic mesangial and endothelial cells (stars). (F) Regenerative changes of some tubular epithelium (stars).

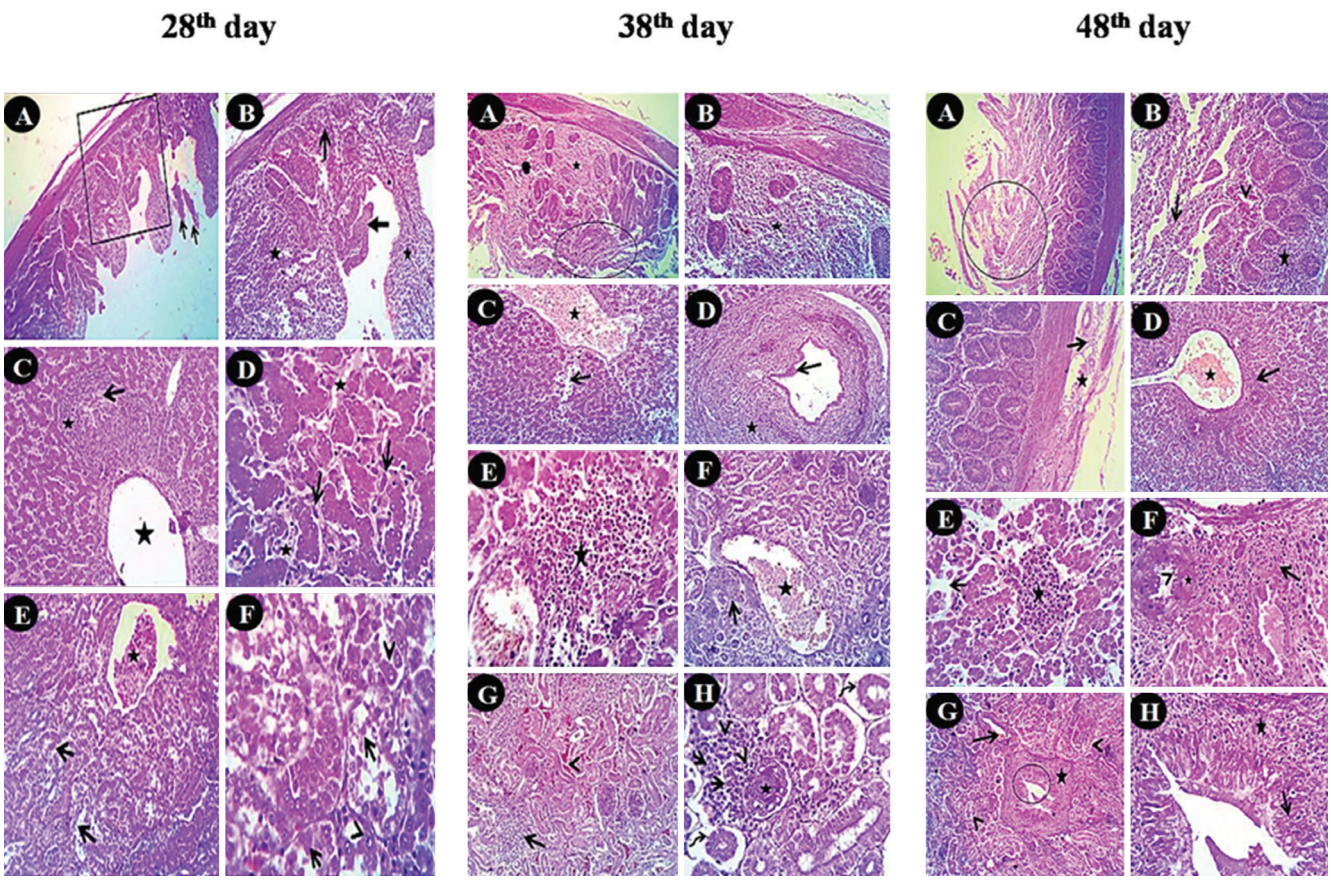

Figure 6: Photomicrograph of chiken's small intestine, liver and kidney of infected and treated with 10 mg amoxicillin and 25 mg allicin/kg b.wt. H&E

At 28th day (X 100, 200, 400); Intestine: (A) showing villous atrophy and desquamated epithelium (open arrows), (B) villous necrosis (closed arrow), gland distortion (open arrow), round cells infiltration in the mucosa and submucosa (star) and intermuscular edema (arrow head). Liver: (C) showing portal biliary hyperplasia (open arrow) with massive round cell infiltration (star), congested hepatic blood vessels (star), (D) dilated sinusoids (stars) and hypertrophied Kupffer cells (open arrow). Kidney: (E&F) showing moderate congestion of renal blood vessels (star) and focal degenerative (arrow heads) and necrotic changes (open arrows) in some tubular epithelium.

At 38th day (X 100, 200); Intestine: (A&B) showing villous necrosis and epithelial sloughing (circle). Moderate infiltration of the mucosa, submucosa and muscular coat by round cells (stars). Liver: (C) showing moderately congested hepatic blood vessels (star) and sinusoids (arrow). (D&E) Severe biliary hyperplasia (open arrow) with periductal fibrosis and round cell infiltration (star). Kidney: (F&G) showing moderate to severe congestion of renal blood vessels (star), intertubular capillaries (arrowhead) with multifocal interstitial round cell aggregations (open arrow). (H) Focal degenerative and necrotic changes in renal tubular epithelium with detached basement membrane (curved arrows) and interstitial aggregation of macrophages (arrow) and lymphocytes (arrowheads) and hyaline casts within renal tubules (star).

At 48th day (X 100, 200, 400); Intestine: (A&B) showing widespread villous necroses (circle) with moderate round cells infiltration in the mucosa and submucosa (star) and dilated blood vessels(arrowhead), (C) exudative edematous changes in the tunica muscularis (open arrow) with congested blood vessels (star). Liver: (D) showing mild to moderate vascular congestion (star) with mild to moderate round cells infiltration in the portal area (open arrow) and (E) in the interstitial tissue (star) beside dilated sinusoids (open arrow). (F) Some of the hepatic arterioles showing vacuolated endothelium (arrowhead) and thick hyalinized walls (star) beside hyalinized fibrosis in the portal area (open arrow). Kidney: (G) showing necrotic changes in the tubular epithelium (arrowhead), hyperplastic changes in the ducts (open arrows) which surrounded by a moderate number of round cells (stars).

Histopathological changes were observed in the intestine, liver, and kidneys of all groups at 28, 38, and 48 days of age; however, in comparison to the control group (Figure 1), all groups infected with *Clostridium perfringens* showed different grades of lesions. Lesions were the most severe in the group, infected but not treated with either amoxicillin and/or allicin (Figure 2), while they were milder in groups treated with amoxicillin and/or allicin (Figure3, Figure 4, Figure 5, Figure 6).

Lesions in the intestines consisted of villous necrosis, epithelial desquamation, distorted crypts and glands, congestion, oedema and mild infiltration of the propria with lymphocytes and macrophages. Liver showed congestion of hepatic blood vessels, portal aggregation of lymphocytes and macrophages, mild degenerative changes (cloudy swelling, hydropic degeneration) in most hepatocytes, and hypertrophied Kupffer cells. Kidney lesions included diffuse haemorrhagic areas containing scattered necrotic tubules, and interstitium infiltrated with erythrocytes and lymphocytic aggregations.

Discussion

Broiler chickens experimentally infected with *Clostridium perfringens* had significant decrease in body weight and weight gain, and increase in feed conversion rate. Similar results were reported in several studies (32, 33), in which the authors described hepatitis, associated with high incidence of *Clostridium perfringens* infections in broiler flocks. Beside hepatitis, they have also observed in the same study a decrease in the growth rate, an increased feed conversion rate and necrotic enteritis. Clostridial toxins induce damage in intestinal tissue and the liver, which leads to a decrease in a nutrient absorption and metabolism, and consequently reduces growth performance (33).

The effects of the treatments in our study, which are presented in Table 1, Table 2 and Table 3 and Figures 2 to 6, revealed that the administration of amoxicillin and/or allicin for the treatment of *Clostridium perfringens* infection resulted in improved growth performance parameters, and milder lesions in the intestines, liver and kidney. This improvement is likely due to the antimicrobial effect of the antibiotic used, resulting in decreased intestinal colonization in diseased broilers, prevention of necrotic enteritis and consequently increased body weight, weight gain, and improved feed conversion rate (34). Furthermore, the infected chickens showed an improvement in body weight gain and FCR when treated with amoxicillin (35). The activities of intestinal mucosa enzymes and nutrient digestibility were increased after garlic supplementation and represent an alternative to antibiotics in broiler nutrition (36). It was reported before that diets supplemented with garlic at a dose of 1 and 1.5 gm/kg diet prevent subclinical necrotic enteritis and improved performance of broiler chickens (37).

A considerable decrease was noted in the total number of erythrocytes, haemoglobin concentration and packed cell volume percentage in infected broiler chickens when compared to non-infected, untreated broiler chickens. These results might be due to excessive destruction of erythrocytes by the clostridial toxin (38). The results of our study also indicate that *Clostridium perfringens* infection in broiler chickens induced a significant increase in the total number of leukocytes. Changes in leukocytes in broiler chickens infected with *Clostridium perfringens* are likely a reflection of the inflammatory response in the intestinal tract due to infection. Interestingly, significant increases in the PCV, Hb, and RBCs of chicken feed with garlic had been previously reported (39).

Infected and untreated chickens displayed significant elevation in liver enzyme activity (AST, ALT, and ALP) in comparison to non-infected untreated chickens. This elevation might be due to pathological changes in liver post infections or due to clostridial toxin-induced alteration in cellular permeability, which allows the escape of liver enzymes into the serum (38). Infected broiler chickens treated with amoxicillin and/or allicin displayed significant elevation in the activity of AST, ALT, and ALP at the 28th day when compared to healthy non-treated broiler chickens. These results are similar to those reported by Bryan C et al. (40) who reported that improved liver enzymes post-treatment infection in chickens might be due to an antimicrobial effect of the drugs used in suppression microorganisms invading the host and retarding its metabolic activity and liver enzyme activity. These findings might be attributed to the antioxidant effect of garlic (41). In infected and untreated chickens, there was a significant reduction in total protein and albumin levels in blood. Hypoalbuminemia could be due

to the destructive effect of the microorganism and clostridial toxins on the liver cells producing albumin. The reduction in total protein and albumin in the infected broiler chickens might be due to the malabsorption of nutrients from the inflamed intestines**.** Another explanation for the reduction in total protein and albumin in broilers infected with *Clostridium perfringens* comes from a study of Lovland A et al., in which the authors reported similar changes of protein picture in broilers infected with *Clostridium perfringens* (33).

Infected chickens treated with amoxicillin displayed an insignificant decrease in total protein blood content in comparison to the healthy non-treated chickens but had significant decreases in albumin concentration. This improvement in serum protein might be due to the improved state of the liver in the treated chickens as a synthesis of albumin; the largest individual protein fraction in avian plasma takes place in the liver, or alternatively, treatment alters the renal secretion by changing the state of the kidney (38). The infected and allicin treated group showed non-significant changes in total proteins and albumin at 2nd-week post-treatment in comparison to the control group. These results indicate an improvement in the hepatic functions due to the antioxidant effect of the phytophenolic compounds in garlic (42).

A marked increase in creatinine and uric acid levels was recorded after experimental infection. Increase in uric acid, creatinine in the infected birds might be a result of degenerative changes in the kidney tubules, preventing the excretion of uric acid and creatinine, increasing their levels in serum. Our data are also in accordance with the finding of Harrison et al. (43), who reported an increase in creatinine level in case of renal disease. Garlic reduced urea, uric acid, and creatinine levels after lead toxicity in broiler chickens (44).

The pathological lesions in chickens infected with *Clostridium perfringens* were similar as were observed and described before (47, 48). Findings in the liver and kidneys in our study were similar to those reported before for these organs in chickens 12 h after inoculation of broth culture or toxins of *Clostridium perfringens* (49).

Conclusions

The combination of both drugs (amoxicillin and allicin) proved to be the better treatment of *Clostridium perfringens* infection than each drug alone, indicating a synergistic effect. No significant differences between the two doses of amoxicillin with allicin were detected. This combination improved the health state, body weight gain, feed conversion rate, blood parameters and biochemical indices, and reduced the severity of histopathological changes in the intestines, liver and kidney.

Acknowledgement

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EFFICACY OF AMOXICILLIN (ATCOMOX®) AND/OR ALLICIN ON PERFORMANCE, HAEMATOLOGICAL, BIOCHEMICAL, AND HISTOPATHOLOGICAL CHANGES IN *CLOSTRIDIUM PERFRINGENS* INFECTED CHICKENS

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Povzetek: V študiji smo ugotavljali učinkovitost amoksicilina in/ali alicinina pri zdravih pitovnih piščancih in pitovnih piščancih poskusno okuženimih z bakterijo *Clostridium perfringens*. Piščanci so bili razdeljeni v šest skupin in so zdravila dobivali peroralno preko vode pet dni zapored. V prvi skupini so bili neokuženi in nezdravljeni piščanci, v drugi okuženi in nezdravljeni, v tretji okuženi in zdravljeni z amoksicilinom (20 mg/kg telesne mase), v četrti skupini okuženi in zdravljeni z alicinom (25 mg/kg telesne mase) v peti skupini okuženi in zdravljeni z amoksicilinom (20 mg/kg teže) in alicinom (25 mg/kg telesne mase) in v šesti skupini okuženi in zdravljeni z amoksicilinom (10 mg/kg teže) in alicinom (25 mg/kg telesne mase). Spremljali smo prirast piščancev ter njihove hematološke in biokemične parametre. Pri okuženih piščancih smo v krvi opazili znatno znižanje skupnih beljakovin, albuminov, RBC, Hb in PCV ter znatno povečanje WBC, AST, ALT, ALP, kreatinina in sečne kisline. Uporaba amoksicilina in/ali alicina za zdravljenje okužbe s *Clostridium perfringens* je povzročila izboljšanje hematoloških in biokemičnih sprememb po okužbi. Odmerek amoksicilina 10 mg/kg in alicina 25 mg/kg telesne mase za zdravljenje okužbe s *Clostridium perfringens* pri pitovnih piščancih brojlerjih se je izkazal kot najbolj učinkovit, verjetno zaradi sinergističnega učinka obeh zdravil, in je povzročil zmanjšanje smrtnosti pitovnih piščancev.

Ključne besede: amoksicilin; alicin; učinkovitost; brojlerji; biokemjski parametri; hematološki parametri