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Efficacy of both antibiotic and probiotic for control of necrotic enteritis in chickens experimentally

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ABSTRACT

Clostridium perfringens (*C. perfringens*) is the pathogenic bacterium caused necrotic enteritis (NE) which is the most threatening problem in poultry sector. This work aimed to study the occurrence of *Clostridium perfringens*, find out its *in-vitro* antibiotic sensitivity pattern to various antimicrobial drugs and the experimentally efficacy of antibiotic (amoxicillin) alone or including a probiotic against necrotic enteritis NE. The occurrence rate of *C. perfringens* in examined 150 tissue samples was 38.6%. Antibiogram analysis revealed that the organisms are very susceptible to amoxicillin (74.1%) followed by bacitracin (70.6%). Almost of isolates were multidrug resistant (MDR). Toxinotyping of *C. perfringens* isolates by PCR showed that all tested isolates belonged to *C.perfringens* type A. In the experiment, one day-old Cobb broiler (N=120) were divided into six equal-sized groups. Group (A) was provided with balanced ration without treatment (control negative), group (B) was fed on balanced ration and infected with *C. perfringens* (control positive), group (C) was infected with *C. perfringens* and treated with selected antibiotic (amoxicillin) in drinking water for 3 consecutive days by dose of 1gm/ L 24 hours post infection, group (D) was received probiotic in the drinking water from one day-old and then infected with *C. perfringens*, group (E) was received probiotic in the drinking water from one day-old and then infected with *C. perfringens* then treated with selected antibiotic (amoxicillin) by the same dose 24 hours post infection and group (F) was infected with *C. perfringens* and treated with selected antibiotic (amoxicillin) and probiotic by the same dose 24 hours post infection. The results showed that birds received probiotic displayed

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higher BW, BWG and most improved FCR over the whole rearing period. Chickens infected with *C. perfringens* had characteristic effect in hematological and biochemical parameters with significant decrease in total protein and HDL. The treated groups revealed significant improvement in above mentioned parameters. Experimentally infected chickens with *C. perfringens* developed atypically clinicopathological pictures of NE with 30% mortality. A milder disease picture has been recorded in treated groups with amoxicillin and or probiotic. According to our research, adding probiotic supplements to a chicken's diet can improve performance, gut health, and blood components to protect the bird against *C. perfringens* infection.

INTRODUCTION:

A common anaerobic bacterium that is easily found in soil, dust, excrement, feed, poultry litter, and intestinal contents is Clostridium perfringens, which is the source of the disease necrotic enteritis (NE). Broilers chickens that are 2 to 5 weeks old are most commonly affected. **(Osman and Elhariri, 2013).**

Clostridium perfringens bacteria can infiltrate the delicate intestinal tissues of broilers attributable to their well-known endo- and exotoxins. When enterotoxins from *C. perfringens* bind to a protein called claudin and negatively affect birds, weight gain, FCR, and crude protein digestibility, the tight junction is broken and the barrier function is impaired (**Saleh et al. 2023).** The main toxin responsible for necrotic enteritis in chicken is the α -toxin **(Timbermont et al. 2009).** Due to cholangiohepatitis and intestinal mucosal necrosis, the disease is characterized by a decreased growth rate and low feed conversion rate. **(Eraky and Abd El-Ghany, 2022).** In the early stages of the disease, adequate antibiotic treatment may be able to control the infection **(Lyras et al. 2009).**

Since the use of antimicrobial feed additives for prevention has been restricted, NE has become more important **(Opengart and Songer, 2013).**

Recently, the economic production of safe chicken products without the use of antibiotics has been assisted by a number of natural alternative feed additives. Probiotic cultures have recently been used to maintain a healthy gut microbiome in humans and animals to prevent the attachment of harmful microbes, especially in the early stages of growth **(Abd El-Hack et al. 2022).** Furthermore, probiotic substances have good impacts on liver histopathology, intestinal morphology and histopathology besides a considerable reduction in both of their lesion ratings **(Hussein et al. 2020).** Additionally, probiotics can be used as supplements to achieve health benefits including hypocholesterolemia and lowering blood sugar since they have a considerable impact on biochemical markers **(Abd Al-Fatah., 2020).**

Owing to the economic importance of this disease, this work was designed for isolation, identification , studying the antimicrobial susceptibility of *C. perfringens* isolated from chickens, screening toxigenic attributes of circulating strains by multiplex PCR and evaluating the impact of dietary supplementation with probiotics and /or antibiotic on prevention of *C.perfringens* infection and reduction of NE severity as well as their effect on growth performance, hematobiochemical parameters and the pathological changes.

MATERIALS AND MEHODS

Ethical approval

This study was declared by the Local Committee of the (ARC-IACUC) committee Institute: Animal Health Research Institute Ethical Committee Approval Number: ARC/ AH/23/45. All steps were carried out in accordance with the Animal Health Research Institute's recommendations in compliance with

OIE criteria for the use of animals in research and teaching.

Samples

A total of 150 intestinal samples from diseased broiler chickens aged from 2 to 5 weeks from different farms with history of diarrhea, depression and reduced growth performance were collected in the study. Samples were aseptically transferred in ice boxes for isolation and identification of *C.perfringens* in the Laboratory.

Bacterial isolation and identification:

Tissue samples were inoculated into tubes containing cooked meat medium (Oxoid, UK) **(Willis, 1977)** and incubated in anaerobic jar for 24 hrs at 37°C. Aliquots of 0.1 ml were streaked over Perfringens agar (Tryptose Sulphite Cycloserine TSC Agar, Oxoid) and 10% sheep blood agar, each containing 200 ug/ml of neomycin sulphate then incubated anaerobically at 37°C for 24 hrs (**Carter and Cole, 1990).** On blood agar, the C. perfringens colonies had a flat, olive color and a distinct double hemolysis zone, with the inner clear zone caused by beta toxin and the outside zone by alpha toxin **(Vaikosen and Muller, 2001).** Due to *C. perfringens'* capacity for sulphite reduction, colonies on TSC agar were black in colour. Gram positive non-motile bacilli were found through microscopic analysis. Biochemical identification of *C. perfringens* isolates showed catalase and indole negative, nitrate reduction positive, lecithinase (phospholipase C; α- toxin) activity on egg yolk Agar with opalescence **(Cruickshank et al. 1975; Koneman et al. 1988 and Macfaddin, 2000).**

Antibiogram

Antimicrobial susceptibility testing of isolates were applied by agar disk diffusion method according to British Society for Antimicrobial Chemotherapy **(BSAC), (2011).** The susceptibility testing were applied against antimicrobial agents of the commonly used in the field amoxicillin (AX:10 µg), clindamycin (DA:2 µg) bacitracin (B:10 µg), lincomycin (L:30 µg), amikacin (AK:30 µg), sulfamethoxazole/trimethoprim (SXT: 25 µg) neomycin (N:30 µg), cefotaxime (CTX:30 µg), ciprofloxacin (CIP:5 µg), spectinomycin (SPC:100 µg) and colistin (CT: 10 µg) by using commercial disks from Oxoid laboratories. In order to encourage the development of anaerobic bacteria, antimicrobial susceptibility tests were performed on 10% sheep blood agar media **(Perelman et al., 1991)**.

Molecular characterization of *C. perfringens* toxigenic attributes

Tested isolates:

Eight *C.perfringens* isolates were randomly selected out of the twenty four isolates that demonstrated multidrug resistance phenotypes by agar disc diffusion for typing by PCR.

DNA extraction:

QIAamp DNA mini kit (Qiagen, Germany, GmbH) was used.

Oligonucleotide primer:

Primers were supplied from metabion (Germany), primers' sequences; thermal profiles for PCR were shown in table (1).

PCR amplification:

Multiplex PCR for *C. perfringens* toxins**,** primers were utilized in a 50- µl reaction containing 25 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 12 µl of water, and 5 µl of DNA template. The reaction was performed in an Appliedbiosystem 2720 thermal cycler, Germany.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH). A generuler 100 bp ladder (Fermentas, thermo, Germany) were used to determine the fragment sizes. A gel-based documentation system (Alpha Innotech, Biometra) took pictures of the gel, and computer software was used to analyze the data.

Antibiotic

Amoxicillin: Arabcomox® obtained commercially from Arab Company, Egypt. Each

100 gm contains Amoxicillin trihydrate 23 gm (equivalent to Amoxicillin base 20gm) by dose 1gm/ L drinking water for 3 days (recommended dose).

Probiotics

Gro2max® obtained commercially from Bionatural America institute (USA) contains a source of live, naturally occurring microorganisms (*Bacillus subtillis* 7×10^6 CFU/gm, *Lactobacillus acidophilus* 3×10^6 CFU/gm, *Pediococcus acidilactici* 2×10^4 CFU/gm, *Pediococcus pentosaceus* 2×10^4 CFU/gm and *Saccharomyces cervisiae* 1×10^6 CFU/gm) was used in a dose of 3.5 gm/gallon (3.8 L) in drinking water as recommended by the producer.

Bacterial inoculum (Bathhoko, 2009)

One of the PCR confirmed toxigenic *C. perfringens* type A isolated field strain in the current study was used in the challenge. At 19 days old; the birds were challenged via oral gavages by inoculation of 1ml of $1.5x10^8$cfu daily, for 3 consecutive days [19^{th}-20^{th} –and 21^{st} day of age].

Experimental design:

One hundred and twenty; one day-old Cobb broiler chickens were divided into six equal groups (A, B, C, D, E and F) of 20 each. The chicks were vaccinated at 7^{th} and 17^{th} days with Newcastle and at 11^{th} and 22th against Gumboro in drinking water. Group (A) was provided with balanced ration without treatment (control negative), group (B) was fed on balanced ration and infected with *Closrtidium perfringens* (control positive), group (C) was infected with *C. perfringens* and treated with selected antibiotic (amoxicillin) in drinking water for 3 consecutive days by dose of 1gm/ L 24 hours post infection, group (D) was received probiotic in the drinking water from one day-old by dose 3.5 gm/gallon and then infected with *C. perfringens*, group (E) was received probiotic in the drinking water from one day-old by the same dose and then infected with *C. perfringens* then treated with selected antibiotic (amoxicillin) by the same dose 24 hours post infection and group (F) was infected with *C. perfringens* then treated with selected antibiotic (amoxicillin) and probiotic by the same dose 24 hours post infection. The chicks were reared on floor rearing, temperature was adjusted according to chicks age and subjected to drinking water and feed ad-libitum **(NRC, 1994),** for 38 days (the experimental period).

Count of *C.perfringens*

The intestinal tract of sacrificed birds of each group at 7 days post treatment was collected for bacterial count. Approximately 1-2 gram of intestinal contents from each of three birds from the challenged groups of chickens were collected, samples from each group were pooled for bacterial reisolation and count post challenge **(Soad et al. 2015).** Samples were diluted in buffered peptone water for an initial of 10^{-1} dilution, then tenfold serial dilutions were applied, one ml of each dilution was spread in duplicates of 10% sheep blood agar containing 200 ug/ml neomycin sulphate. All plates were incubated in gas pack anaerobic jar at 37˚C for 48 hours. Plates that produced 30-300 colonies were selected for enumeration. Count was expressed as log10 CFU per gram of intestinal contents, **(Cruickshank et al. 1975).**

Monitoring the Bird's Growth Performance:

The body weight (BW) and the average daily feed intake (FI) were determined to calculate the body weight gain (BWG) and feed conversion ratio (FCR) at the end of each period **(Cengiz et al. 2015).**

Blood samples:

Blood samples were collected from wing vein puncture under aseptic precautions after 7 and 14 days post treatment. The first sample was 1 ml of blood collected on EDTA for hematological examination. The second blood sample was 3 ml of blood taken without anticoagulant in a clean and dry centrifuge tube, left to clot at room temperature and centrifuged at 3000 rpm for 5 min. Serum samples were putted in dry clean capped tubes and kept in deep freeze at- 20ºC for biochemical analysis.

The hematological studies:

Blood samples with anticoagulant were subjected for detection of cellular blood constituents according to **Feldman et al. (2000).**

Biochemical studies:

All biochemical parameters were measured using commercially available kits, and the manufacturer's manual's recommended technique was followed for each parameter. The liver transferases (alanine aminotransferase ALT and aspartate aminotransferase AST) activities were estimated according to **Murray (1984).** Serum uric acid was determined according to **Sanders et al. (1980) and according to Henry (1974),** the serum creatinine was estimated. Serum total protein was measured according to **Tietz (1995).** The procedure outlined by **Lott and Turner (1975)** was used to measure serum glucose. Immunoglobulin G (IgG) measured according to **Dati (1989).** Total lipids analysis using commercially available kits from Diamond Diagnostics in Egypt, total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were tested calorimetrically per the manufacturer's instructions according to **Saleh (2013).**

Histopathological examination:

Samples of 3 cm long that obtained from intestine (1 cm cut from the midpoint), liver and kidney from each group were collected at 7^{th} and 14^{th} day post treatment. Tissue specimens were collected and flushed with phosphate buffered saline (PBS, pH 7.4), and fixed in neutral buffered formaldehyde (10%). The fixed specimens were processed by the conventional paraffin embedding technique including dehydration through graded concentrations of ethanol, clearing in 3 washes of xylene and melted paraffin, and finally embedding in paraffin wax at 65°C. 5µm thick sections were stained by Hematoxylin and Eosin (**Bancroft and Layton, 2013).** and then stained sections were examined under light microscope.

Statistical analysis:

The analysis of variance (ANOVA) method of statistics was used. Differences in treatment mean were determined using Duncan's Multiple Range at a significance level of 0.05. The SPSS program was used to run all of the statistics (**Kinnear and Gray, 2006).**

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target bacteria	Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
C. perfringens	*Alpha toxin*	GTTGATAGCGCAG-GACATGTTAAG CATGTAG-TCATCTGTTCCAGCATC	402 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Yoo et al., (1997)**
	Beta toxin	ACTATACAGA-CAGATCATTCAACC TTAGGAGCAGTTAGAAC-TACAGAC	236 bp						
	Epsilon toxin	ACTGCAAC-TACTACTCATACTGTG CTGGTGCCTTAA-TAGAAAGACTCC	541 bp						
	Iota toxin	GCGATGAAAAGCC-TACACCACTAC GGTATATCCTCCAC-GCATATAGTC	317 bp						

RESULTS

Recovery rate of *C. perfringens*.

C. perfringens was isolated from 58 of 150 pooled tissue samples (38.6%). Standard microbiological methods were used to identify them.

Antimicrobial susceptibility testing.

Antimicrobial sensitivity profiling of the 58 confirmed *C. perfringens* isolates, indicated that higher rates of sensitivity to amoxicillin 43 (74.1%) followed by Bacitracin 41 (70.6 %), Amikacin 40 (68.9%) and clindamycin 39 (67.2%). On the other hand, out of examined isolates 42 (72.4%) were found resistant to Sulfamethoxazole/trimethoprim followed by (67.24%) to Colistin and (65.54%) to Neomycin (Fig. 1). All of the *C. perfringens* isolates under examination shown resistance to the common antimicrobials. Overall, results showed that most isolates were resistant to at least three of the tested antimicrobial agents from different groups, making them multidrug resistant (MDR)

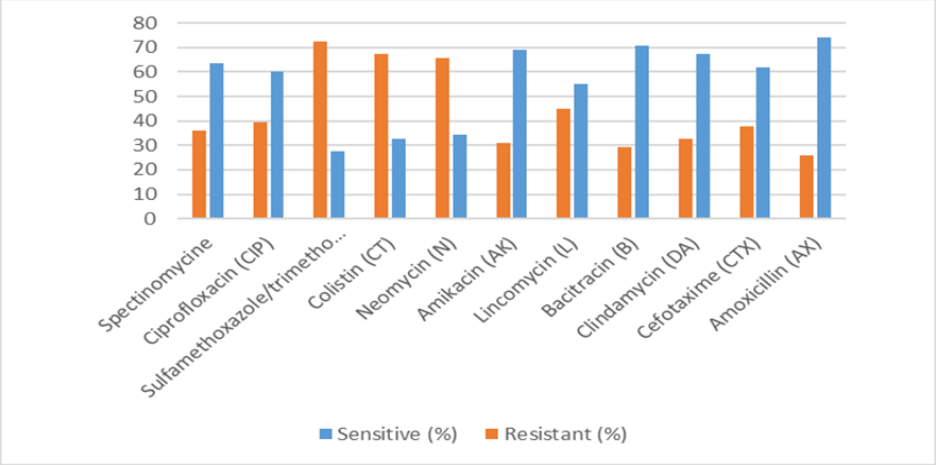

Figure 1. Antimicrobial susceptibility profiles of C. perfringens isolates.

Toxinotyping of *C. perfringens* isolates by PCR.

Toxinotyping of isolates by PCR was applied on 8 randomly selected MDR *C. perfringens* isolates. PCR targeted the detection of toxigenic genes cpa gene encodes for alpha toxin, cpb gene encodes for beta toxin, εtx gene encodes for epsilon toxin and iA gene encodes for iota toxin, respectively. The results revealed that all (100%) of PCR examined isolates belonged to *C. perfringens* type A and produced positive PCR result for only cpa gene that encodes alpha toxin with the creation of a particular 402 bp amplicon. On the other hand, none of the 8 tested isolates was positive for cpb gene, etx gene, or iA gene (Fig. 2).

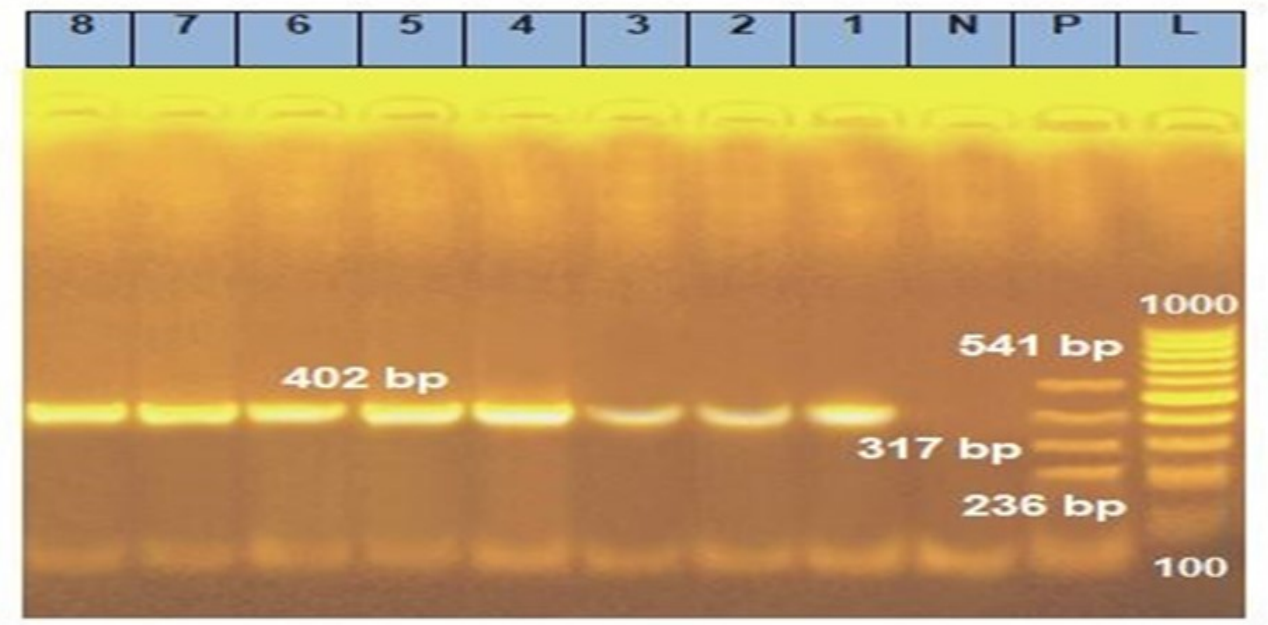

Figure (2): Multiplex PCR for Toxinotyping of *C.perfringens* isolates. Lane Neg.: negative control; (E.coli). lane POS: Positive control for *C.perfringens* type A with positive PCR result for cpα, cpβ, εtx genes and iA genes encode for alpha, beta, epsilon and iota toxins, with production of specific amplicons at 402 bp, 236 bp 541 bp and 317 bp, respectively, lane L: gene ruler ladder 100-1000 bp. Lanes 1- 8 : positive for cpa gene encodes for alpha toxin with production of specific amplicon at 402 bp.

Growth Performance parameters

The results concerning growth performance parameters of broiler chickens at starter, grower (preinfection) and finisher periods (postinfection) are presented in Table (2). During starter and growing periods, the BW and BWG of broiler chickens were significantly increased in groups (D&E). Broilers received probiotic in the drinking water showed improved FCR when compared with other experimental groups. At the end of experimental period, *C. perfringens* challenge caused significant growth retardation in all experimental groups, except for broilers received probiotic in the drinking water from one day-old compared to control positive group (B).

Table 2. Effect of antibiotic and or probiotic supplementation on growth performance under clostridia infection (M±S.E) (n=5).

Groups parameters		Gr(A)	Gr(B)	Gr(C)	Gr(D)	Gr(E)	Gr(F)
Initial body weight (gm)		44±3.25	45±2.14	44±3.01	44.00±2.78	45±2.35	45±3.88
starter(1-10 days)	BW (g/bird)	266±16.95^{b}	270±20.37^{b}	250±17.14^{b}	318±11.38^{a}	315±12.08^{a}	275±15.75^{b}
	BWG (g/bird)	222±16.53^{b}	225±27.55^{b}	206±18.95^{b}	274±16.00^{a}	270±14.22^{a}	230±18.24^{b}
	FC (g/bird)	414	420	398	425	415	400
	FCR	1.86±0.08^{a}	1.87±0.05^{a}	1.93±0.03^{a}	1.55±0.03^{b}	1.54±0.04^{b}	1.74±0.05^{a}
Grower (11-22 days)	BW (g/bird)	1100±6.20^{b}	1115±16.96^{b}	1110±19.30^{b}	1210±14.44^{a}	1200±20.57^{a}	1135±14.66^{b}
	BWG (g/bird)	834±20.55^{b}	845±17.15^{b}	860±20.12^{ab}	892±12.75^{a}	885±17.92^{a}	860±18.94^{ab}
	FC (g/bird)	1225	1232	1226	1120	1140	1210
	FCR	1.47±0.17^{a}	1.46±0.13^{a}	1.42±0.17^{a}	1.26±0.11^{b}	1.29±0.09^{b}	1.41±0.15^{a}
Finisher (23-38 days)	BW (g/bird)	1895±30.00^{b}	1630±40.48^{d}	1820±25.49^{c}	1980±25.45^{a}	2030±37.41^{a}	1925±25.00^{b}
	BWG (g/bird)	795±24.87^{ab}	515±28.30^{d}	710±21.32^{c}	770±16.90^{b}	830±17.92^{a}	790±16.97^{b}
	FC (g/bird)	1800	1650	1750	1750	1800	1800
	FCR	2.26±0.05^{c}	3.20±0.06^{a}	2.46±0.04^{b}	2.27±0.04^{c}	2.17±0.03^{d}	2.28±0.03^{c}

Different letters at the same rows means that there was a significant change at $p<0.05$

Hematological study

The changes in Erythrogram and leukogram parameters were demonstrated in table (3) showed that the broilers infected with *C. Perfringens* resulted in significant decrease RBCs count, Hb concentration, TLC, heterophils and monocytes measures while lymphocytes showed significant decrease in infected group. A significant improvement in RBCs count, Hb content and leukogram in infected treated groups compared to the infected untreated group

Table 3. Effect of antibiotic and or probiotic supplementation on Erythrogram and leukogram under clostridia infection at 7 and 14 days post treatment (M±S.E) (n=5).

Groups parameters	At 7 days post treatments						At 14 days post treatments					
	Gr(A)	Gr(B)	Gr(C)	Gr(D)	Gr(E)	Gr(F)	Gr(A)	Gr(B)	Gr(C)	Gr(D)	Gr(E)	Gr(F
RBCs x10^{6} /µl	2.66^{ab} ±0.03	2.20^{c} ±0.07	2.59^{b}± 0.04	2.55^{b}± 0.03	2.73^{a}± 0.04	2.62^{b}± 0.05	2.78^{a} ±0.04	2.52^{c} ±0.05	2.65^{b} ±0.03	2.63^{b} ±0.03	2.82^{a} ±0.02	2.63^{b} ±0.05
Hb (gm/dl)	13.00^{b} ±0.23	12.58^{c} ±0.30	12.90^{b} ±0.18	12.66^{b} ^{c}±0.21	13.65^{a} ±0.29	13.15^{b} ±0.15	13.67^{a} ±0.25	12.90^{c} ±0.37	13.25^{b} ±0.25	13.28^{b} ±0.36	13.75^{a} ±0.33	13.28^{b} ±0.29
PCV %	30.85 ±0.38	30.50 ±0.32	30.53 ±0.30	30.62 ±0.51	31.74 ±0.40	31.55 ±0.32	31.00 ±0.55	30.95 ±0.48	30.88 ±0.44	31.15 ±0.38	32.00 ±0.50	31.05 ±0.45
TLCx10^{3} /µl	20.19^{c} ±1.09	23.03^{a} ±1.15	22.52^{b} ±1.08	22.12^{b} ±0.48	20.75^{c} ±1.20	22.23^{b} ±0.98	20.56^{c} ±0.83	22.83^{a} ±1.05	22.74^{ab} ±1.28	22.30^{b} ±1.12	21.06^{c} ±1.27	22.36^{b} ±1.08
hetero-philsx10^{3} /µl	5.63^{d} ±0.13	8.87^{a} ±0.11	6.68^{b} ± 0.44	8.00^{a} ±0.15	6.15^{c} ±0.38	6.75^{b} ±0.22	5.50^{c}± 0.16	8.85^{a} ±0.18	6.80^{b} ± 0.21	8.70^{a} ±0.25	6.08^{c} ±0.09	6.33^{bc} ±0.18
Lympho-cytesx10^{3} / µl	10.73^{b} ±0.38	9.22^{c} ±0.25	11.73^{a} ± 0.35	9.20^{c} ±0.28	10.25^{b} ±0.40	11.23^{a} ±0.25	11.19^{a} ±0.18	9.34^{c} ±0.25	11.82^{a} ± 1.08	8.98^{c} ±0.30	10.85^{b} ±0.32	11.75^{a} ±0.48
Mono-cytesx10^{3} / µl	2.75^{b} ±0.06	3.82^{a} ±0.08	3.00^{b} ± 0.20	3.80^{a} ±0.11	3.25^{b} ±0.18	3.15^{b} ±0.15	2.76^{c} ±0.11	3.55^{a} ±0.08	3.04^{b} ±0.15	3.50^{a} ±0.18	2.98^{b} ±0.09	3.20^{ab} ±0.15
Eosino-philsx10^{3} /µl	1.08 ±0.02	1.10 ±0.01	1.11 ± 0.03	1.12 ±0.01	1.10 ±0.05	1.10 ±0.03	1.11 ±0.01	1.09 ±0.03	1.08 ± 0.04	1.12 ±0.02	1.15 ±0.01	1.08 ±0.03

Different letters at the same row means that there was a significant change at $p<0.05$,

Biochemical study

The impact of dietary supplementation with antibiotic and /or probiotics on serum biochemical parameters and lipid profile are documented in table (4) and (5) respectively. AST, ALT, uric acid, creatinine, glucose and IgG values revealed significant decrease in treated groups at 7 and 14 days post treatments compared with control positive group (B). While serum total protein showed significant increase in treated groups compared to control positive group (B). Lipid profile analysis (total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol) revealed significant improvement in the treated groups (D, E and F) compared to control positive group (B) at the two periods. Most of the mention parameters returned to the normal levels in treated groups ((D, E and F) compared to control negative group (A) at 14 days.

Table 4. Effect of antibiotic and or probiotic supplementation on some biochemical parameters under clostridia infection at 7 and 14 days post treatment (M±S.E) (n=5).

Groups parameters	At 7 days post treatments						At 14 days post treatments					
	Gr(A)	Gr(B)	Gr(C)	Gr(D)	Gr(E)	Gr(F)	Gr(A)	Gr(B)	Gr(C)	Gr(D)	Gr(E)	Gr(F)
AST U/L	93.4^{f} ±0.3	191.60^{a} ±0.9	147.1^{b} ±1.3	135.90^{c} ±0.6	103.4^{e} ±0.1	106.8^{d} ±0.3	95.00^{d} ±0.15	207.9^{a} ±1.1	138.9^{b} ±0.5	120.0^{c} ±0.36	94.80^{d} ±0.35	95.80^{d} ±0.57
ALT U/L	9.30^{e} ±0.3	20.70^{a} ±0.5	16.00^{b} ±0.1	14.90^{c} ±0.1	11.50^{d} ±0.1	12.20^{d} ±0.1	9.98^{d} ±0.18	23.30^{a} ±0.19	14.00^{b} ±0.42	12.70^{c} ±0.20	10.00^{d} ±0.30	10.20^{d} ±0.3
Uric acid mg/dl	2.20^{f} ±0.06	4.00^{a} ±0.09	3.00^{b} ±0.02	2.80^{c} ±0.04	2.40^{e} ±0.03	2.60^{d} ±0.009	2.32^{c} ±0.05	4.14^{a} ±0.12	2.86^{b} ±0.07	2.54^{c} ±0.09	2.40^{c} ±0.05	2.35^{c} ±0.08
Creatinine mg/dl	0.24^{e}± 0.002	0.41^{a} ±0.005	0.32^{b}± 0.004	0.29^{c} ±0.003	0.25^{d} ±0.003	0.26^{d}± 0.002	0.25^{c} ±0.002	0.47^{a} ±0.01	0.28^{b} ±0.00 4	0.26^{c} ±0.00 3	0.24^{c} ±0.003	0.25^{c} ±0.003
Total protein g/dl	3.65^{a} ±0.16	2.36^{e} ±0.00	2.60^{d} ±0.04	2.91^{c} ±0.01	3.15^{b} ±0.06	2.99^{b}± 0.00^{bc}	3.86^{a} ±0.16	2.34^{c} ±0.01	2.95^{b} ±0.01	3.12^{b} ±0.05	3.84^{a} ±0.09	3.63^{a} ±0.17
Glucose mg/dl	180.6^{f} ±0.7^{f}	302.40^{a} ±1.9	269.2^{b} ±0.8	220.00^{c} ±0.6	192.9^{e} ±1.3^{e}	206.1^{d} ±2.5	181.3^{d} ±0.8	290.8^{a} ±7.9	243.4^{b} ±4.9	210.3^{c} ±0.40	190.9^{d} ±1.6^{d}	192.10^{d} ±1.7
IgG mg/dL	493.2^{e} ±1.3	714.90^{a} ±6.2	652.9^{b} ±1.8	610.80^{c} ±8.3	504.4^{de} ±3.8	513.6^{d} ±4.2^{d}	493.7^{d} ±1.3	711.4^{a} ±6.4	565.8^{b} ±8.7^{b}	533.3^{c} ±8.15	493.8^{d} ±1.5	495.20^{d} ±2.2

Different letters at the same row means that there was a significant change at $p<0.05$,

Table 5. Effect of antibiotic and or probiotic supplementation on Lipid profile under clostridia infection at 7 and 14 days post treatment (M±S.E) (n=5).

Groups parameters	At 7 days post treatments						At 14 days post treatments					
	Gr(A)	Gr(B)	Gr(C)	Gr(D)	Gr(E)	Gr(F)	Gr(A)	Gr(B)	Gr(C)	Gr(D)	Gr(E)	Gr(F)
Total Cholesterol mg/dl	117.3^{e} ±1.1	164.0^{a} ±1.4	155.5^{b} ±0.6	138.0^{c} ±0.7^{c}	122.1^{d} ±0.1	125.3^{d} ±1.6	117.90^{d} ±1.10	164.90^{a} ±1.38	149.7^{b} ±0.05	132.4^{c} ±1.48	120.20^{d} ±0.32	120.5^{d} ±0.37
Triglyceride mg/dl	77.50^{e} ±0.5	136.8^{a} ±0.8	115.6^{b} ±1.0	100.8^{c} ±1.3	89.50^{d} ±0.5	91.70^{d} ±0.4	78.10^{f} ±0.59	140.50^{a} ±1.96	106.6^{b} ±1.77	98.30^{c} ±0.80	83.00^{e} ±1.34	87.30^{d} ±1.36
LDL mg/dl	47.70^{f} ±2.9	95.50^{a} ±0.9	80.60^{b} ±0.8	63.30^{c} ±1.1	52.20^{e} ±0.5	57.90^{d} ±0.1	48.10^{d} ±3.04	96.10^{a} ±1.08	77.70^{b} ±1.01	59.20^{c} ±1.80	47.80^{d} ±2.74	49.00^{d} ±2.03
HDL mg/dl	50.70^{a} ±0.1	41.60^{e} ±0.5	44.60^{d} ±0.6	47.80^{c} ±0.1	49.40^{b} ±0.4	47.90^{c} ±0.2	51.10^{a} ±0.33	40.90^{d} ±0.65	46.50c ±0.68	48.80^{b} ±0.26	49.90^{ab} ±0.33	49.60^{b} ±0.24

Different letters at the same row means that there was a significant change at $p<0.05$

Clinical Signs, Mortalities, gross Lesions and count of *C. perfringens*.

The clinical signs observed in infected group B (non-treated) post challenge with a toxigenic *C. perfringens* type A were severely decreased appetite, depression, emaciation, ruffled feather and brownish diarrhea. The severity of these signs was markedly decreased in the treated chickens especially those of group (E-F). The highest mortality rate was recorded in group B as 30%, followed by group C as 15%, group D as 10% and both groups (E &F) as 5% at 7th day post treatment. The macroscopic examination of chicken intestine in group B showed that small intestine was thin, dilated wall, filled with gas (Ballooning of intestine) and ulceration with some focal necrosis. These gross lesions were moderate in group D and very mild in groups E &F.

Concerning clostridial enumeration at 7th day post treatment revealed that, group (A) that was control negative showed clostridial count ($2X10^3$ CFU/ml). Group (C) treated with amoxicillin had clostridial count ($5X10^5$CFU/ml) lower than positive control group (B) which had ($4X10^9$ CFU/ml). Furthermore, the groups (E, F) (treated with antibiotic and prebiotics) had clostridial count ($1X10^3$ CFU/ml) and ($1.2X10^4$ CFU/ml) respectively. Interestingly, group (D) received probiotic only had clostridial count ($3X10^4$ CFU/ml).

Histopathological findings

The microscopical examination of intestine in group (B) (challenged-non treated) at 7th day post challenge revealed focal areas of necrotic enteritis invests an area of severe mucinous degeneration and congestion of submucosal blood vessels (Fig.3a). Focal colonization of microbial bacilli invaded submucosa was seen (Fig.3b), while liver appeared with severe congestion of hepatic blood vessels, perivascular fibrosis and cholestasis (Fig.3c). Hyperplasia of biliary epithelium, endotheliosis with periductal fibrosis were detected (Fig.3d). Kid-

ney exhibited focal hemorrhagic areas at renal medulla (Fig.3e) .while intestine at 14th day post challenge showed complete degeneration of some intestinal villi (Fig.3f) and complete degeneration of some submucosal glands with sloughing of some intestinal villi (Fig.3g). Liver appeared with perivascular leucocytic cells infiltration (Fig.3h) while kidney at the same day showed congestion of renal blood vessels (Fig.3i). Organs of infected chickens with *clostridium perfringens* and treated with antibiotic group (C) at 7th day post treatment showed some changes revealed intestine with atrophy of some submucosal glands (Fig.4a). Liver with focal area of leucocytic cells infiltration (Fig.4b), and kidney with focal area of leucocytic cells infiltrate renal cortex (Fig.4c), while at 14th day post treatment intestine showed fusion of some intestinal villi (Fig.4d). Liver with congestion of blood vessels and perivascular fibrosis (Fig.4e). Kidney with focal cystic dilation of some renal tubule (Fig.4f).

Organs of chickens treated with probiotic from 1st day then infected with *clostridium perfringens* group (D) showed intestine at 7th day post treatment with mucinous degeneration of villus epithelium (Fig. 5a). Liver with severe congestion of blood vessels, normal tissue architecture and cellular details (Fig.5b), while kidney showed renal cortex suffered congestion of blood vessels and inflammatory cells infiltration (Fig.5c). At 14th day post treatment revealed intestine with hyperplasia of submucosal glandular epithelium was detected (Fig. 5d). Liver exhibited mild congestion of blood vessels, endotheliosis and focal billary necrosis (Fig.5e). Kidney with degeneration of some renal tubules (Fig.5f).

Organs of chickens treated with probiotic from 1st day then infected with *clostridium perfringens* and treated with antibiotic group (E) at 7th day post treatment revealed intestine with focal necrosis of some villus tips (Fig.6a) . Liver with diffuse congestion of blood vessels, perivascular fibrosis and dilated sinusoids (Fig.6b). Kidney showed focal area of renal degeneration represented in cloudy swellings (Fig.6c) while intestine at 14th day post treatment appeared with normal mucosa and submucosa. (Fig. 6d) and liver with mild congestion of blood vessels, focal area inflammatory cells infiltration (Fig. 6e) Kidney at the same day were apparently normal (Fig. 6f).

Organs of chickens infected with *C. perfringens* then treated with both antibiotic and probiotic group (F) at 7th day post treatment revealed intestine with mild congestion of submucosal blood vessels (Fig.7a), while liver showed apparently normal tissue architecture and cellular details. (Fig.7b). Kidney showed mild congestion and mild atrophy of some renal glomeruli (Fig. 7c). Intestine at 14th day post treatment appeared with normal tissues of mucosa and submucosa (Fig. 7d). Liver showed normal tissue architecture and cellular details (Fig. 7e). Kidney appeared with focal areas of cloudy swellings (Fig.7f).

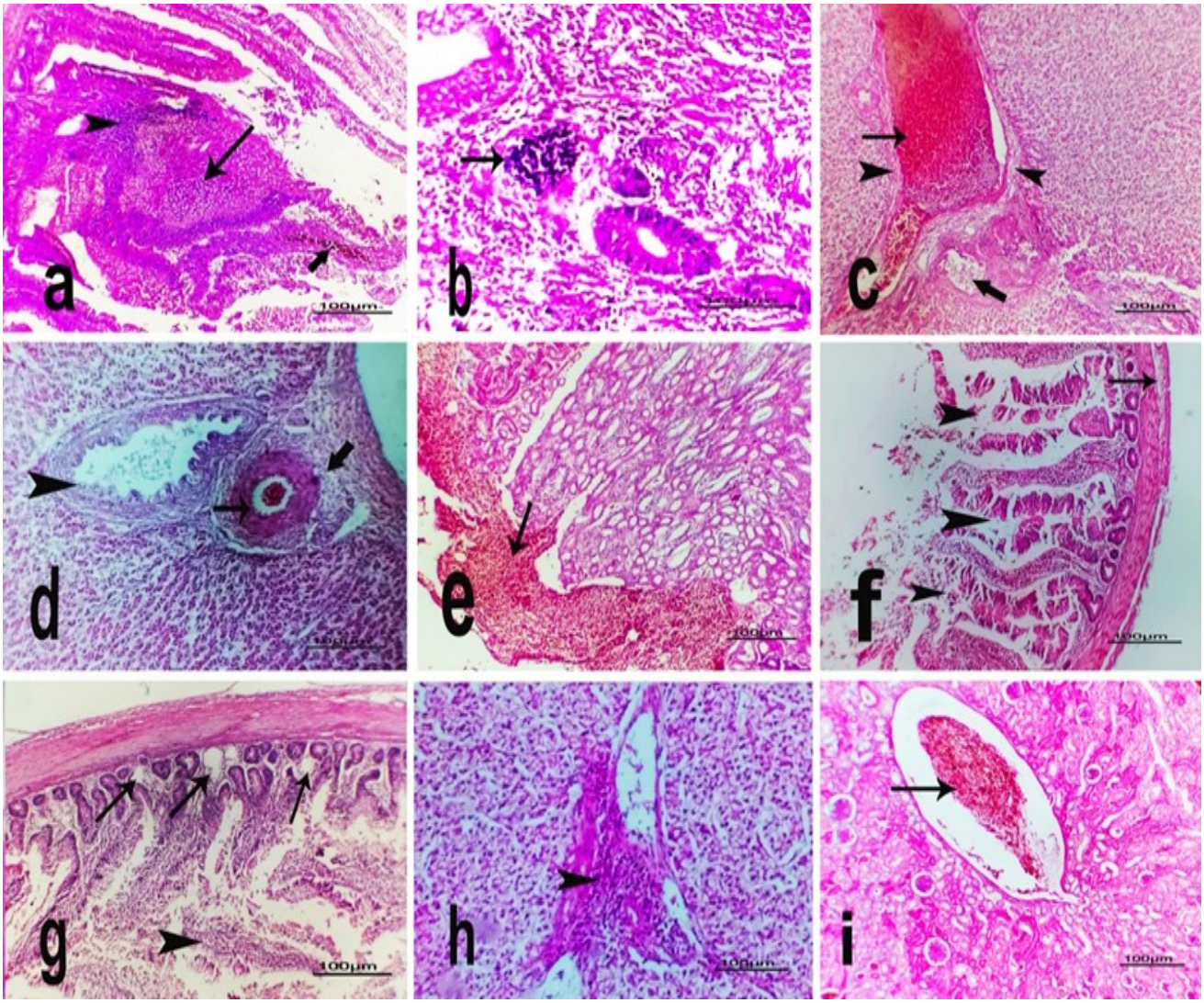

Figure (3):Photomicrograph of H&E stained chickens organs infected with *clostridium perfringens* group (B) showing .(a) intestine at 7th day post infection (PI) with focal areas of necrotic enteritis (arrowhead) invests an area of severe mucinous degeneration (thin arrow) and congestion of submucosal blood vessels (thick arrow).(b) intestine at 7th day PI with focal colonization of microbial bacilli invaded submucosa (arrow) . (c) liver at 7th day PI with severe congestion of hepatic blood vessels (arrow), perivascular fibrosis (arrowhead) and cholestasis (thick arrow).(d) liver at 7th day PI with hyperplasia of bilary epithelium (arrowhead), endotheliosis (thin arrow) and periductal fibrosis (thick arrow). (e) Kidney at 7th day PI with focal hemorrhagic areas at renal medulla (arrow). (f) Intestine at 14th day PI with complete degeneration of some intestinal villi (arrows head). (g) Intestine at 14 th day PI with complete degeneration of some submucosal glands (arrows) and sloughing of some intestinal villi (arrowhead). (h) Liver at 14th day PI with perivascular leucocytic cells infiltration (arrowhead) (i) kidney at 14th day PI with congestion of renal blood vessels (arrows) (scale bar=100µm).

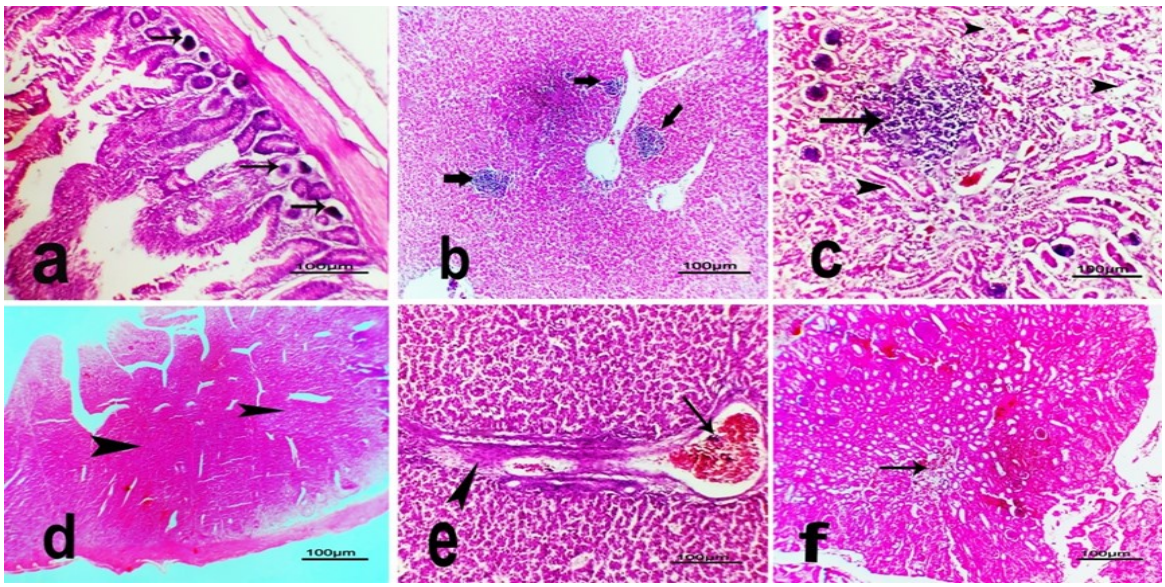

Figure (4):Photomicrograph of H&E stained chickens organs infected with *clostridium perfringens* and treated with antibiotic gp.(C) showing (a) intestine at 7th day post treatment with atrophy of some submucosal glands (arrows).(b) liver at 7th day post treatment with focal area of leucocytic cells infiltration (arrows) . (c) kidney at 7th day post treatment with focal area of leucocytic cells infiltrate renal cortex (arrow).(d) intestine at 14th day post treatment fusion of some intestinal villi (arrowhead). (e) liver at 14th day post treatment with congestion of blood vessels (arrow) and perivascular fibrosis (arrowhead).(f) kidney at 14th day post treatment with focal cystic dilation of some renal tubules (arrow) (scale bar=100µm).

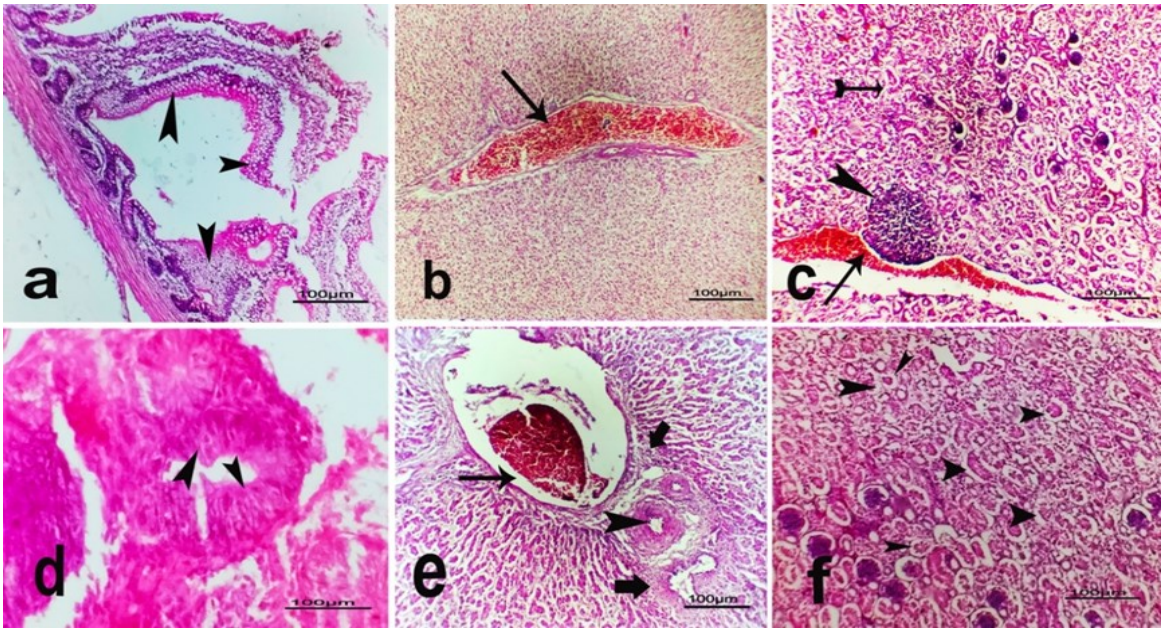

Figure (5): Photomicrograph of H&E stained chickens organs treated with probiotic from 1st day then infected with *clostridium perfringens* gp.(D) showing (a) intestine at 7th day post treatment with mucinous degeneration of villus epithelium (arrows head). (b) Liver at 7th day post treatment with severe congestion of blood vessels (arrow) and normal tissue architecture and cellular details (arrows). (c) Kidney at 7th day post treatment with of renal cortex represented in congestion of blood vessels (arrow) and inflammatory cells infiltration (arrowhead). (d) Intestine at 14th day post treatment with hyperplasia of submucosal glandular epithelium (arrowhead). (e) Liver at 14th day post treatment with mild congestion of blood vessels (thin arrow), endotheliosis (arrowhead) and focal billary necrosis (thick arrow). (f) kidney at 14th day post treatment with degeneration of some renal tubules (arrows head) (scale bar=100µm).

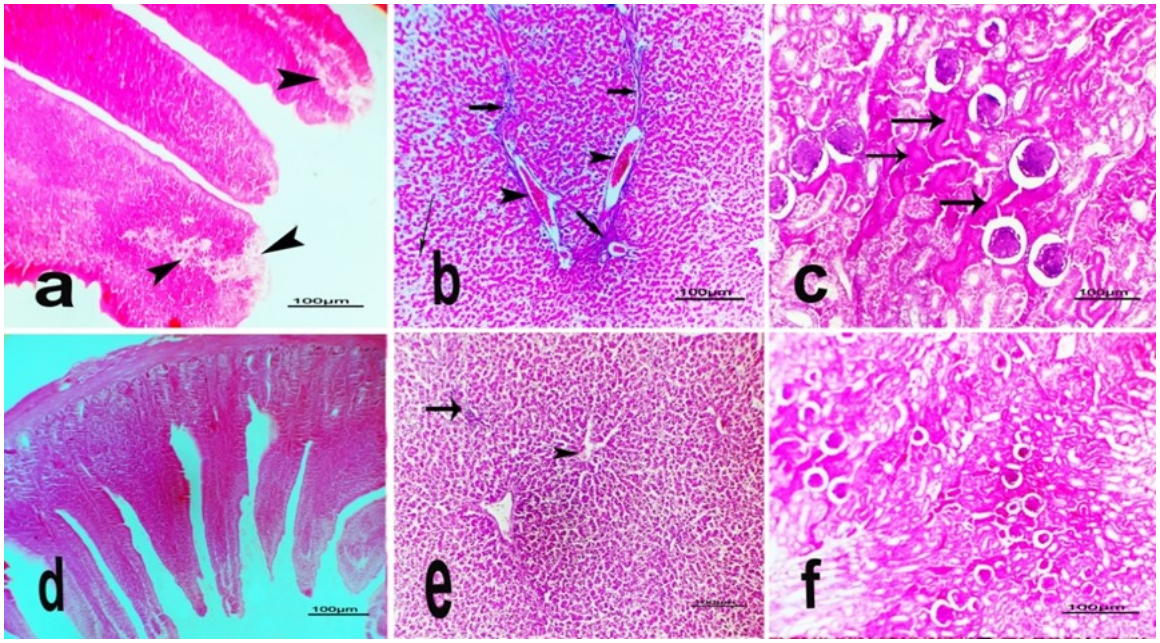

Figure (6): Photomicrograph of H&E stained chickens organs treated with probiotic from 1st day then infected with *clostridium perfringens* and treated with antibiotic gp.(E) showing (a) intestine at 7th day post treatment with focal necrosis of some villus tips (arrows head). (b) Liver at 7th day post treatment with diffuse congestion of blood vessels (arrows head), perivascular fibrosis (thick arrows) and dilated sinusoids (thin arrow). (c) Kidney at 7th day post treatment with focal area of renal degeneration represented in cloudy swellings (arrows) (d) intestine at 14th day post treatment with normal tissues of mucosa and submucosa. (e) Liver at 14th day post treatment with mild congestion of blood vessels (arrowhead) and focal area inflammatory cells infiltration (arrow). (f) Kidney at 14th day post treatment with apparently normal renal cortex (scale bar=100µm)

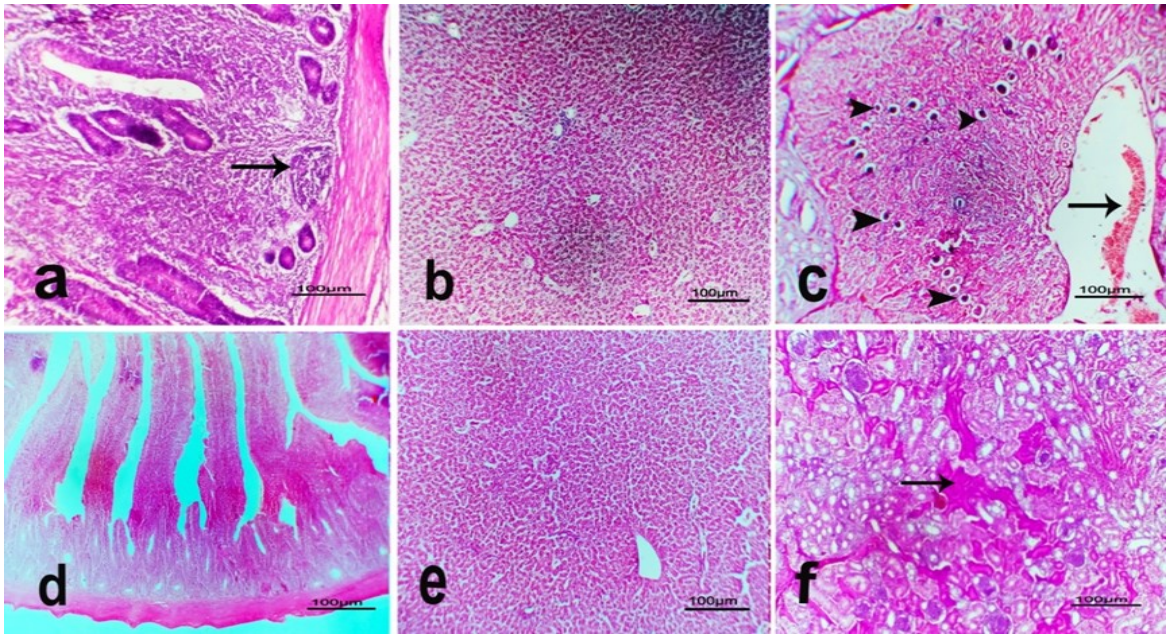

Figure (7): Photomicrograph of H&E stained chickens organs infected with *clostridium perfringens* then treated with both antibiotic and probiotic gp.(F) showing (a) intestine at 7th day post treatment mild congestion of submucosal blood vessels (arrow). (b) Liver at 7th day post treatment with apparently normal tissue architecture and cellular details. (c) Kidney at 7th day post treatment with mild congestion (arrow) and mild atrophy of some renal glomeruli (arrowshead). (d) intestine at 14th day post treatment with normal tissues of mucosa and submucosa. (e) liver at 14th day post treatment normal tissue architecture and cellular details.(f) kidney at 14th day post treatment with focal areas of cloudy swellings (arrow) (scale bar=100µm).

DISCUSSION

In the poultry industry, one of the most critical problems is necrotic enteritis (NE) **(Wang et al. 2017),** because of severe economic losses and increased mortalities. Another significant aspect is that it is involved in food-borne intoxication, which evolved from consumption of different raw and canned foods, particularly chicken meat and meat products **(Stringer, 2018).**

In our study the recovery rate of *C. perfringens* was 38.6% which isolated from intestinal samples collected from diseased broiler chickens. These finding is in accordance to previous studies of **EL-Helw et al., (2014) and Heidy et al. (2015)** who stated *C. perfringens* prevalence rate 33.33%, and 45.9%, respectively. On the other hand these recovery rate lower than previous outcomes of **Abd El-Hamid et al.(2015), Asmaa et al. (2017) and Prerana et al. (2018),** who recorded *C. perfringens* prevalence rates (70%), (65.1%) and (55.9%) respectively. These variances are rather typical when you consider the various management and sanitary practices and procedures used on various farms. Additionally, chickens with *C. perfringens* regularly shed it into the environment and poultry litter, which presents a risk of reinfection for chickens. In chickens and other animals, cross-infection through faeces, litter, waste from poultry, feed and water is a primary source of infection **(Praveen et al. 2019).** Furthermore, when hens have a concurrent clinical or subclinical coccidial infection in the gut, the prevalence of *C. perfringens* may considerably rise **(Mohiuddin et al. 2016).**

In the present study, antimicrobial sensitivity profiling of the 58 confirmed *C. perfringens* isolates, indicated that higher rates of sensitivity to amoxicillin (74.1%) followed by Bacitracin (70.6 %) and Amikacin (68.9%). these results nearly similar to the published data from **Salem et al. (2020).** Amoxicillin is believed to be the most effective treatment for chickens infected with *C. perfringens*, according to other studies conducted in the United States, China, and Norway **(Llanco et al. 2012).** On the other hand, 72.4% of examined isolates were resistant to Sulfamethoxazole/trimethoprim followed by 67.24% to Colistin and 65.54% to Neomycin. Similarly, **Gad et al. (2011)** recorded highest resistance against neomycin and colistin but found that most *C. perfringens* strains were sensitive to Sulfa trimethoprim. Also, **Prerana et al. (2018)** found that 86.8% of isolates were resistant to colistin. The resistance of examined isolates to sulfa-trimethoprim agreed with those obtained by **Llanco et al. (2012) and Osman and El-hariri, (2013).** Our results illustrated that the overall examined isolates were resistant to at least 3 of the tested antimicrobial agents from different groups, making them multidrug resistant (MDR) which agreed with study results of **Osman and Elhariri (2013).** The discrepancy in the resistance pattern from one study to another can be attributed to the differences in *C.perfringens* exposure to different levels of antibiotic stress (as feed additive ,prophylaxis and therapeutic agent) in different localities.

One of the typical commensals in the intestinal flora of both humans and animals is *C. perfringens*. Therefore, it is important to distinguish between toxic and non-toxic strains. In this regards, toxigenic attributes of 8 isolates that had multidrug resistant phenotypes were studied by multiplex PCR. PCR results revealed that all examined isolates (100%) belonged to *C.perfringens* type A that carry cpa gene, encodes for alpha toxin beside to absence of other major lethal toxin genes. These results were in accordance with the findings of **Eaftekhar et al. 2023 and Salem et al. (2020). Van Immerseel et al. (2009)** reported that alpha toxin was the major virulence factor in the pathogenesis of necrotic enteritis in poultry.

The present study was designed to investigate the efficacy of amoxicillin and probiotic in control of necrotic enteritis in broiler , the clinical signs of infected chicken showed depression, emaciation, sever decrease appetite, ruffeled feather and brownish diarrhea which agree with **(Islam, et al. 2009, Saleh, et al .2011, Abd El-hamid et al. 2015, Wafaa et al. 2022)** These clinical signs may be due to the effect of colistridium toxins **(Anders, 2006).** The main characteristic lesion is necro-

sis at the intestinal level, whose clinical signs include depression, dehydration, diarrhea, and a decreased food consumption also lesions are observed throughout the gastrointestinal tract with the presence of gas and occasionally, lesions occur in other organs like liver, and kidney these results were similar to previous studies by **Van Immerseel et al. (2009) Cooper et al. (2013) Paiva et al. (2014) and Caly et al. (2015).**

Our results showed that the highest mortality rate (30%) with highest clostridial count ($4X10^9$) were recorded in infected non treated group (B) that matched with Umar et al., (2018) and lower than recorded in previous studies by **Aboubaker and Elbadawy (2017) and Salem et al. (2020)** who recorded mortality rate 40% and 50%, respectively .It's possible that the effects of *C. perfringens'* toxins are to blame for the mortalities observed in infected birds **(Sameh et al. 2005).** On contrary, **Pedersen et al. (2008)** reported that there is no mortality detected during experimentally infected with *C. perfringens* and only a transient colonization with challenge strains had been obtained. Also, **Malmarugan et al. (2010)** showed that a *C. perfringens* infection trial with chickens exhibited no clinical symptoms of NE and no deaths. **Vijay and Dustan, (2007)** attributed these differences in mortalities caused by *C. perfringens* infection were due to many stresses factor that birds are exposed to it as wet litter, high temperature, ventilation management, crowdedness, type of ration and other management protocols. On the other hand, amoxicillin treated group (c) had mortality rate (15%) with clostridial count ($5X10^5$) which lower than recorded in infected non treated group (B). these finding can be justified as a result to antimicrobial effect in suppression of *C. perfringens* and decreased its intestinal colonization which lead to prevention of necrotic enteritis as mentioned by **Watkins et al. (1997).**

The current findings show that dietary supplementation with probiotic displayed higher BW, BWG and most improved FCR over the whole rearing period these results correlated **with Hussein et al. (2020) and Shah et al. (2021).** Dietary inclusion of probiotics significantly increased body weight gain (BWG) and improved feed conversion ratio (FCR) during the starter and overall periods in Japanese quails **(Siadati et al. 2017).**

Herein, the groups (E, F) (treated with antibiotic and probiotics) had mortality rate 5% with clostridial count ($1X10^3$ CFU/ml) and ($1.2X10^4$ CFU/ml) respectively. These results agree with the published data from **Sokale et al. (2019)** who found that the supplementation of broiler chicks with *Bacillus subtilis* alone resulted in improved production, growth performance and reduced mortality after a *C. perfringens* challenge. Therefore, supplementation with *Bacillus subtilis* can not only be used to control NE diseases but also enhances gut health in broiler chicks **(Jayaraman et al. 2013).** *Clostridium perfringens* infection has been evidenced to decrease feed efficiency and increase gut lesions (Injured mucosa) leads to decreased digestion and absorption, reduced weight gain and increased feed conversion ratio and mortality rates, which account for higher productive losses in poultry **(Van Immerseel et al. 2009).** Using multi-strain probiotics appears to be the most effective strategy to increase the effects of probiotics. These probiotics have a positive impact on the host by strengthening growth-promoting bacteria in the intestinal tract, which is combined with a viable antibiosis of harmful bacteria **(Lukic et al. 2017)**. The biological role of probiotics in the change of intestinal pH may be responsible for the improvement of all performance metrics since it benefits the bacterial population, enhances nutrient absorption, and boosts feed utilization efficiency. **(Dunne 2001).**

The hematological parameters changes in broilers suffering from necrotic enteritis might be due to bacterial toxin **(Liu et al. 2010).** The decrease in erythrogram might be due to intravascular hemolysis induced by *C. perfringens* **(Topley and Wilsons 1999), also Allam et al., (2013)** reported that Clostridial toxins caused breakdown of phospholipids of erythrocytes membrane and cause hemolysis by damaging circulating erythrocytes. A significant improvement in RBCs count and Hb content of *C. Perfringens* infected broilers then treated with amoxicillin and/or probiotic and compared to the infected non treated group. **Nagar-**

alli et al. (2002) Saied that amoxicillin act by inhibition of cell wall mucopeptide biosynthesis during bacterial multiplication. **Mikkelsen et al. (2009)** mentioned that *C. perfringens* growth was suppressed by organic acids supplementation. Our results agree with **El-Gharbawy (2014) and Sayed et al. (2016)** in which an improvement of erythrogram parameters in *C. Perfringens* infected broilers then treated with amoxicillin or probiotic were detected.

Variations of leukogram in our study may be due to the bacterial infection and inflammation that lead to leukocytosis, heterophilia and monocytosis **(Nasr El –Deen et al. 2019).** Our results were matched with **Gheith et al.,(2011) and Saleh et al. (2011)** who reported that a leukocytosis and heterophilia as a characteristic feature of in *C. Perfringens* infected broilers. Treated birds with amoxicillin or probiotic or with a combination of amoxicillin and probiotics showed improvement in the leukogram compared to non-treated group. Similar results were also obtained by **El-Shahat (2014) and El-Sheikh et al. (2018).** The immune system has been demonstrated to benefit from taking probiotics in a number of ways, including increased lymphocyte, macrophage, and natural killer (NK) cell activity, increased heterophil oxidative burst, and increased immunoglobulin synthesis. The usage of probiotics, which help to stabilize the stomach and regulate the immune system, may help to maintain a healthy balance between anti-inflammatory and pro-inflammatory cytokines **(Shumaila et al. 2022).** In light of these findings, it has been found that probiotics may increase the quantity of intestinal epithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) in the small intestine while also preventing the growth of pathogenic microorganisms **(Dhama and Singh, 2010).**

Regarding to the recorded results of serum biochemical parameters. Infected non treated group revealed significant increase in all biochemical parameters with significant decrease in total protein and HDL. The liver enzymes (AST and ALT) activities were increased in *C.perfringens* infected group (B) which may be due to denoting hepatic damage and biliary stasis caused by clostridial toxins **(Nasr El –Deen et al. 2019).** Serum uric acid and creatinine were significantly increased in infected non treated group that may be a result of renal injury and cellular necrosis. As renal tubule degeneration inhibited the excretion of uric acid and creatinine, their levels in the serum of infected birds increased. **(Nasr El –Deen et al. 2019).** Similar results obtained by **(Allam et al. 2013 and Saleh et al. 2023).** Hypoproteinemia observed in infected non treated group may be due to the decreased feed intake, the loss through the intestine and the kidneys, or may be due to liver damage by clostridial toxins. The treated groups with amoxicillin and or probiotic revealed significant improvement in serum biochemical parameters of the challenged groups. Diet supplementation with *Bacillus subtilis* and *E. faecium* results in normal liver function as a result of a significant decrease in the ALT and AST activities in the blood **(Hatab et al. 2016).** On the other hand Hussein (2014) found that probiotics (*Saccharomyces cerevisiae*) did not have any significant effect on serum AST and ALT activities in broiler chicks fed a supplemented diet compared to the control group. Also probiotics decrease urease activity in gut subsequently, reducing the concentration of non-protein nitrogen, uric acid, ammonia and urea that result in lowering the ammonia formation in litter **(Abd Al-Fatah 2020).** While serum uric acid levels were significantly increased with the increasing of probiotics levels in broiler **(Sultan and Abdul- Rahman 2011).** However, there were no any changes in kidneys of mice that treated with probiotics may be as a result to serum uric acid level was at tolerance level **(Salahuddin et al. 2013).**

Glucose is an important cellular source of energy and serves as a metabolic substrate **(Nahavandinejad et al. 2014).** Significant increase in serum glucose level in infected non treated group also detected by **Nasr El –Deen et al. (2019).** Regarding to effect of probiotics on blood glucose levels, our results conflict with **Hussein et al. (2020**) found that serum glucose was highest when probiotics were used against *Clostridium perfringens* infection in broiler chickens. Some studies investigated a significant improvement in blood glucose

level after ingestion of probiotics for weeks **(Ejtahed et al. 2012). Shah et al. (2021**) added that probiotic treatments significantly lowered plasma glucose levels compared to treatment by antibiotic growth promoters. While IgG recorded the high values for control positive group. IgM and IgG isotypes are indicators for resistance of diseases in poultry **(Star et al. 2007).** Probiotics have a significant role in development of immune response against Newcastle disease, tetanus toxoid and *Clostridium perfringens* alpha-toxin **(Haghighi et al. 2006). Llanco et al. (2012)** suggested amoxicillin was effective against necrotic enteritis infection. The inclusion of native and commercial probiotics had a significant effect on serum glucose, total protein, globulin, uric acid, LD and LDL/HDL ratio of Japanese quails **(Siadati et al. 2017).** Probiotics' diverse roles in sup-pressing cancer, lowering serum glucose, and lowering serum cholesterol are leading to increased accep-tance of probiotics **(Adhikari and Kim 2017). Shah et al. (2021)** detected minor depletions in levels of total cholesterol, HDL, and triglycerides in broiler supplemented with probiotic. **Dibaji et al. (2012)** found that feeding probiotics decreased serum LDL, but not HDL levels in broilers. On the other hand, **Hussein et al. (2020)** found that total protein, lipids and albumin in broiler chicks was not affected by probiotic supplementation.

Important information on the condition of the digestive tract can be gleaned from the small intestine's structure. (**Teirlynck et al. 2009).**

For proper food absorption and the development of the gut microbiota, the mucosa and intestinal villi with their microvilli are vital, while the mucosa and microbiota, together with the gut-associated lymphoid tissue (GALT), provides an immune complex that will work as a gastric defense mechanism. **Alshamy et al. (2018), Sun and Jia (2018)** discovered that aggressiveness towards any of these elements, particularly during the first few weeks of life, could cause changes to the integrity of the intestinal epithelium, nutritional bioavailability, and absorption, fostering an inflammatory response inside the gut.

The histopathology of *C. perfrigenes* experimentally infected chicken revealed necrotic enteritis with submucosal congestion and degeneration of intestinal villi similar to that obtained by **(Abd El-hamid et al. 2015, Abd El-Ghany et al. 2022) .Caly et al. (2015)** who found that the infected broiler chickens showed severe lesions of the small intestine presenting a degenerated mucosa. Congestion of blood vessel and flattening of villi in the lamina propria and submucosa have also been noted. Subsequently, there is a necrosis in the intestinal mucosa and villi of infected broiler chickens that noted by **Mora et al. (2020). Hussein et al. (2020)** reported that broiler chickens challenged with Clostridium showed signs of injury to intestine tissue, including the degeneration and necrosis of intestinal villi.

However, in our result these histopathological effects were significantly improved in chickens of group E and F the intestinal tissue appeared with normal mucosa and submucosa, then C and D respectively. These results are also in accordance with the note of **Llanco, et al. (2012); Sarker, et al. (2013) and Lensing et al. (2010)** that proved the efficacy of amoxicillin in controlling of necrotic enteritis infection. However, the assumption that probiotics can protect the body from gut diseases by enhancing gut immunity **Vieira et al. (2013),** improving gut development **Chen et al.(2015),** and altering gut microbiota **Ubeda et al. (2012)** has been well accepted with our result especially in combination with the antibiotic.

The liver of infected non treated group showed cholestasis with inflammatory cell infiltrations and congestion of hepatic blood vessel. These results were similar to those obtained by **Cooper et al. (2009).** In our results we showed hyperplasia of bilary duct these result similar to that recorded with **Mora et al. (2020) Van Immerseel et al. (2009), Redondo et al. (2016), Smyth et al. (2016)** who found that the histopathological lesions can be observed in the intestinal tract, including ulcers, bile duct hyperplasia, and inflammation. The chronic subclinical disease process allows

bacteria to reach the bile duct and bloodstream, therefore, the pathogen can be found in the liver. The bacterial infection is the most important cause of lobular localization and may be a preamble to hepatocyte necrosis **Gkretsi et al. (2007)**. Our results are partial in agreement with **Hussein et al. (2020)** who found that the liver of chicks challenged with Clostridium were associated with several hepatocellular damages, including the congestion of portal blood vessels with numerous lymphocytic aggregations, as well as edema and coagulative necrosis. The liver in group D (treated with probiotic) normal tissue architecture and cellular details with mild congestion of blood vessels. **Hussein et al (2020)** reported that a normal hepatocellular structure with mild perivascular lymphocytic aggregation in broiler chicks with probiotic supplementation. The supplementation of probiotics may enhance the enrollment of pro-inflammatory immune cells to systemic lymphoid tissues, including the liver and other organs **Gkretsi et al. (2007).** Moreover, the role of probiotics on the transfer of immune cells in the liver was reported by **Zhang et al. (2008).** The liver showed mild congestion of blood vessels and small focal aggregation of inflammatory cells in some cases of group E and F while in the most cases the liver showed apparently normal tissue architecture and cellular details which indicates that the combination of the antibiotic and or the probiotic in drinking water, are effective in restoring the drawbacks of NE on liver and this improvement agree with our clinicobiochemical results.

The presence of nephrotoxicity was confirmed by the histopathological results of kidney which showed congestion of renal blood vessels and hemorrhage in infected non treated group, the histopathological lesions of kidney were significantly improved with amoxicillin treatment in combination with probiotic in group E and F. the supplementation of probiotics significantly improves the intestinal health and decreases the histological damage caused by *C. perfringens* these result similar to the results obtained by **Menconi et al. (2020).** Our histopathological results showed that the applied antibiotic was the most effective on the control of chickens infected with *C. Perfringens* which accords with **Abd El-hamid et al. (2015) and Wafaa et al. (2022).**

CONCLUSION

C. perfringens infection in broiler chickens induced adverse effects on growth performance, hemato-biochemical and immumological profile of birds which could be reversed or ameliorated by using amoxicillin and or probiotic which had a beneficial effect in control of the infection. So, the study recommended the use of probiotic not only promote the growth of chickens but also it helps in the prophylaxis against bacterial infection hand in hand with antibiotics.

Conflict of interest: none.

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