Zagazig University
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CLINICOPATHOLOGICAL STUDIES ON THE EFFECT OF CLOSTRIDIUM INFECTION IN CHICKENS

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Dedicated to ....

My Parents

My Husband

My Brothers

My Sons, Omar and Kareem

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LIST OF ABBREVIATIONS

α	:	Alpha
β	:	Beta
ε	:	Epsilon
γ	:	Gamma
%	:	Percentage
ALP	:	Alkaline phosphatase
ALT	:	Alanine aminotransferase
AST	:	Aspartate aminotransferase
B.W.G	:	Body weight gain
B.wt	:	Body weight
C. albicans	**:**	*Candida albicans*
CFU	:	Colony forming unit
Cl. perfringens	:	*Clostridium perfringens*
E.Coli	:	*Escherichia coli*
EDTA	:	Ethylene diamine tetraacetic acid
ELISA	:	Enzyme-linked immunosorbent assay
FCR	:	Feed conversion ratio
Fig.	:	Figure
Gp.	:	Group
H&E	**:**	Hematoxylin and Eosin
Hb	:	Hemoglobin
HBSS	:	Hank's balance salt solution
I/M	:	Intramuscular
IgG	:	Immunoglobulin G
IgM	:	Immunoglobulin M
FCS	:	Foetal calf serum
MCH	:	Mean corpuscular hemoglobin
MCHC	:	Mean corpuscular hemoglobin concentration
MCV	:	Mean corpuscular volume
PBS	:	Phosphate buffer saline
PCV	:	Packed cell volume
pH	:	Hydrogen ion concentration
RBCs	:	Red blood cells
RPMI	:	Rose well park memorial institute
rpm	:	Revolutions per minute
S.E	:	Standard error
Tab.	:	Table
TLC	:	Total leukocytic count
WBCs	:	White blood cells

INTRODUCTION

Poultry industry in Egypt is very important as it contributes to a great extent in overcoming the meat shortage and provides consumers with a cheaper source of animal protein. The basic role of poultry production is turning feed stuffs into meat. Broilers are efficient in this role, and so on growth rate and feed conversion rate. Any slight alteration from the optimal condition is mostly accompanied by disruption of the growth process ***(Baha et al., 1997).***

Poultry industry is always under the threat of major losses by poultry diseases. Infectious diseases are major constraint of poultry rearing causing 30% mortality of chickens per year ***(Hasan et al., 2010).***

Clostridium perfringens Type A is a bacterial pathogen causing necrotic enteritis in broilers and responsible for visible and invisible economic losses through mortality, morbidity, weight loss, low feed conversion ratio and poor performance ***(Hafez, 2011).***

To combat the disease as well as to minimize the losses due to this bacterial infection in poultry, proper investigation, and identification of the organism and antibiogram studies are very essential ***(Sarkar et al., 2013).***

Over the last few decades, necrotic enteritis in poultry has been controlled and treated by addition of antimicrobials to feed or water. One of the antibiotics widely used by veterinarians is amoxicillin ***(Koutoulis et al., 2015).***

The antibacterial spectrum of amoxicillin includes majority of gram-positive and gram-negative organisms. Therefore, amoxicillin is the antibiotic

of choice for treating most of bacterial infections in poultry because of its good absorption, broad spectrum and rapid bactericidal activity ***(Anadon et al., 1996).***

Increasing awareness of treatment failures, the risk of bacterial resistance, residues in tissues and the possibility of undesirable effects on natural defense mechanisms have increased the interest in alternative non-antibiotic methods of treatment ***(Salim et al., 2013).***

Combined with good hygiene management for poultry houses, consideration of diet composition and application of antibiotic alternatives might be effective to some extent in maintaining production and controlling necrotic enteritis. Among the candidate replacements for antibiotics are probiotics, prebiotics, organic acids, enzymes, bacteriophages and vaccination ***(Dahiya et al., 2006).***

However, due to rising consumer concerns, many countries ban the routine use of antimicrobials in feed. Thus, alternative prevention methods, such as organic acids, are needed ***(Kerry, 2007)***. Organic acids can act either as a source of carbon and energy, or as inhibitory agents depending upon the concentration of the acid, its ability to enter the cell and capacity of the organisms to metabolize the acid ***(Cherrington et al., 1991[a]).***

The present work aims to

1- Study the effect of *Cl. perfringens* infection on broiler chickens.

2- Comparing between the effect of amoxicillin and / or organic acids on *Cl. perfringens* infection through the evaluation of growth or body performance, hemogram, blood chemistry, cellular and humoral immune response and associated histopathological lesions.

REVIEW OF LITERATURE

I- Clostridium Infection:

Necrotic enteritis was first described and attributed to *Clostridium perfringens* by ***Parish*** in 1961. It is a common inhabitant of chicken intestinal tract, with no apparent impact on the host ***(Ficken and Wages, 1997).***

Clostridium perfringens (*Cl. perfringens*) is a widespread spore-forming, Gram-positive, anaerobic, non-motile rod shape bacteria. It is recognized as an enteric bacterial pathogen in humans, poultry and other farm animals worldwide ***(Songer, 1996 and Craven et al., 2003).***

Overcrowding of chickens, inadequate hygiene routines, wet litter and feed composition are the main predisposing factors ***(Van Immerseel et al., 2004).*** The risk of necrotic enteritis is low when chickens are kept on wire floor that minimizes contact with feces ***(Mark, 2008)***.

1) Pathogenicity of Cl. perfringens:

Yoo et al., (1997) reported that the pathogenicity of *Cl.perfringens* is associated with several toxins. These toxins are classified into five types (A - E) on the basis of their ability to produce the major lethal toxins α, β and ε and according to specific animal hosts. Only *Cl.perfringens* types A and C are pathogenic for poultry.

Lovland and Kaldhusdal (1999) indicated that *Cl. perfringens* and its toxins may reach the liver and lead to bile stasis and inflammation of the biliary tract.

Smedley et al., (2004) reported that *Cl. perfringens* strains type B and type C produce β-toxin and cause necrotic enteritis characterized by haemorrhagic mucosal ulceration or superficial mucosal necrosis of the small intestine in animals**.**

Anders (2006) indicated that *Cl. perfringens* enterotoxin interacts with epithelial cells tight junction proteins, leading to diarrhea and intestinal cramping caused by leakage of water and ions .Production of enterotoxin is co-regulated with sporulation, as the toxin released when vegetative cells undergo lysis. Epsilon is necrotizing, lethal toxin and the most potent clostridial toxin after botulinum and tetanus neurotoxins.

McDevitt et al., (2006) mentioned that 75 - 95% of birds are colonised by *Cl. perfringens*, but only a small proportion of these shows symptoms of the disease.

Thompson et al., (2006) proved that α-toxin destroys cell membranes by oxidation and hydrolysis of membrane phospholipids and also enters the blood stream, causing systemic effects and death**.**

2) Clinical signs of Cl. perfringens infection:.

Sahar (2001) reported that 3 weeks old chickens infected with 2ml *Cl. perfringens* type "A" (1.9×10^9 organism/ml) on alternate days showed depression, decreased appetite, diarrhea and emaciation. Mortality rate was 20% within 10 days after last inoculation.

Islam et al., (2009) revealed that birds infected with necrotic enteritis showed severe depression, diarrhea (shooting type), huddling,

reluctance to move, ruffled feathers, and sudden death in an investigation during 2007 to 2008. The deaths occurred inspite of history of a good body condition and good appetite**.**

Miah et al., (2011) isolated *Cl. perfringens* from 2-5 weeks old broiler chickens. They recorded that the mortality rate was between 2-10% and sometimes as high as 40-50%. They added that the recorded symptoms were resemble to coccidiosis and may be misdiagnosed.

Saleh et al., (2011) reported that birds infected with *Cl. perfringens* type A, C or AC by adding thioglycolate broth media containing Log 10 CFU/ml of *Cl. perfringens* to drinking water at a ratio of 1:2 (1 L of fluid media/2 L water) for 3 successive days at 35 days old showed marked depression, anorexia, reluctance to move, ruffled feathers, dropping of wings and head in addition to diarrhea. The birds gradually became dehydrated. No deaths occurred among affected chickens.

Asmaa (2016) revealed necrotic enteritis typical clinical signs including depression, ruffled feather, dropping wings, loss of appetite and bloody diarrhea with mortality rate up to 15% in chickens infected with *Cl. perfringens* type C (1.5×10^9 organism/ml) at 21 days old.

Aboubakr and Elbadawy (2017) reported loss of appetite, dropping wings, depression, ruffled feathers, diarrhea, dehydration, polydipsia and emaciation in broiler chickens infected with *Cl. perfringens* type C (1.5×10^9 organism/ml) at 21 days old.

El-Sheikh et al., (2018) recorded that broilers infected with necrotic enteritis caused by *Cl. perfringens* type A broth culture ($1x10^9$ CFU /mL) at 14 days old suffered from loss of appetite, diarrhea and anorexia in addition to a 30% mortality rate.

3) Effect of Cl. perfringens infection on body performance:

Abudabos and Yehia (2013) noticed that broilers challenged with *Cl. perfringens* (4×10^8 CFU) at 18, 19 and 20 days old had a significant decrease in body weight gain when compared to the normal control.

Jayaraman et al., (2013) found a significant reduction in weekly body gain in *Cl. perfringens* infected chickens at a dose of 10^8 CFU/mL per bird on 19^{th} day of the trial.

El-Bayoumi et al., (2014) proved that chickens challenged with *Cl. perfringens* (10^7 CFU/ml) at 18, 19 and 20 days old showed a significant decrease in body weight gain coupled with an increase in feed conversion rate.

Asmaa (2016) observed that experimentally infected chickens with *Cl. perfringens* type C (1.5×10^9 organism/ml) at 21 days old showed a significant decrease in feed consumption, body weight and body gain with a higher feed conversion ratio.

Aboubakr and Elbadawy (2017) reported a significant decrease in body weight and body weight gain and increase in feed conversion rate of chickens experimentally infected with *Cl. perfringens* type C (1.5×10^9 organism/ml) when compared with healthy untreated chickens.

Marwa (2017) indicated that *Cl. perfringens* infected chickens (1.5 × 10^9 organism/ml) at 15^{th}, 17^{th} and 19^{th} days old showed low feed consumption, weight loss and a higher feed conversion rate.

El-Sheikh et al., (2018) found a significant reduction in feed consumption, body weight and body weight gain, besides an increase in feed conversion rate in *Cl. perfringens* type C (1.5 × 10^9 organism/ml) infected broilers.

4) Effect of Cl. perfringens infection on hematological parameters:

Sahar (2001) recorded a significant decrease in red blood cells (RBCs) count, hemoglobin (Hb) concentration, packed cell volume (PCV) and mean corpuscular hemoglobin concentration (MCHC) in *Cl. perfringens* type "A" (1.9 × 10^9 organism/ml) infected chickens. In contrast there was a significant increase in mean corpuscular volume (MCV), total leukocytic count (TLC), heterophils count and lymphocytes. The resulting anemia was macrocytic hypochromic anemia starting from the 4^{th} week till the end of her experiment when compared with normal control.

Gheith et al., (2011) recorded a significant leukocytosis, lymphocytosis and monocytosis in chickens infected with *Cl. perfringens* type A (9×10^8 CFU) at 10 days old. They added that eosinophils and basophils count showed non-significant changes when compared with normal control.

Saleh et al., (2011) indicated that RBCs count, Hb concentration, PCV and Heterophil's percentage significantly increased in chickens infected with *Cl. perfringens* type A and C. In contrast total leukocytic

count, eosinophils, basophils and lymphocytes percentage decreased significantly when compared with healthy control. Moreover, Monocytes did not change significantly in challenged groups.

El-Shahat (2014) reported a significant decrease in erythrocytic count, hemoglobin content and packed cell volume in broiler chickens post infection with *Cl. perfringens* type C (1.5×10^9 organism/ml) at 21 days old with development of macrocytic hypochromic anemia. A significant increase in leukocytic count, heterophils, lymphocytes, esinophils, basophils and monocytes were recorded along the experimental periods compared with the normal control.

Asmaa (2016) indicated a significant decrease in RBCs count, Hb content and PCV resulting in macrocytic hypochromic anemia with a significant increase in WBCs in *Cl. perfringens* type C (1.5×10^9 organism/ml) infected chickens at 21 days old.

Marwa (2017) revealed a significant decrease in erythrocytic count, Hb concentration and PCV post *Cl. perfringens* type C (1.5×10^9 organism/ml) infection. She also recorded macrocytic hypochromic anemia in infected chickens when compared with normal control chickens.

El-Sheikh et al., (2018) recorded a significant reduction in RBCs count, Hb concentration and PCV with development of macrocytic hypochromic anemia in *Cl. perfringens* type C (1.5×10^9 organism/ml) infected broilers compared with normal control. Moreover, a significant increase in TLC, heterophils, lymphocytes, monocytes, esinophils and basophils.

5) Effect of Cl. perfringens infection on biochemical parameters:

Sahar (2001) revealed that chickens infected with *Cl. perfringens* showed significant increases in serum activities of aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP), uric acid and creatinine levels all over the experimental periods. In contrast, the serum albumin level was decreased significantly compared with the normal control group.

Thrall (2004) recorded a significant hypoproteinemia and hypoalbuminemia beside a significant increase in serum activities of AST, ALT, ALP, uric acid and creatinine levels in chickens naturally infected with *Cl. perfringens.*

Saleh et al., (2011) observed a significant increase in serum levels of total proteins, albumin, globulins, AST, ALT, ALP and uric acid in chickens infected with *Cl. perfringens* type A, C or AC (10 CFU/ml) compared with the normal control group.

El-Shahat (2014) recorded a significant increase in serum levels of globulins, AST, ALT, ALP, uric acid and creatinine in *Cl. perfringens* type C (1.5×10^9 organism/ml) infected chickens. He added that serum levels of total proteins and albumin were significantly decreased along the experimental period compared with the normal control group.

Asmaa (2016) reported a significant decrease in total proteins with a significant increase in serum activities of AST, ALT, ALP, uric acid and creatinine levels in *Cl. perfringens* type C (1.5×10^9 organism/ml) infected broilers.

Aboubakr and Elbadawy (2017) recorded a significant elevation in globulins, serum liver enzymes activity, uric acid and creatinine levels with a reduction in serum total proteins and albumin all over the experimental periods after *Cl. perfringens* infection (1.5 x 10^9 organisms/ml) on alternate days at 21 days old.

Marwa (2017) reported a significant increase in serum activities of AST, ALT, ALP, uric acid and creatinine levels with a reduction in total proteins levels of chickens experimentally infected with *Cl. perfringens* type C (1.5 × 10^9 organism/ml).

El-Sheikh et al., (2018) found a significant decrease in serum levels of total proteins and albumin in *Cl. perfringens* type C (1.5 × 10^9 organism/ml) infected broiler chickens. Serum levels of globulins, AST, ALT, ALP, uric acid and creatinine were significantly increased when compared with non-infected non-treated group.

6) Effect of Cl. perfringens infection on avian immune system:

Sahar (2001) reported a significant increase in serum levels of α and β-globulins in *Cl. perfringens* infected chickens along the experimental periods but the γ-globulin was significantly increased at the end of the 5th and 6th weeks only. On the other hand, phagocytic % and index and lymphocytes transformation rate were significantly decreased along the experimental periods compared with normal control.

Lovland et al., (2003) found that serum level of IgG was increased significantly in broilers with a history of subclinical necrotic enteritis.

Thrall (2004) recorded a significant increase in serum levels of α and β –globulins in chickens naturally infected with *Cl. perfringens.*

Gheith et al., (2011) proved a significant decrease in serum Alpha 1 and Beta globulins of broiler chickens infected with *Cl. perfringens*.

Hala et al., (2015) mentioned that chickens infected with *Cl. perfringens* (1.9×10^9 organism/ml) at 20 days old showed a significant increase in serum levels of α, γ-globulins and total globulins coupled with a significant decrease in phagocytosis and phagocytic index.

Dalia et al., (2016) found a significant decrease in phagocytic % and index at the end of the 4^{th} and 6^{th} weeks old in broilers infected with *Cl. perfringens* (1.5×10^9 organism/ml) at 18 days old on three alternate days.

El-Sayed (2018) reported that chickens experimentally infected with *Cl. perfringens* ($1x10^9$ CFU /ml) at the 14^{th} day old showed a significant decrease in IgM and phagocytic % and index coupled with a significant increase in serum levels of alpha globulins and total globulins. Non- significant increase in serum levels of beta and gamma globulins was noticed allover the experimental period.

El-Sheikh et al., (2018) indicated a significant decrease in serum levels of phagocytic percent and index, α, β and γ-globulins in *Cl. perfringens* type A ($1x10^9$ CFU /mL) infected chickens.

7) Histopathological changes associated with Cl. perfringens infection:

Das et al., (1997) reported that chickens infected by *Cl. perfringens* type "A" in 21-day-old broiler chickens after 3 oral inoculations of 2ml (1.9×10^9 organism/ml) on alternate days showed thickening and corrugation of the mucosa of small intestine with development of a pseudo-membrane in some cases. Microscopically, the lesions were congestion, necrosis, marked catarrhal enteritis and diffuse hemorrhage in intestinal villi and submucosa, marked infiltration of lymphoid cells and thickening of gut wall.

Ficken and Wages (1997) recorded acute coagulative necrosis with little associated hemorrhage or inflammatory response in the liver of *Cl. perfringens* infected broilers.

Lovland and Kaldhusdal (1999) reported that the livers of *Cl. perfringens* infected broilers may appear pale and firm, with or without small whitish foci. Histologically, there was bile duct hyperplasia, fibrosis, and focal granulomatous inflammation**.**

El-Bayoumi et al., (2014) recorded severe necrosis in the mucosa of the intestine of *Cl. perfringens* (10^7 CFU/ml) infected chickens with inflammatory cells infiltration. Liver showed vacuolar degeneration of the hepatocytes, severe congestion of the portal veins and coagulative necrosis.

Asmaa (2016) indicated a cystic dilatation of intestinal glands with edema under the submucosa and increased thickening of the intestinal wall. Focal coagulative necrosis of the liver and hyperplasia of the bile

duct were recorded. The renal blood vessels showed severe congestion with leukocytic infiltration in chickens infected with *Cl. perfringens*.

Dalia et al., (2016) mentioned that *Cl. perfringens* infected broilers showed necrosis of the intestinal mucosa with leukocytic infiltration and haemorrhage in lamina propria. Severe congestion of hepatic blood vessels and hepatic sinusoids with leukocytic infiltrations at the end of the 4th week were noticed. They also detected sloughing of some intestinal villi and necrosis of some intestinal glands. Focal hemorrhage in the hepatic parenchyma, hyperplasia of epithelial lining bile duct and caseous necrosis of hepatic tissue with leucocytic infiltration were encountered at the end of the 6th week.

II- AMOXICILLIN

Amoxicillin is a broad spectrum β-lactamase sensitive, semisynthetic penicillin and provides bactericidal activity against wide range of Gram+ve and Gram-ve bacteria. It's given orally in the form of amoxicillin trihydrate and parentrally in the form of amoxicillin sodium ***(Alello and Mays 1998)***.

1) Mechanism of action:

Nagaralli et al., (2002) proved that amoxicillin has bactericidal activity by inhibition of biosynthesis of cell wall mucopeptide during bacterial multiplication.

Simar et al., (2011) reported that amoxicillin originally introduced in the early 1970's for oral use in United Kingdom. Its mechanism of action involves interference with synthesis of bacterial cell walls. As, when the bacterial cell grows, more cell wall must be synthesized to

accommodate the additional protoplasmic material. Amoxicillin prevent this synthesis in growing cells resulting in rupture of bacterial cells due to the increased internal pressure**.**

2) Dosage:

Brander et al., (1993) mentioned that the therapeutic dose of amoxicillin for chickens is 25mg/kg.B.wt. for 5 consecutive days.

Anadon et al., (1996) indicated that 10 mg/kg.B.wt. amoxicillin administrated orally at 24 h intervals for 3-5 consecutive days is effective in treating a variety of systemic infections in poultry.

Elviss et al., (2009) used amoxicillin at a dose of 15 mg/Kg B.wt orally in drinking water for 5 consecutive days for treatment of bacterial infections in broilers**.**

3)Effect of amoxicillin on Clostridium:

Anadon et al., (1996) reported that the antibacterial spectrum of amoxicillin includes important Gram-positive organisms (*Clostridium, Staphylococcus, Streptococcus and Corynebacterium*) and Gram-negative organisms (*Bordetella bronchiseptica, Escherichia coli, Proteus mirabilis, Pasteurella, Salmonella and Haemophilus*).

Abd El-Gwad and Abd El-Kader (2001) revealed that *Cl. perfringens* isolates were highly sensitive to amoxicillin in chickens experimentally infected with *Cl. perfringens* (1×10^8 CFU/ml) at 3 days old.

Brennan et al., (2001) reported that necrotic enteritis can be prevented by the use of antibiotics, among these antimicrobials; amoxicillin and metronidazole.

Lanckriet et al., (2010) concluded that amoxicillin (100g/1000 L drinking water for 4 days) was effective against *Cl. perfringens* (4 x 10^8 CFU/ml).

Sarkar et al., (2013) mentioned that the results of antimicrobial sensitivity test from *Cl. perfringens* naturally infected chickens revealed that 57% of the isolates were sensitive to amoxicillin.

Fan et al., (2016) recorded that most of *the Cl. perfringens* isolates were susceptible to amoxicillin.

4) Effect of amoxicillin on body performance:

Abaza et al., (2006) reported a significant decrease in the feed intake of hens fed diets supplemented with amoxicillin at level of 0.02%. They added that feed conversion rate did not change significantly.

El-Bayoumi et al., (2014) recorded an improvement in body weight and FCR in healthy chickens treated with amoxicillin (40 mg/kg.B.wt). Also, a significant increase in body weight, weight gain and feed intake with a significant decrease in FCR in *Cl. perfringens* (10^7 CFU/ml) infected chickens and treated with amoxicillin when compared with the infected group.

El-Shahat (2014) observed a significant increase in body weight and body weight gain with an improvement in feed consumption and feed

conversion rate in healthy broilers administrated amoxicillin (25mg/kg.B.wt.) for 5 successive days compared with normal control group. While *Cl. perfringens* ($1.5x10^9$organisms/ml) infected broilers at 21 days old and treated with amoxicillin showed a significant increase in body weight and body weight gain with an improvement in feed consumption and feed conversion rate compared with infected group.

Hao et al., (2014) indicated that antibiotic as amoxicillin destroy or inhibit bacteria and is used to improve body weight as growth promoters when administrated at a low, sub-therapeutic dose.

Omnia (2017) mentioned that *Cl. perfringens* type C (1.5×10^9 organism/ml) infected chickens after treatment with amoxicillin (25 mg /kg.B.wt) for 5 consecutive days showed a significant increase in body weight and body weight gain with an improvement in feed consumption and feed conversion rate when compared with the infected group.

Aboubakr and Elbadawy (2017) observed a significant increase in body weight and body weight gain with an improvement in feed conversion rate of chickens administrated amoxicillin (20 mg/kg B. wt) for 5 consecutive days orally or I/M when compared with healthy untreated chickens. Also, a significant increase in body weight and body weight gain with an improvement in feed consumption and feed conversion rate of chickens infected with *Cl. perfringens* (1.5×10^9 organisms/ml) and treated with amoxicillin (20 mg/kg B. wt) when compared with the infected group.

El-Sheikh et al., (2018) recorded that broilers infected with *Cl. perfringens* type A broth culture ($1x10^9$ CFU /mL) and treated by

ampicillin (1.25 gm/litre) in drinking water for 5 consecutive days showed a significant increase in feed consumption, body weight and body weight gain with a significant decrease in FCR when compared with the infected group.

5) Effect of amoxicillin on hematological parameters:

Ali and Jalaa (2005) proved a significant decrease in all hematological values (RBCs, Hb, PCV, MCV, MCH, MCHC and WBCs) after 5 days of continuous treatment with amoxicillin 20% resulting in microcytic hypochromic anemia in comparison with normal control chicks.

Turcu et al., (2011) found a significant leukocytosis, heterophilia and eosinopenia in amoxicillin treated broiler chickens (46 mg /kg B.wt/day) compared with normal control. Moreover, erythrocytic count, hemoglobin concentration, MCV and MCHC showed non-significant changes.

El-Shahat (2014) reported a significant decrease in the count of RBCs, Hb concentration and PCV with development of macrocytic hypochromic anemia. In addition to a significant increase in total leukocytic count, heterophils, lymphocytes, eosinophils, basophils and monocytes at the 1^{st} and 10^{th} days post amoxicillin administration (25mg/kg.B.wt.) for 5 successive days in healthy broilers compared with normal control group. While *Cl. perfringens* ($1.5x10^9$ organisms/ml) infected broilers at 21 days old and treated with amoxicillin showed a significant increase in RBCs, Hb concentration and PCV compared with infected non-treated group. In addition to a significant decrease in total

leukocytic count, heterophils, lymphocytes, eosinophils, basophils and monocytes.

Ayana et al., (2016) recorded a significant decrease in the counts of RBCs, monocytes and lymphocytes after amoxystin application for healthy broilers in water at sub-therapeutic dose (10 mg/kg.B.wt.) for 41 days with development of macrocytic hypochromic anemia. Also a significant decrease in Hb concentration was recorded.

Omnia (2017) reported that *Cl. perfringens* infected chickens and treated with amoxicillin displayed a significant increase in RBCs count and Hb concentration compared with the infected non-treated group. On the other hand, non-significant changes were recorded in total leukocytic count and PCV.

El-Sheikh et al., (2018) indicated that broilers infected with *Cl. perfringens* type A broth culture and treated by ampicillin (1.25 gm/litre) in drinking water for 5 consecutive days showed a significant increase in RBCs count, Hb concentration and PCV with a significant decrease in total leukocytic count, eosinophils, basophils, monocytes, lymphocytes and heterophils when compared with the infected non-treated group.

Madhuchhanda et al., (2018) observed that administration of amoxicillin (10 and 20 mg/kg.B.wt.) for 14 consecutive days to layer poultry caused non-significant increase in Hb concentration, total leukocytic count and heterophils accompanied by a decrease in lymphocytes and eosinophils.

6) Effect of amoxicillin on biochemical parameters:

Al Harthi, (2004) found that serum AST and ALT activities did not change significantly by the addition of 40 mg amoxicillin/kg.B.wt. from 22 to 44 weeks old to the laying hens diet.

Abaza et al., (2006) recorded a significant increase in serum level of total proteins and albumin levels in amoxicillin (0.02%) treated laying hens compared with normal control. Moreover, serum globulins level did not change significantly.

El-Shahat (2014) proved a significant increase in serum levels of AST, ALT, ALP, uric acid and creatinine coupled with insignificant increase in serum total globulins one-day post amoxicillin administration (25mg/kg.B.wt.) for 5 successive days in healthy chickens. Also, insignificant increase of serum levels of total proteins and albumin was recorded one-day post treatment. Moreover, at the 10^{th} day post treatment the serum levels of AST, ALT, ALP, uric acid and creatinine returned to the normal values. While *Cl. perfringens* ($1.5x10^9$ organisms/ml) infected broilers at 21 days old and treated with amoxicillin showed a significant decrease in serum levels of total globulins, AST, ALT, ALP, uric acid and creatinine with a significant increase in serum levels of total proteins and albumin compared with infected non-treated group.

Ayana et al., (2016) reported a significant decrease in serum levels of total proteins, AST and ALT with no deviation in uric acid after amoxystin application for broilers in water at sub-therapeutic dose (10 mg/kg.B.wt.) for 41 days.

Aboubakr and Elbadawy (2017) revealed that administration of amoxicillin (20 mg/kg B. wt) to healthy broilers displayed a significant increase in serum activities of AST, ALT, ALP, uric acid and creatinine levels with insignificant increase in serum levels of total proteins, total globulins and albumin compared with normal control. On the other hand, a significant decrease in serum activities of AST, ALT, ALP, total globulins, uric acid and creatinine levels was recorded in chickens infected with *Cl. perfringens* and treated with amoxicillin when compared with infected non-treated group. Moreover, a significant increase in serum total proteins and albumin was noticed.

Omnia (2017) concluded that broilers infected with *Cl. perfringens* and treated with amoxicillin displayed a significant decrease in serum activities of total globulins, AST, ALT, ALP, uric acid and creatinine levels when compared with infected non-treated group. Non-significant change in serum levels of albumin and total proteins was noticed.

El-Sheikh et al., (2018) mentioned that *Cl. perfringens* infected broilers and treated by ampicillin showed a significant decrease in serum activities of AST, ALT, ALP, uric acid and creatinine levels with a significant increase in serum total proteins and albumin when compared with infected non-treated group.

Madhuchhanda et al., (2018) detected a significant increase in plasma ALT activity in layers administrated amoxicillin in therapeutic (20 mg/kg B. wt) and sub-therapeutic dose (10 mg/kg.B.wt.) for 14 consecutive days.

7) Effect of amoxicillin on immune system:

Al-Ankari and Homeida (1996) reported that phagocytic activity of healthy chickens administrated ampicillin (0.001 g/kg) didn't change significantly compared to control group.

Ayhan (1999) found that amoxicillin (1 g/L) at 12 days old for 3 days can depress the cellular and humoral immunity in healthy chickens.

El-Sheikh et al., (2018) indicated a non-significant decrease in serum levels of α, β, and γ globulins with a significant increase in phagocytic percentage and index in broilers administrated ampicillin (1.25 gm/litre) as a treatment for *Cl. perfringens* type A infection ($1x10^9$CFU /mL) when compared with the infected group.

8) Histopathological changes associated with administration of amoxicillin:

Dalia (2007) indicated few necrotic areas on the top of intestinal villi with few round cell infiltrations after treatment of *E.coli* infected chickens with amoxicillin (10 gm/L) for 3 consecutive days.

El-Bayoumi et al., (2014) indicated that chickens administrated amoxicillin (40 mg/kg.B.wt) from one day old then challenged with *Cl. perfringens* (10^7 CFU/ml) showed slight inflammatory cells infiltration in the intestinal mucosa and congestion of the portal vein. While chickens infected with *Cl. perfringens* then treated with amoxicillin showed congestion of the blood vessels in the submucosa and inflammatory cells infiltration for the mucosa of the intestine with severe hydropic degeneration of hepatocytes.

Koutoulis et al., (2015) reported a thin walled mucosa with a flaccid appearance of the intestine and disappearance of the necrotic pseudomembrane 3 days post amoxicillin treatment (20 mg kg/ B.wt) in the drinking water for five consecutive days in chickens naturally infected with *Cl. perfringens*. Moreover, full recovery of the intestinal mucosa was seen 14 days post amoxicillin treatment.

Salah-Eldin et al., (2015) observed inflammatory cells infiltrations with hemorrhages in the intestine of broilers infected with *Cl. perfringens* type A ($6x10^8$ CFU) at 19^{th} day old. The liver showed necrotic hepatocytes and congestion of blood vessels. While after treatment with amoxicillin (10µg concentration) at 22-26 days old, the intestine showed mild inflammatory cells infiltration and the liver exhibited extravasations of erythrocytes besides congestion of blood vessel and hepatic sinusoids.

Aboubakr and Elbadawy (2017) recorded thin wall of the intestine with mild necrosis and ulceration in broilers infected with *Cl. perfringens* and treated with amoxicillin.

Omnia (2017) observed that broilers infected with *Cl. perfringens* and treated with amoxicillin showed normal intestine and liver without necrotic foci.

Yu et al., (2017) reported mild congestion of liver, hepatocytes shape was irregular with disruption of cell membrane and the cytoplasm was reduced in chickens administrated amoxicillin (100mg/kg) once a day for 3 consecutive days.

Madhuchhanda et al., (2018) reported acute cellular and vacuolar degeneration of the liver in healthy layers administrated amoxicillin (10 mg/kg.B.wt.) for 14 consecutive days. While coagulative necrosis of the liver was recorded at amoxicillin therapeutic dose (20 mg/kg.B.wt.).

III- ORGANIC ACIDS

Organic acids are used nowadays as animal feed additives in animal husbandry and in abattoirs and food-processing because of their antimicrobial activities ***(Cherrington et al., 1991[b])***.

Public pressure and concerns about food made researchers think about alternatives as organic acids which have polyvalent antibacterial and immuno-stimulatory activities ***(Diarra and Malouin, 2014).***

1) Mechanism of action:

Brul and Coote (1999) concluded that the growth of bacteria is inhibited by organic acids. This inhibitory effect has been through a lower environmental pH caused by its weak acidic properties, a prebiotic effect on intestinal microflora, or a direct effect on the bacterial cell integrity. Organic acids may cross the bacterial cell membrane and dissociate in the higher intracellular pH inside the bacteria, resulting in the release of charged anions and protons that cannot cross the cell membrane. This decrease in bacterial intracellular pH and inhibition of essential metabolic reactions reduces bacterial growth.

Viola and Vieira (2007) suggested that acidifiers are efficient as antibiotics in maintaining the performance and morphology of the small intestines of broiler chickens.

2) Effect of organic acids on Cl. perfringens:

Skrivanová et al., (2005) proved that caprylic acid, capric acid, lauric acid, myristic acid, and oleic acid all reduced the growth of *Cl. perfringens* in vitro**.**

Islam et al., (2009) mentioned that the use of electrolytes to correct ionic balance of the body fluids and acidification of gastrointestinal tract with acidifier (vinager@10ml per litre of water daily for 5 days) along with the course of antibiotics were effective in treating *Cl. perfringens* infection in broilers.

Mikkelsen et al., (2009) proved that acidifiers (4.5 g/kg feed) limit the incidence of necrotic enteritis and the associated losses.

Timbermont et al., (2010) mentioned that feeding a diet supplemented with lauric acid (0.5 mg/ml) reduced the intestinal lesion scores in chickens suffered from necrotic enteritis (4 x 10^8 CFU/ml) at 19 days old.

Casagrande et al., (2013) reported that the use of antibiotic alternative such as a mixture of propionic acid, formaldehyde and organic acids (8 gm/kg animal meal) controlled *Cl. perfringens* (10^6 CFU/ml) when used for five days treatment course in chickens**.**

Franciszek et al., (2013) indicated that the administration of acidifiers (3, 6 and 9 gm/kg) from 1 - 42 days old and additives that reduce digesta pH seem the most important factor regulating the status of intestinal microflora in broilers**.**

El-Bayoumi et al., (2014) concluded that the use of acidifier (0.5-2 ml /l drinking water) in chickens as safe alternative for antibiotic medication with long treatment period is preferable as it acts as growth promotor either by enhancing digestibility or competitive inhibition for colonization of pathogenic bacteria such as *Cl. perfringens* (10^7 CFU/ml) at 18, 19 and 20 days of age twice daily.

3) Effect of organic acids on body performance:

Awaad et al., (2011) observed a significant increase in body weight, weight gain and improvement in feed intake and FCR in chickens subcutaneously inoculated with *Cl. perfringens* (0.5×10^8 CFU) at 21 days old and treated with organic acids (250 g / T) when compared with the infected group.

Eman et al., (2012) reported that organic acids contained formic acid and lactic acid (3ml/liter via drinking water) are capable of reducing colonization of pathogenic microorganism and improving body performance in chickens.

Franciszek et al., (2013) proved that dietary acidification which contained 20.7% butyric acid, 17.5% ammonium propionate, 12.5% propionic acid and 4.2% ammonium propionate at a dose of (3, 6 and 9 gm/kg feed) significantly increased body weight of apparently healthy chickens by 6.2-8.2% at 21 days of age, and by 2.7- 3.6 % at 42 days of age, respectively. On the other hand, non-significant effect was recorded in feed consumption and feed conversion rate.

Luckstadt and Kuhlmann (2013) indicated that growth performance improved significantly by the use of water acidifier (1 ml/ 1 liter) for 24 days before slaughter in broiler chickens.

Azza and Naela (2014) showed a significant increase in growth performance of broiler chickens in different groups fed acidified diets consisted of 3% butyric acid or 3% fumeric acid or 3% lactic acid from 7-42 days old.

El-Bayoumi et al., (2014) showed an improvement in body weight and FCR in chicks early supplemented with acidifier mixtures together with copper sulphate at a dosage of (0.5-2.0 ml /liter) in drinking water compared with normal control. Also, a significant increase in body weight, weight gain and feed intake with a significant decrease in FCR were recorded in *Cl. perfringens* (10^7 CFU/ml) infected chickens and treated with acidifier mixtures when compared with infected non-treated group.

Abudabos et al., (2015) reported that broilers received organic acids (1 ml/kg drinking water) from 1-42 days old led to significant increase in body weight with significant decrease in FCR.

Dalia et al., (2016) revealed a significant increase in body weight, weight gain and feed intake with a significant decrease in FCR at the end of the 4^{th} and 5^{th} weeks in chickens infected with *Cl. perfringens* and treated with acidifier (1.5 L/ 1000 L) when compared with infected non-treated group.

El-Sheikh et al., (2018) recorded that broilers infected with *Cl. perfringens* type A broth culture ($1x10^9$ CFU /mL) and treated by acidifier (2 ml/liter) for 5 consecutive days showed a significant increase in feed consumption, body weight and body weight gain with a significant decrease in FCR when compared with infected non-treated group.

4) Effect of organic acids on hematological parameters:

Cengiz et al., (2012) reported that organic acids (3 kg/ton) from 1 to 10 days of age treatment significantly reduced the heterophils count and heterophils: lymphocytes ratio in chickens compared with normal control.

Al-Saad et al., (2014) recorded a significant increase in WBCs count in blood samples of organic acids (1000 gm/ton) treated broilers from 1 to 42 days of age, compared with the antibiotic treated group but not to the control group. There were no significant differences in RBCs count and hemoglobin concentration between organic acid and antibiotic treated broilers.

Ndelekwute et al., (2016) observed non-significant changes in Hb concentration, MCV, MCHC and WBCs count in organic acids treated broilers at level of 0.25% of their diets for eight weeks.

Zaib et al., (2016) proved that there was non-significant effect on RBCs, Hb concentration, PCV, MCV, MCH, MCHC, WBCs and heterophils in broilers fed organic acids (10 gm/kg) from 8-42 days old. Moreover, a significant increase in lymphocytes was recorded.

El-Sheikh et al., (2018) indicated that broilers infected with *Cl. perfringens* type A broth culture and treated by acidifier (2 ml/liter) for 5 consecutive days showed a significant increase in RBCs count, Hb concentration and PCV with a significant decrease in total leukocytic count, heterophils, lymphocytes, monocytes, esinophils and basophils when compared with infected non-treated group.

Eugenes et al., (2018) reported that feeding of organic acids to broilers at level of 0.25% of diet at the fifth week of age did not alter hematological parameters such as RBCs count, Hb concentration, PCV, MCHC and WBCs count.

5) Effect of organic acids on biochemical parameters:

Yesilbag and Çolpan (2006) proved that serum total proteins and albumin concentrations as well as AST activity were significantly increased in layers treated with organic acid mixture (1.5 %) for 18 weeks. While serum ALT showed non-significant change compared with normal control.

Abdel-Fattah et al., (2008) proved that broilers fed an organic acid supplemented diet at levels of (1.5% and 3.0%) showed higher level of serum globulins.

Kaya and Tuncer (2009) detected a significant decrease in serum total proteins after feeding organic acids (1 kg/ton) to broilers from 1-42 days old compared with normal control.

Franciszek et al., (2013) recorded non-significant changes in plasma level of total proteins in chickens supplemented with organic acid

(3, 6 and 9 gm/kg) from 1-42 days old compared with the normal control group**.**

Azza and Naela (2014) reported a significant hyperglobulinemia in broiler chicken in different groups fed acidified diets consisted of 3% butyric acid or 3% fumeric acid or 3% lactic acid from 7-42 days old. They added that, serum levels of uric acid as well as serum activities of AST and ALT showed non-significant changes compared with normal control.

Abudabos et al., (2015) recorded that serum activities of AST and ALT didn't change significantly by organic acids administration (1 mL/kg drinking water) from 1-42 days old.

Dalia et al., (2016) revealed a significant decrease in serum activities of AST and ALT at the end of the 4^{th} and 6^{th} weeks and serum levels of uric acid and creatinine at the end of the 6^{th} week in chickens infected with *Cl. perfringens* after treatment with acidifier (1.5 L/ 1000 L) when compared with infected non-treated group.

Ndelekwute et al., (2016) proved that serum levels of total proteins, albumin, globulins, uric acid, creatinine, AST, ALT and ALP were not significantly different in broilers fed organic acid (0.25% of their diets) for eight weeks compared with the normal control.

El-Sheikh et al., (2018) mentioned that *Cl. perfringens* infected broilers and treated by acidifier showed a significant decrease in serum levels of AST, ALT, ALP, uric acid and creatinine with an insignificant

increase in albumin and total proteins when compared with the infected group.

Eugenes et al., (2018) reported that there were no significant differences in serum levels of total proteins, ALP, ALT, uric acid and creatinine in chickens fed on organic acid (0.25% of their diets) at the fifth week of age when compared with normal control.

6) Effect of organic acids on immune system:

Lohakare et al., (2005) demonstrated that the infectious bursal disease (IBD) titres measured post-vaccination showed significantly higher value in the ascorbic acid (0.2%) supplemented group compared with normal control.

Park et al. (2009) noticed that IgG level was significantly increased with the addition of organic acids (0.2%) to broiler's diet.

Wang et al., (2009) found that layers fed organic acid supplementation at levels of (0.1%, 0.2% and 0.3%) had significant increase in the lymphocytes percentage when compared with the normal control.

Haque et al., (2010) mentioned that citric acid supplementation (0.5%) enhanced the density of the lymphocytes in the lymphoid organs and enhancing the non-specific immunity in chickens.

Hedayati et al., (2015) concluded that chicken's diet supplemented with acidifier at a level of 0.1% from 1-42 days of age led

to improvement in antibody titers against Newcastle Disease (ND) and Infectious Bursal Disease (IBD).

Dalia et al., (2016) revealed noticeable improvement in immune response (phagocytic activity and antibody titer against ND) at the 2nd week old in healthy broilers administrated acidifier (1.5 L/ 1000 L) all over the experimental period. Moreover, chickens infected with *Cl. perfringens* (1.5×10^9 organism/ml) and treated with acidifier showed a significant increase in phagocytic percentage and phagocytic index when compared with infected non-treated group.

Zaib et al., (2016) indicated that dietary supplementations with organic acids (10 gm/kg) from 8-42 days of age significantly increased the antibody titer against Newcastle Disease (ND) compared with the normal control.

El-Sheikh et al., (2018) recorded non-significant changes in serum levels of α, β, and γ globulins with a significant increase in phagocytic percentage and index in broilers administrated acidifier as a treatment for *Cl. perfringens* infection when compared with infected non-treated group.

7) Histopathological changes associated with administration of organic acids:

Şenköylü et al., (2005) proved that villi height was significantly increased by organic acid supplementation (3 g/kg) into starter and finisher broiler diets.

Garcia et al., (2007) reported that broilers fed diets containing formic acid 1.0% had the longest villi compared with control.

Panda et al., (2009) indicated that organic acids (0.6%) in the broiler's diet, improved the villus length and crypt depth in the duodenum.

Adil et al., (2010) demonstrated that the highest duodenal, jejunal and ileal villus heights were recorded in broilers fed on diets supplemented with 3% butyric acid, 3%fumeric acid and 2% fumeric acid, respectively.

Kum et al., (2010) indicated that organic acid (1.0% sorbic acid and 0.2% citric acid) supplementation to broiler chickens at 14 days of age significantly increased the villus width, height and area of the duodenum, jejunum and ileum.

Adil et al., (2011) mentioned that dietary supplementation of organic acids 3% fumeric acid in chickens increased the villus height in duodenum, jejunum and ileum.

Awaad et al., (2011) found no histopathological changes in chickens subcutaneously inoculated with *Cl. perfringens* (0.5×10^8 CFU) at 21 days old and treated with organic acids (250 g / T) except for slight edema in lamina propria of intestine and vacuolar degeneration of hepatocytes of liver.

El-Bayoumi et al., (2014) indicated that chickens administrated acidifier (0.5-2 ml /l drinking water) from one day old then challenged with *Cl. perfringens* (10^7 CFU/ml) showed slight inflammatory cells infiltration of the the intestinal mucosa and congestion of the portal vein. While chickens challenged with *Cl. perfringens* then treated with acidifier

showed congestion of the blood vessels in the submucosa and inflammatory cells infiltration of the mucosa of the intestine and severe hydropic degeneration of the hepatocytes.

Mohamed et al., (2014) reported that ileum had more crypts in broilers fed organic acids (0.06%) from 10 to 36 days old. Also, numerous blood vessels, sinusoids and lymphocytes of different size are observed. There were large numbers of small and large lymphocytes in both red pulp and white pulp areas extended all over the splenic tissues.

MATERIAL AND METHODS

I- Materials

1- Experimental chicks:

One hundred and sixty, one-day old, commercial Hubbard chicks from Al-Kahira Poultry Company, 10th of Ramadan city, Egypt were used for this investigation. The chicks were reared under standard environmental and hygienic condition and fed on a balanced commercial ration free from antibacterial agents. All chicks were vaccinated against New castle disease at 7 and 18 days old and Gumboro disease at 14 days old ***(Marangon and Busani, 2006).***

2- Amoxicillin (Octacillin®697 mg/g Powder):

The drug (amoxicillin trihydrate) was obtained from Dechra Veterinary Products. The therapeutic dose is 15 mg/kg.B.wt. in the drinking water for 5 consecutive days ***(Elviss et al., 2009).***

3- Organic acids (Mix acid®):

The acidifier was obtained from Nanjing Weite Veterinary Co. The indicated dose is 1 ml/1 liter (***Luckstadt and Kuhlmann, 2013)***. Each 1 liter contains:

- Formic acid (85%) 150gm
- Tartaric acid (99%) 15gm
- Acetic acid (80%) 40 gm
- Propionic acid (99%) 250 gm
- Lactic acid (80%) 50 gm
- Phosphoric acid (75%) 55 gm
- Fumeric acid (99%) 80 gm
- Sorbic acid (99%) 15 gm
- Malic acid (99%) 12 gm

- Benzoic acid (99%) 22gm
- Ammonium formate 90 gm
- Calcium lactate 10 gm
- Copper pentasulphate 10 gm
- Distilled water up to 1 liter

4- Microorganisms:

a- ***Clostridium perfringens*** **strain**: *Cl. perfringens* type "A" was obtained from Anaerobic Bacterial Section, Animal Health Research Institute, Dokki, Giza, Egypt to be used for experimental infection.

b- ***Candida albicans (C. albicans***): was obtained from Department of Bacteriology, Mycology and Immunology, Animal Health Research Institute, Zagazig branch.

5- Test kits:

The biochemical tests were performed using test kits of Diamond- Egypt. Sodium and potassium levels were measured using Spectrum kits.

Immunoglobulins were measured using ELISA kits of Bethyl Laboratories Inc.

6- Materials used in phagocytic assay:

- **Heparin solution ampoules (Nile pharmaceutical Co- Egypt)** was used as anticoagulant. The solution was prepared by dissolving one ampule of 5000 I.U in 50 ml sterile phosphate buffer saline to get 100 I.U/ml for phagocytic activity tests.
- **Sabouraud's dextrose agar** was used for cultivation and isolation of *C. albicans* according to the method described by ***Cruickshank et al., (1975).*** The hydrogen ion concentration (pH) of the

Sabouraud's dextroae agar media adjusted to be around 5.0. This medium was used to obtain *C. albicans* in yeast phase.

- **Hank's balance salt solution (HBSS):** Preparation of HBSS according to ***Cruickshank et al., (1975).***
- **Lymphocytic separation medium "Ficollhypaque" (Sigma Co- USA):** This medium was used for separation of mononuclear leukocyte cells from peripheral blood. It was used as an aqueous solution having a density of 1.077 gm/ml and contained 57 gm ficoll 400 and 9.0 gm diatrazoat sodium in 100 ml distilled water.
- **Rose well park memorial institute 1640 "RPMI-1640" tissue culture medium (Flow laboratories- UK):** The content of a packet (10.4 gm) was reconstituted in one-liter double distilled water and supplemented with sterile bicarbonate (2%) and antibiotic (penicillin 250 IU/ml, streptomycin 100 µg/ml). The prepared medium was sterilized by filtration through Millipore filter and stored at 4^{o}C until use.
- **Sterile foetal calf serum "FCS" (Gibco limited- UK):** Sterile foetal calf serum was kept at -20^{o} C after heat inactivation at 56^{o} C for 30 minutes and added at final concentration of 15% with RPMI-1640 medium for phagocytic activity test.
- **Cedar oil** was used for phagocytic activity and phagocytic index examination.

7- Reagents:

a- Phosphate buffer saline "PBS" was prepared according to the method described by ***Cruickshank et al., (1975).*** It was used for cell washing and for serial dilution of bacterial growth.

b- Dipotassium salt of EDTA (Al Nasr co- Egypt): It was used as anticoagulant at a dose of one drop of 10% solution /5ml blood for hematological examination.

8- Stains:

- **a- Leishman stain:** was used for phagocytosis assay.
- **b- H & E stain:** was used for histopathological examination.

9- Instruments:

a. Fluorescent microscope (Nikon, Japan).
b. Automatic cell counter sysmex XT (2000 IV).
c. Centrifuge (MPS-England) was used for separation of serum sample.
d. Automatic pipettes (Finn pipette – Finland).
e. Gas pack anaerobic jar ***(Brewer and Allegier, 1966)***.
f. Spectrophotometer (Spectronic20 D, Milton Roy, USA)
g. Incubator (Selecta, mode 287) with thermostat to maintain the temperature at 37°C was used for incubating the bacterial strain on the selective bacteriological medium.
h. Deep freezer (-20) was used for preservation of serum samples.
i. ELISA (A Sys- Expert Plus UV) for measuring of IgM and IgG.
j. Microtome for sectioning blocks for histopathological examination.
k. Beakers, flasks, test tubes, glass slides, cover slides, forceps, gloves, marker pens, labels and syringes.

10- Histopathology:

- a- Formalin 10% (El Nasr Co. ARE).
- b- Ethyl alcohol (Merck, Germany).
- c- Xylol, paraffin wax and canada balsam (El Nasr Co., ARE).

II- Methods:

1) Experimental design:

One hundred and sixty, one-day old chicks were divided into 8 equal groups as following:

- **Group (1):** was kept as a normal control.
- **Group (2):** was given amoxicillin (15 mg/kg.b.wt.) for 5 successive days in the drinking water at 25 days old ***(Elviss et al., 2009)***.
- **Group (3):** was administered organic acids (1 ml /l water) from 1st day old till the end of the experiment (***Luckstadt and Kuhlmann , 2013)***.
- **Group (4):** was given amoxicillin (15 mg/kg.b.wt.) for 5 successive days in the drinking water at 25 days old and organic acids (1 ml /l water) for the same period.
- **Group (5):** was given broth culture (1.9×10^9 CFU/ml) of *Cl. perfringens* at 19th days old 3 times orally day after day ***(Sahar, 2001)***.
- **Group (6):** was infected with *Cl. perfringens* as in group (5) and treated with amoxicillin (15 mg/kg.b.wt.) for 5 successive days in drinking water after appearance of clinical signs (25days old)**.**
- **Group (7):** was given organic acids as in the 3rd group and infected with *Cl. perfringens* as in the 5th group.
- **Group (8):** was infected with *Cl. perfringens* as in group (5) and treated with amoxicillin and organic acids as in group (4).

Table (1) : The experimental design .

Groups		No. Of Birds	*Clostridium perfringens*		Amoxicillin		Organic acids		Time of blood sampling in days
			Dose and Route	Age of infection in days	Dose and Route	Age of treatment in days	Dose and Route	Age of treatment in days	
Gp1	**Normal control**	20	-	-	-	-	-	-	25^{th}, 32^{nd} and 39^{th} days old
Gp2	**Amoxicillin**	20	-	-	15mg/kg. B.wt in drinking water	25 - 29	-	-	
Gp3	**Organic acids**	20	-	-	-	-	1 ml/L water	1-39	
Gp4	**Amoxicillin+ organic acids**	20			15mg/kg. B.wt in drinking water	25 - 29	1 ml/L water	25 - 29	
Gp5	***Cl . perfringens***	20	broth culture (1.9 × 10^9 CFU/ml) orally	19-21-23	-	-	-	-	
Gp6	***Cl . perfringens*** → **Amoxicillin**	20	broth culture (1.9 × 10^9 CFU/ml) orally	19-21-23	15mg/kg. B.wt in drinking water	25 - 29	-	-	
Gp7	**Organic acids→** ***Cl . perfringens***	20	broth culture (1.9 × 10^9 CFU/ml) orally	19-21-23	-	-	1 ml /L water	1 - 39	
Gp8	***Cl . perfringens*** → **Amoxicillin+ organic acids**	20	broth culture (1.9 × 10^9 CFU/ml) orally	19-21-23	15mg/kg. B.wt in drinking water	25 - 29	1 ml /L water	25 - 29	

2) Indices for evaluation of body performance:

A) Feed consumption: The ration was weighed daily to determine the feed consumption/ week for each group.

B) Body weight (B. W.): The birds were weighed individually at weekly intervals to determine the average body weight in each group.

C) Body weight gain (B. W. G.): The body gain was determined as the weekly increase in the body weight.

D) Feed conversion rate (FCR): The weekly feed consumption was subdivided on the weekly increase in the body weight (body gain) to obtain the feed conversion rate. This was recorded every week according to ***Wanger et al., (1983).***

$$\textbf{FCR} = \frac{amount\ of\ feed\ consumption\ (g)/\ bird/\ week}{B.wt.gain\ (g)\ /bird/\ week}$$

3) Blood samples:

Five chickens, from each group, were used for collecting blood samples at 25th, 32nd and 39th days old. Each blood sample was divided into 3 portions. The first portion was taken on dipotassium salt of EDTA (1mg/1ml blood) for hematological examination. The second portion was collected into clean centrifuge tube for obtaining serum by centrifugation for biochemical studies. The third portion (2 ml blood) was collected in a sterile plastic centrifuge tube containing heparin (50 i.u/ml) to be used for phagocytic activity.

4) Hematological studies:

Erythrocytic count, Hb, PCV, MCV, MCH, MCHC, total and differential leukocytic count were measured by using the automatic cell counter sysmex XT (2000 IV).

5) Biochemical studies:

a) Liver function tests:

► ***Determination of serum total protein****:*

- Serum total protein level was measured according to ***Grant et al., (1987)***.
- **Principle:** Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant. The intensity of the color formed is proportional to the total proteins concentration in the sample.
- **Calculation:** Total protein g/dl = $\frac{A\ sample}{A\ standard}$ x 6.0 (standard conc.)

► ***Determination of serum albumin:***

- Serum albumin level was determined according to ***Doumas et al., (1981)***.
- **Principle:** Albumin in the presence of bromocresol green at a slightly acid pH, produces a colour change of the indicator from yellow-green to green-blue. The intensity of the color formed is proportional to the albumin concentration in the sample.
- **Calculation:** Albumin g/dl = $\frac{A\ sample}{A\ standard}$ x 4 (standard conc.)

► ***Determination of serum globulins:***

- Serum globulins level was calculated by subtracting the obtained albumin level from total protein level as described by ***Doumas and Biggs (1972).***

► ***Determination and electrophoretic analysis of serum proteins:***

- Electrophoretic analysis was carried out for determination of serum alpha, beta and gamma globulins according to the technique described by ***Davis (1964).***
- **Principle:** Two electrodes are immersed in two separate buffer chambers. The two chambers are connected such that charged particles can migrate from one chamber to the other. By using a power supply, electric potential difference is generated between the two electrodes. As a result, electrons flow from one of the electrodes, the anode, towards the other electrode, the cathode. Electrons from the cathode are taken up by water molecules of the buffer, resulting in a chemical reaction which generates hydrogen gas and hydroxide ions. In the other buffer chamber, water molecules transfer electrons to the anode an in another chemical reaction that generates oxygen gas and protons. (Protons are immediately taken up by water molecules to form hydroxonium ions.) As charged particles can migrate between the two chambers due to the electric potential difference, positive ions (cations) move towards the negatively charged cathode while negatively charged ions (anions) move towards the positively charged anode.

► ***Determination of serum aspartate aminotransferase (AST):***

- Serum aspartate aminotransferases (AST) activity was determined colorimetrically according to ***Reitman and Frankel (1957)***.
- **Principle:** Colorimetric determination of AST activity according to the following reaction:

$$\alpha\text{-oxoglutarate} + \text{DL-alanine} \xrightarrow{GOT} \text{L-glutamate} + \text{oxaloacetate}.$$

The oxaloacetate formed is measured in its derivative form 2, 4 dinitrophenyl hydrazine.

- **Calculation:** The absorbance of the unknown is converted to AST activity from a table.

► ***Determination of serum alanine aminotransferase (ALT):***

- Serum alanine aminotransferases (ALT) activity was determined colorimetrically according to ***Reitman and Frankel (1957)***.

- **Principle:** Colorimetric determination of ALT activity according to the following reaction:

$$\alpha\text{-oxoglutarate} + \text{DL-alanine} \xrightarrow{GPT} \text{L-glutamate} + \text{pyruvate}.$$

The pyruvate formed is measured in its derivative form 2, 4 dinitrophenyl hydrazine.

- **Calculation:** The absorbance of the unknown is converted to ALT activity from a table.

► ***Determination of serum alkaline phosphatase (ALP):***

- Serum alkaline phosphatase activity was determined according to modified method of ***Kind and King (1954).***
- **Principle:** Colorimetric determination of alkaline phosphatase activity according to the following reaction:

$$\text{Phenylphosphate} \xrightarrow[pH\ 10]{ALp} \text{phenol} + \text{phosphate}$$

Phenol liberated is measured in the presence of 4-aminoantipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

- **Calculation:** $\frac{\text{OD serum sample} - \text{OD serum blank}}{\text{OD standard}}$ x n

n= 20 (Kind and King U/100ml)

b) Kidney function tests:

► ***Determination of serum uric acid:***

- **Principle:** Colourimetric determination of serum uric acid after deproteinization by phosphotungstate and reduction in an alkaline medium (sodium carbonate) was carried out by spectrophotometer using specific kits after ***Caraway (1955).***
- **Calculation:** $\frac{\text{OD serum sample}}{\text{OD standard}}$ x n= mg/100, **n**=10mg/100ml.

► ***Determination of serum creatinine:***

- Serum creatinine level was determined colorimetrically according to the method of ***Husdan and Rapoport (1968).***
- **Principle:** The assay is based on the reaction of creatinine with sodium picrate. Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents. The intensity of the color formed is proportional to the creatinine concentration in the sample.
- **Calculation:** Creatinine mg/dl = $\frac{A\ specimen}{A\ standard}$ x 2

► ***Determination of serum sodium (Na):***

- Serum sodium level was determined colorimetric according to the method of ***Burtis and Bruns (2007).***
- **Principle:** The present method is based on reaction of sodium with a selective chromogen producing a chromophone whose absorbance varies directly as the concentration of sodium in the test specimen.
- **Calculation:** Serum sodium conc. m.Eq/l = $\frac{A\ sample}{A\ standard}$ x 150

► ***Determination of serum potassium (K):***

- Serum potassium level was determined using Turbidimetric Tetraphenylborate method by ***Burriel and Ramirez (1957).***
- **Principle:** At an alkaline pH Potassium ions and TPB form a turbid emulsion, the increase of which can be measured quantitatively in a photometer at 578 nm. The increase of the absorbance (A) is directly proportional to the concentration of Potassium in the sample.
- **Calculation:** Serum Potassium conc. m.Eq/l = $\frac{\text{A sample}}{\text{A standard}}$ x 5

6) Cellular immune response:

► **Phagocytic activity and phagocytic index:**

Measurement of phagocytic activity of peripheral blood monocytes using *Candida albicans* was adapted as described by ***Anthony et al., (1985)*** and ***Chu and Dietert (1989).***

a) Separation of peripheral mononuclear cells (PBMC):

Peripheral blood mononuclear cells (PBMC) were isolated using "Ficoll-Hypaque" density gradient according to the method described by ***Boyum (1986)*** and ***Goddeeris et al., (1986)***.

1- 2ml heparinized blood was harvested from each chicken and then diluting the blood 1:2 in heparinized (10 I.U heparin/ml) PBS.

2-The diluted blood was carefully layered on the surface of lymphocyte separation medium Ficoll-Hypaque (1:1) in sterile centrifuge tubes, and was centrifuged for 30 minutes at 2400 rpm.

3- After centrifugation, the mononuclear cells form white opaque band at Ficoll- plasma interference and was aspirated by sterile pasteur pipette and placed in sterile plastic tube contain PBS.

4-The PBMC were washed three times in heparinized PBS, pelleted by centrifuge at 2500, 1500 and 1000 rpm respectively each for 10 minutes.
5- The cells were suspended in RPMI-1640 media containing 1% of foetal calf serum.

b) Phagocytic Assay (Wilkinson, 1976):

1- 0.25ml of adjusted viable leukocytes suspension was added to 0.25ml heat-inactivated *C. albicans* in sterile plastic tubes.
2- The tubes were incubated at 37°C for 30 minutes in a humidified Co_2 incubator.
3- Subsequently, the tubes were centrifuged at 2500 rpm for 5 minutes and the supernatant were removed with Pasteur pipette leaving a drop into which the sediment was re-suspended.
4- Smears were prepared from the deposit, dried in air and stained with Leishman's stain.

c) Evaluation of phagocytic activity (Wilkinson, 1976):

Under a light microscope using oil immersion lens, a total number of 100 phagocytic cells were counted randomly in about ten microscopic fields.

$$\text{Phagocytic \%} = \frac{Total\ number\ of\ phagocytes\ containing\ C.albicans}{Total\ number\ of\ phagocytic\ cells} \times 100$$

$$\text{Phagocytic index} = \frac{Total\ number\ of\ ingested\ C.albicans}{Total\ number\ of\ phagocytic\ cells\ containing\ C.albicans}$$

7) Humoral immune response:

a) Determination of IgM:

Serum IgM was measured using chicken IgM ELISA kit according to ***Granfors (1979)*.**

- **Principle:** The assay is based on sandwich ELISA. Chicken IgM present in the test sample is captured by anti-chicken IgM antibody that has been pre-absorbed on the surface of microtiter wells. After sample binding, unbound proteins and molecules are washed off, and a biotinylated detection antibody is added to the wells to bind to the captured IgM. A strepavidin-conjugated horseradish peroxidase is then added to catalyze a colorimetric reaction with the chromogenic substrate (tetramethylbenzidine).
- The colorimetric reaction produces a blue product, which turns to yellow when the reaction is terminated by addition of dilute sulfuric acid. The absorbance of the yellow product is proportional to the amount of IgM analyte present in the sample.

- **Calculation:** Chicken IgM is determined in unknown sample using the prepared standard curve by noting the chicken IgM concentration (X axis) that correlates with the absorbance value (Y axis) obtained from the unknown sample.

b) Determination of IgG:

Serum IgG was measured using chicken IgG ELISA kit according to ***Granfors (1979).***

- **Principle:** The assay is based on sandwich ELISA. Chicken IgG present in the test sample is captured by anti-chicken IgG antibody that has been pre-absorbed on the surface of microtiter wells. After sample binding, unbound proteins and molecules are washed off, and a biotinylated detection antibody is added to the wells to bind to the captured IgG. A strepavidin-conjugated horseradish

peroxidase is then added to catalyze a colorimetric reaction with the chromogenic substrate (tetramethylbenzidine). The colorimetric reaction produces a blue product, which turns to yellow when the reaction is terminated by addition of dilute sulfuric acid. The absorbance of the yellow product is proportional to the amount of IgG analyte present in the sample.

- **Calculation:** Chicken IgG is determined in unknown sample using the prepared standard curve by noting the chicken IgG concentration (X axis) that correlates with the absorbance value (Y axis) obtained from the unknown sample.

8) Histopathological Studies: The sacrificed chickens were subjected to postmortem examination, specimens were collected from kidney, liver, small intestine and spleen then fixed in 10% neutral formalin and embedded in paraffin wax. Sections of five microns thickness were prepared, stained by haematoxylin & eosin stain and examined microscopically ***(Suvarna et al., 2013).***

9) Statistical analysis:. The obtained data was statistically analyzed by using computerized SPSS program version 16 using one way ANOVA. The comparison of means was carried out with Duncan's multiple range test ***(Tamhane and Dunlop, 2000).***

Results

I- Clinical Signs and mortality rate:

Groups (1- 4) appeared healthy without clinical signs of illness and no mortality was recorded during experimental period. On the other hand, group (5) showed decrease in appetite, depression, emaciation and ruffled feather **(photo 1).** In some cases brownish diarrhea and sudden death were recorded two days post infection. The mortality rate was 25%. However, the clinical signs were improved post treatment in groups (6- 8) and the mortality rate was 15%, 10% and 10% respectively.

Table (2): Mortality rate of chickens in different groups during the experimental periods.

Gps.	*Subject*	*No. of chickens*	*No. of dead chickens*	*Mortality Rate*
Gp. (1)	-ve control	20	Zero	Zero%
gp.(2)	Amoxicillin	20	Zero	Zero%
gp.(3)	Organic acids	20	Zero	Zero%
gp.(4)	Amoxi + Org.	20	Zero	Zero%
gp. (5)	Clostridium	20	5	25%
gp.(6)	*Cl.*→Amoxi.	20	3	15%
gp.(7)	Org. → *Cl.*	20	2	10%
gp.(8)	*Cl.*→ Amoxi.+Org.	20	2	10%

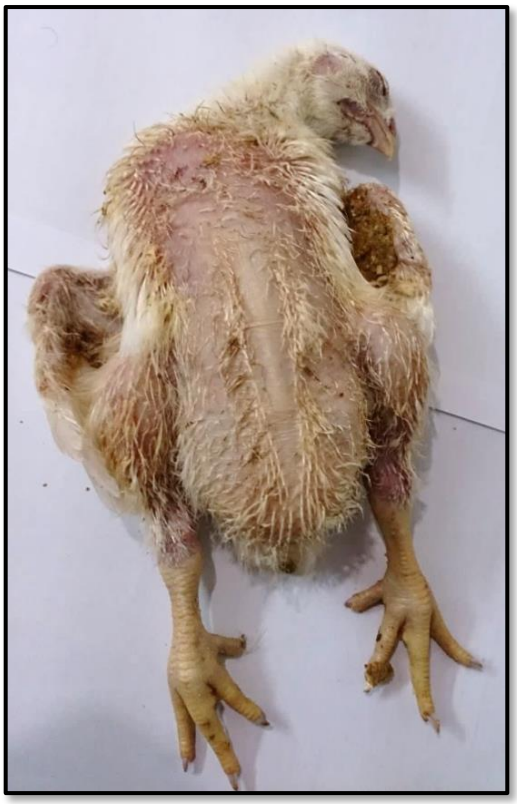


Photo (1): *Cl. perfringens* infected chicken showing ruffled feather and death in gp. (5).

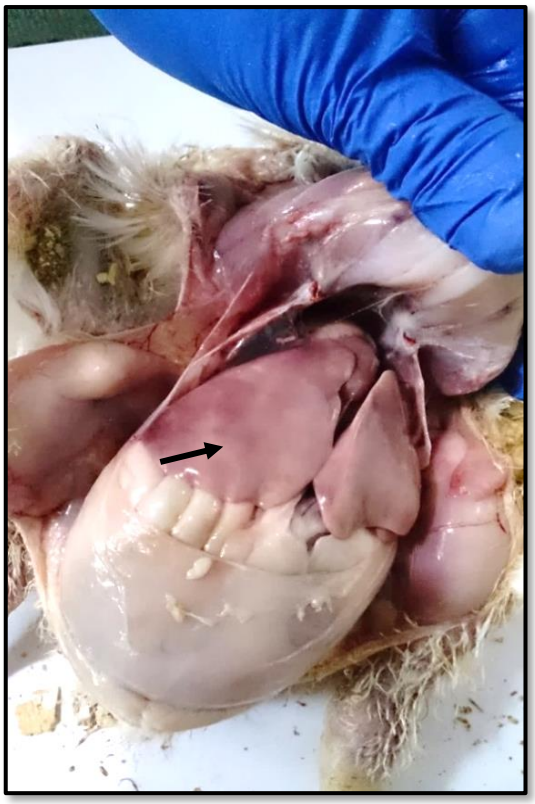


Photo (2): Liver of *Cl. perfringens* infected chicken showing area of discolouration in gp. (5).

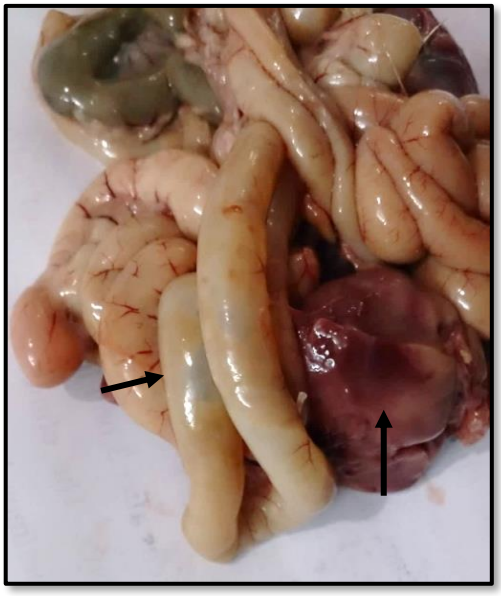


Photo (3): *Cl. perfringens* infected chicken showing distension of intestine with gases and congestion of the liver in gp. (5).

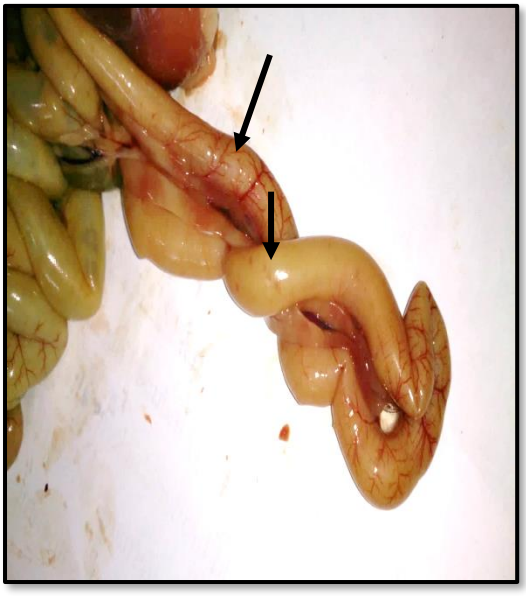


Photo (4): *Cl.perfringens* infected chicken showing congestion of the blood vessels of the intestine with necrotic spots on its surface in gp. (5).

II-Body performance:

Feed consumption:

The feed consumption in groups (2- 4) did not change the feed consumption all over the experimental periods. On contrary, groups (5-7) revealed a decrease in feed consumption during the different experimental periods. However, the feed consumption was decreased in group (8) but returned near to its normal level at 39 days old when compared with the normal control group **(tabs. 3- 5 and fig. 1).**

Body weight and body weight gain:

At 25, 32 and 39 days old, the B.W. and B.W.G. of chickens in groups (2 and 4) showed non-significant changes at 25 days old but at 32 and 39 days old a significant increase in body weight and B.W.G. was recorded when compared with the normal control group. While the 3rd group showed a significant increase in these parameters along the experimental periods. Moreover, the B.W. and B.W.G. of chickens in groups (5-8) were significantly ($P<0.05$) decreased except B.W.G. of gp. (8) at 39 days old showed non-significant change compared with normal control. The lowest values were recorded in gp. (5) **(tabs. 3- 5 and figs. 2 and 3).**

Feed conversion rate:

At 25, 32 and 39 days old, groups (2- 4) showed a significant decrease in FCR in all duration except groups (2 and 4) at 25 days old showed non-significant changes when compared with the normal control group. The best feed conversion rate was recorded in gp. (3). Groups (5-8) showed a significant increase in FCR along the experimental periods except birds in gp. (8) showed non-significant change at 39 days old

when compared with the normal control group. The highest level in FCR was noticed in gp. (5) **(tabs. 3- 5 and fig. 4).**

Table (3): Body performance (mean values ± S.E) of chickens in different groups at 25 days old (N=5).

Parameters / Groups	Feed consumption g/ bird	Initial body weight at 18 days old g/bird	Body weight g/ bird	Body weight gain g /bird	FCR
Control Gp (1)	539	805.0 [b] ± 4.46	1149.00 [b] ± 1.87	344.00 [b] ± 2.53	1.57 [b] ± 0.03
Amoxicillin Gp (2)	545	792.0 [b] ± 3.39	1134.00 [b] ± 1.87	342.00 [b] ± 2.55	1.59 [b] ± 0.01
Organic acids Gp (3)	530	821.0 [a] ± 4.30	1176.00 [a] ± 7.48	355.00 [a] ± 5.00	1.49 [c] ± 0.02
Amoxicillin+Organic Gp (4)	541	796.0 [b] ± 6.21	1141.00 [b] ± 3.32	345.00 [b] ± 2.24	1.57 [b] ± 0.02
Clostridium Gp (5)	380	796.0 [b] ± 4.30	1002.00 [d] ± 7.18	206.00 [d] ± 4.30	1.85 [a] ± 0.04
Cl. ➡ Amoxicillin Gp (6)	375	792.0 [b] ± 4.36	990.00 [d] ± 4.18	198.00 [d] ± 2.55	1.84 [a] ± 0.02
Organic acids ➡ *Cl.* Gp (7)	365	822.0 [a] ± 5.15	1040.00 [c] ± 7.58	218.00 [c] ± 2.55	1.86 [a] ± 0.02
Cl. ➡ Amoxi. + Organic Gp (8)	395	792.0 [b] ± 2.55	999.00 [d] ± 2.92	207.00 [d] ± 2.55	1.91 [a] ± 0.03

Different letters in the same column indicate significant changes ($P < 0.05$).

Table (4): Body performance (mean values ± S.E) of chickens in different groups at 32 days old (N=5).

Parameters / Groups	Feed consumption g/ bird	Body weight g/ bird	Body weight gain g /bird	FCR
Control Gp (1)	710	1583.00 ^{c} ± 5.39	434.00 ^{c} ± 4.30	1.64 ^{c} ± 0.02
Amoxicillin Gp (2)	730	1616.00 ^{b} ± 6.00	482.00 ^{ab} ± 6.04	1.52 ^{d} ± 0.02
Organic acids Gp (3)	760	1678.00 ^{a} ± 5.15	502.00 ^{a} ± 3.39	1.51 ^{d} ± 0.01
Amoxicillin+Organic Gp (4)	740	1629.00 ^{b} ± 6.40	468.00 ^{b} ± 18.75	1.52 ^{d} ± 0.02
Clostridium Gp (5)	536	1281.00 ^{f} ± 5.34	279.00 ^{f} ± 2.92	1.92 ^{a} ± 0.02
Cl. ➡ Amoxicillin Gp (6)	590	1324.00 ^{e} ± 8.28	334.00 ^{de} ± 5.34	1. 77 ^{b} ± 0.03
Organic acids ➡ *Cl.* Gp (7)	598	1359.00 ^{d} ± 8.28	319.00 ^{e} ± 4.30	1. 88 ^{a} ± 0.03
Cl. ➡ Amoxi. + Organic Gp (8)	612	1353.00 ^{d} ± 6.04	354.00 ^{d} ± 4.30	1. 73 ^{b} ± 0.02

Different letters in the same column indicate significant changes (P <0.05).

Table (5): Body performance (mean values ± S.E) of chickens in different groups at 39 days old (N=5).

Parameters / Groups	Feed consumption g/ bird	Body weight g/ bird	Body weight gain g /bird	FCR
Control Gp (1)	905	2103.00 ^{c} ± 8.46	520.00 ^{c} ± 6.89	1.74 ^{c} ± 0.02
Amoxicillin Gp (2)	885	2159.00 ^{b} ± 6.78	543.00 ^{b} ± 6.25	1.63 ^{d} ± 0.02
Organic acids Gp (3)	915	2259.00 ^{a} ± 4.85	581.00 ^{a} ± 4.00	1.58 ^{e} ± 0.01
Amoxicillin+Organic Gp (4)	885	2174.00 ^{b} ± 7.31	545.00 ^{b} ± 5.00	1.62 ^{d} ± 0.02
Clostridium Gp (5)	659	1632.00 ^{g} ± 3.00	351.00 ^{f} ± 5.09	1.88 ^{a} ± 0.03
Cl. ➡ Amoxicillin Gp (6)	850	1799.00 ^{e} ± 8.43	475.00 ^{d} ± 5.00	1.79 ^{b} ± 0.02
Organic acids ➡ *Cl.* Gp (7)	760	1779.00 ^{f} ± 12.59	420.00 ^{e} ± 5.70	1.81 ^{b} ± 0.02
Cl.➡ Amoxi. + Organic Gp (8)	895	1879.00 ^{d} ± 7.48	526.00 ^{c} ± 4.30	1.70 ^{c} ± 0.01

Different letters in the same column indicate significant changes ($P < 0.05$).

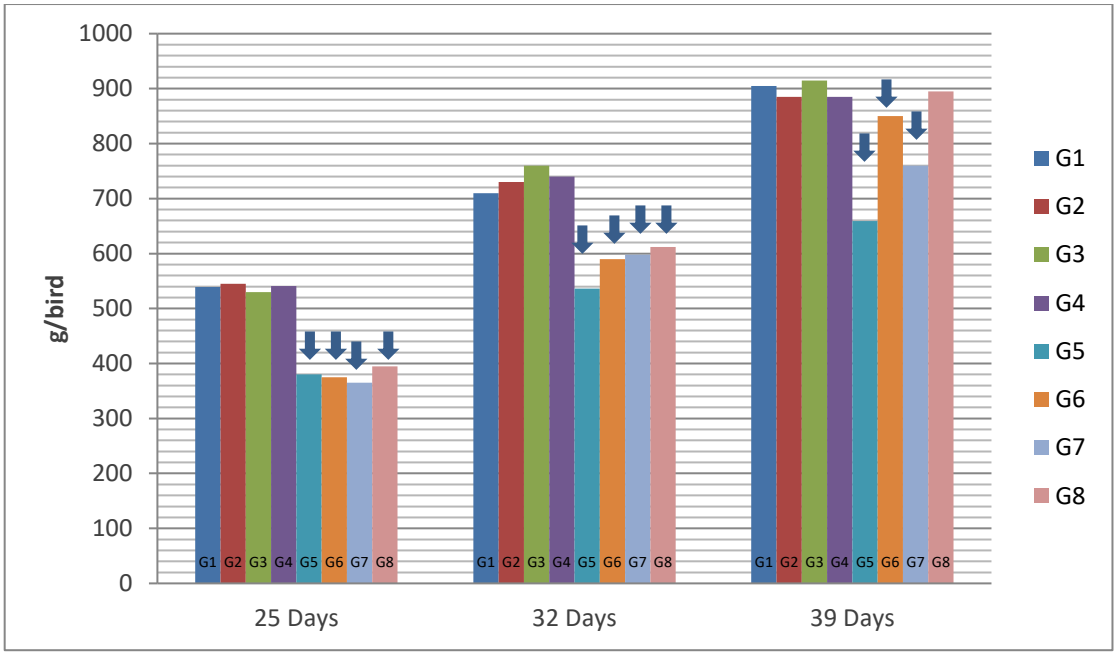


Fig. (1): Changes in **feed consumption** (g/bird) among different groups at different ages (25, 32 and 39 days old)

⬆ Significant increase - ⬇Significant decrease

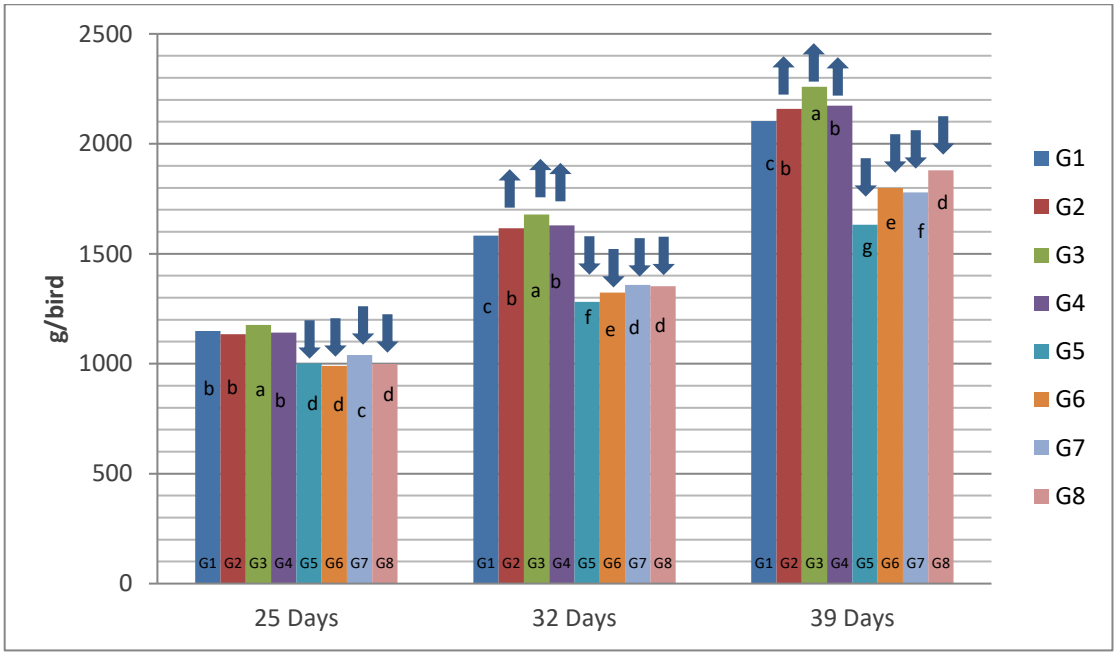


Fig. (2): Changes in **body weight** (g/bird) among different groups at different ages (25, 32 and 39 days old)

⬆ Significant increase - ⬇Significant decrease

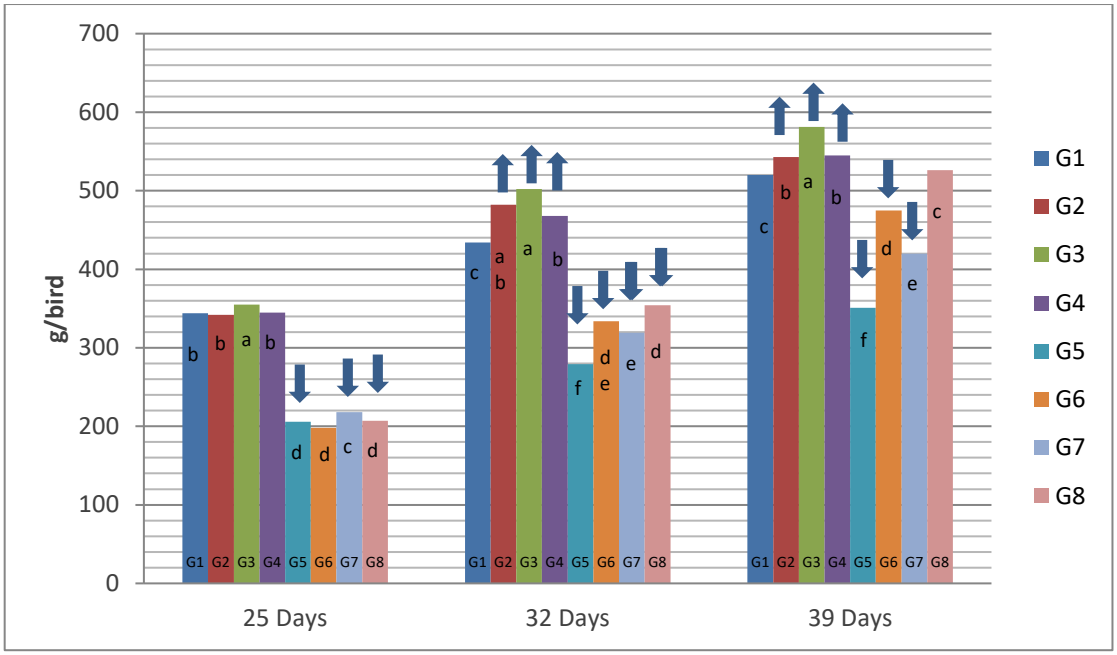


Fig. (3): Changes in **body weight gain** (g/bird) among different groups at different ages (25, 32 and 39 days old)

⬆ Significant increase - ⬇Significant decrease

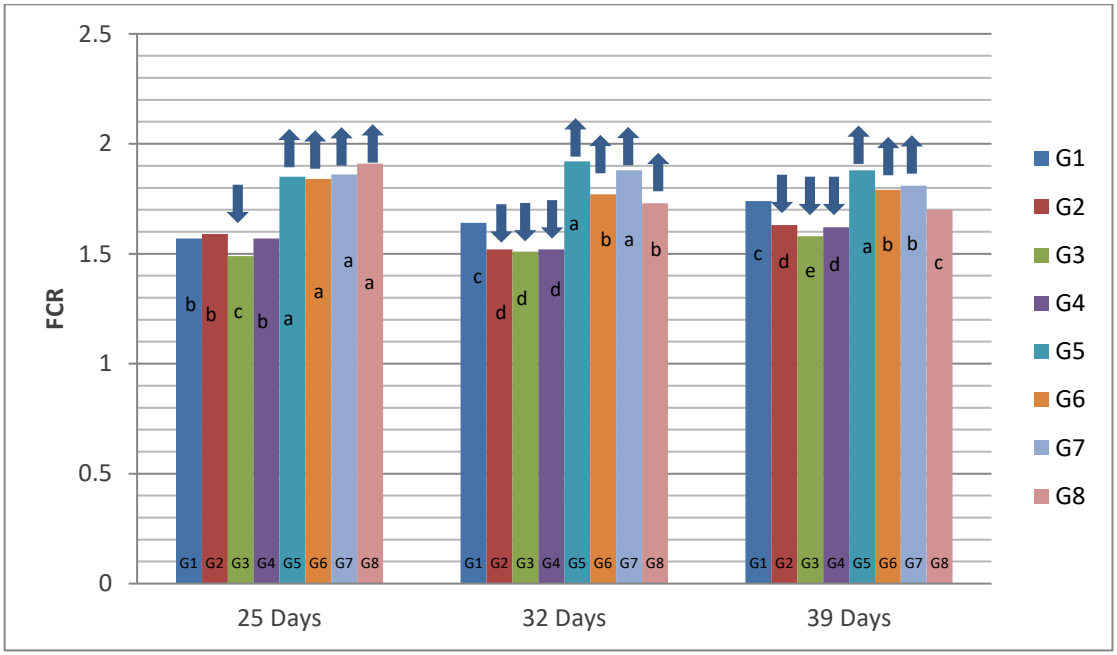


Fig. (4): Changes in **feed conversion ratio** among different groups at different ages (25, 32 and 39 days old)

⬆Significant increase - ⬇Significant decrease

III- Hematological results:

Groups (2-4) revealed non-significant changes in hemogram when compared with normal control along the experimental periods **(tabs. 6-11 and figs. 5- 16).**

1) Erythrogram:

At 25 days old, chickens in groups (5 and 7) showed a significant ($P<0.05$) decrease in RBCs count, Hb concentration and PCV with non-significant changes in MCV, MCH and MCHC when compared with the normal control group (normocytic normochromic anemia). The lowest values were recorded in gp. (5) **(table 6 and figs. 5- 10).**

At 32 days old, the results obtained from groups (5- 8) denoted a significant ($P<0.05$) decrease in RBCs count, Hb concentration, PCV and MCHC with non-significant changes in MCH. Moreover, a significant ($P<0.05$) increase was recorded in MCV in the fore-mentioned groups (macrocytic hypochromic anemia) when compared with the normal control group **(table 7 and figs. 5- 10).**

At 39 days old, chickens in groups (5 - 7) showed a significant decrease in RBCs count, Hb concentration, PCV and MCHC with insignificant changes in MCH. The lowest values were recorded in gp. (5). Moreover, a significant ($P<0.05$) increase was recorded in MCV in the fore-mentioned groups when compared with the normal control group with development of macrocytic hypochromic anemia. On the other hand, gp. (8) showed non-significant differences in RBCs count, Hb concentration, PCV, MCV, MCH and MCHC when compared with the normal control group (**table 8 and figs. 5- 10).**

Table (6): Erythrogram (mean values ± S.E) of chickens in different groups at 25 days old (N=5).

Parameters / Groups	RBCs $X10^6/\mu l$	Hb gm/dl	PCV %	MCV fl	MCH pg	MCHC %
Control Gp (1)	2.33^{a} ± 0.04	8.60^{a} ± 0.06	34.35^{a} ± 0.40	147.42^{a} ± 2.67	36.91^{a} ± 0.51	25.03^{abc} ± 0.17
Organic acids Gp (3)	2.39^{a} ± 0.07	8.70^{a} ± 0.14	34.22^{a} ± 0.78	143.18^{a} ± 1.04	36.40^{a} ± 0.47	25.42^{ab} ± 0.17
Clostridium Gp (5)	2.00^{b} ± 0.07	7.15^{c} ± 0.02	29.40^{b} ± 0.77	147.00^{a} ± 2.41	35.75^{a} ± 0.91	24.31^{c} ± 0.58
Organic acids ➡ *Cl.* Gp (7)	2.10^{b} ± 0.04	7.68^{b} ± 0.04	30.35^{b} ± 0.25	144.52^{a} ± 1.75	36.57^{a} ± 0.47	25.30^{ab} ± 0.11

Different letters in the same column indicate significant changes ($P < 0.05$).

Table (7): Erythrogram (mean values ± S.E) of chickens in different groups at 32 days old (N=5).

Parameters / Groups	RBCs X10^6/µl	Hb gm/dl	PCV %	MCV fl	MCH pg	MCHC %
Control Gp (1)	2.42 ^{ab} ± 0.04	8.88 ^{a} ± 0.03	34.65 ^{a} ± 0.22	143.32 ^{c} ± 0.73	36.69 ^{ab} ± 0.76	25.62 ^{a} ± 0.11
Amoxicillin Gp (2)	2.39 ^{b} ± 0.08	8.78 ^{a} ± 0.05	34.34 ^{a} ± 0.34	143.68 ^{c} ± 5.46	36.74 ^{ab} ± 1.12	25.57 ^{a} ± 0.33
Organic acids Gp (3)	2.40 ^{ab} ± 0.04	8.82 ^{a} ± 0.05	34.86 ^{a} ± 0.17	145.25 ^{bc} ± 1.77	36.75 ^{ab} ± 0.68	25.30 ^{a} ± 0.23
Amoxicillin+Organic Gp (4)	2.51 ^{a} ± 0.01	8.75 ^{a} ± 0.07	34.48 ^{a} ± 0.07	137.73 ^{c} ± 3.1	34.86 ^{b} ± 0.99	25.37 ^{a} ± 0.24
Clostridium Gp (5)	1.83 ^{e} ± 0.06	6.85 ^{d} ± 0.05	28.77 ^{e} ± 0.12	157.21 ^{a} ± 4.17	37.43 ^{a} ± 0.90	23.81 ^{d} ± 0.09
Cl. ➡ Amoxicillin Gp (6)	2.15 ^{c} ± 0.02	8.02 ^{c} ± 0.02	32.44 ^{c} ± 0.10	150.88 ^{b} ± 1.69	37.30 ^{a} ± 0.40	24.72 ^{b} ± 0.02
Organic acids ➡ *Cl.* Gp (7)	2.01 ^{d} ± 0.01	7.52 ^{c} ± 0.04	31.28 ^{d} ± 0.25	155.62 ^{a} ± 0.59	37.41 ^{a} ± 0.02	24.04 ^{c} ± 0.10
Cl. ➡ Amoxi. + Organic Gp (8)	2.20 ^{c} ± 0.01	8.20 ^{b} ± 0.06	33.59 ^{b} ± 0.21	152.68 ^{b} ± 1.70	37.27 ^{a} ± 0.06	24.41 ^{b} ± 0.26

Different letters in the same column indicate significant changes (P <0.05).

Table (8): Erythrogram (mean values ± S.E) of chickens in different groups at 39 days old (N=5).

Parameters / Groups	RBCs X10^6/µl	Hb gm/dl	PCV %	MCV fl	MCH pg	MCHC %
Control Gp (1)	2.54 ^{a} ± 0.054	9.14 ^{a} ± 0.037	35.55 ^{ab} ± 0.247	140.00 ^{d} ± 2.113	35.98 ^{abc} ± 0.621	25.71 ^{a} ± 0.106
Amoxicillin Gp (2)	2.50 ^{a} ± 0.071	8.92 ^{a} ± 0.027	34.75 ^{b} ± 0.165	139.00 ^{d} ± 3.537	35.68 ^{abc} ± 0.988	25.67 ^{a} ± 0.173
Organic acids Gp (3)	2.55 ^{a} ± 0.041	9.00 ^{a} ± 0.043	35.94 ^{a} ± 0.111	140.94 ^{d} ± 1.946	35.29 ^{c} ± 0.435	25.04 ^{ab} ± 0.286
Amoxicillin+Organic Gp (4)	2.51 ^{a} ± 0.037	8.92 ^{a} ± 0.184	34.96 ^{b} ± 0.296	139.28 ^{d} ± 0.918	35.54 ^{bc} ± 0.213	25.51 ^{a} ± 0.317
Clostridium Gp (5)	1.75 ^{c} ± 0.040	6.50 ^{d} ± 0.027	27.11 ^{e} ± 0.384	154.91 ^{a} ± 1.370	37.14 ^{ab} ± 0.862	23.98 ^{c} ± 0.252
Cl. ➡ Amoxicillin Gp (6)	2.30 ^{b} ± 0.043	8.35 ^{b} ± 0.052	33.81 ^{c} ± 0.256	147.00 ^{b} ± 1.923	36.30 ^{ab} ± 0.501	24.70 ^{bc} ± 0.259
Organic acids ➡ *Cl.* Gp (7)	2.15 ^{b} ± 0.026	8.00 ^{c} ± 0.077	32.05 ^{d} ± 0.234	149.07 ^{b} ± 1.193	37.21 ^{ab} ± 0.089	24.96 ^{bc} ± 0.285
Cl. ➡ Amoxi. + Organic Gp (8)	2.47 ^{a} ± 0.031	8.89 ^{a} ± 0.047	35.35 ^{ab} ± 0.276	143.12 ^{cd} ± 1.438	35.00 ^{abc} ± 0.300	25.15 ^{ab} ± 0.095

Different letters in the same column indicate significant changes (P <0.05).

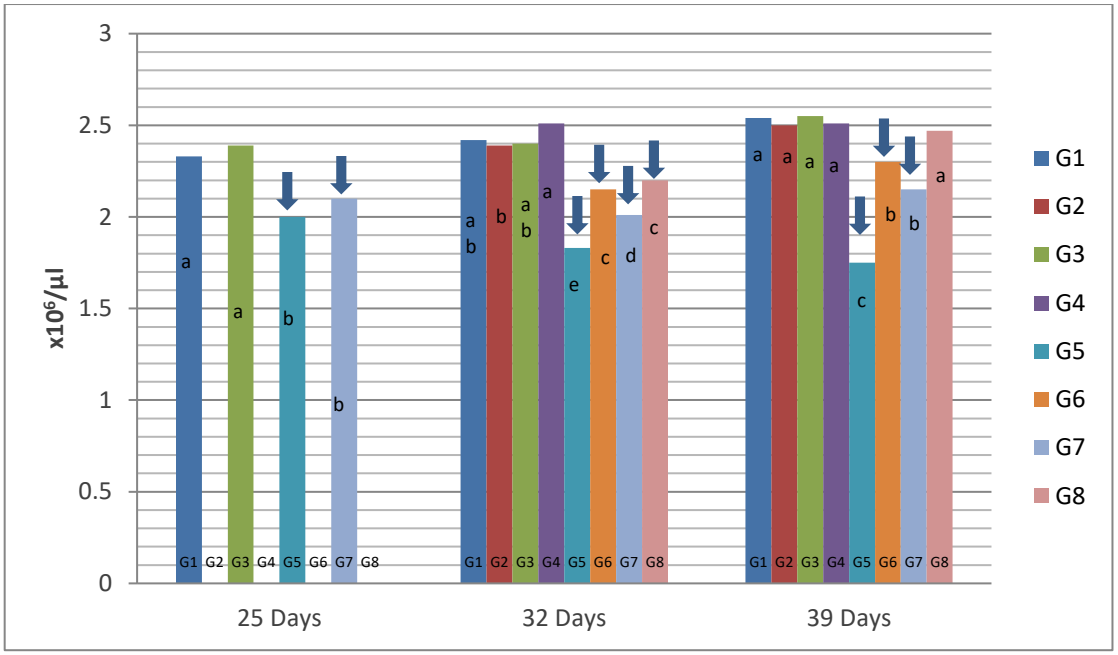


Fig. (5): Changes in **RBCs count** ($x10^6/\mu l$) among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

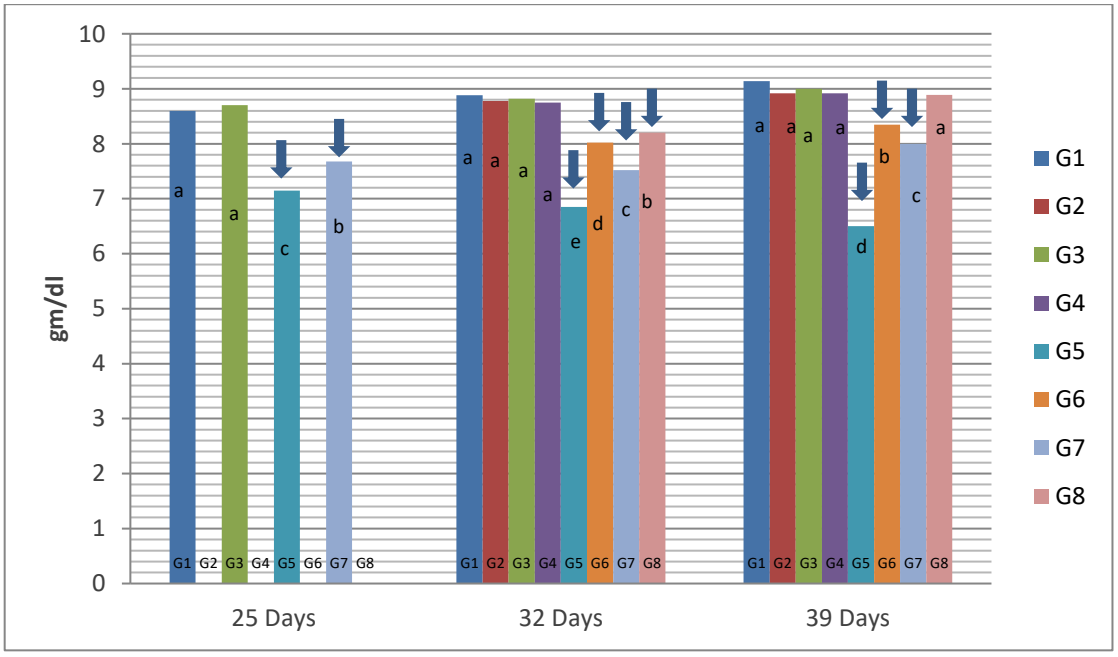


Fig. (6): Changes in **Hb levels** (gm/dl) among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

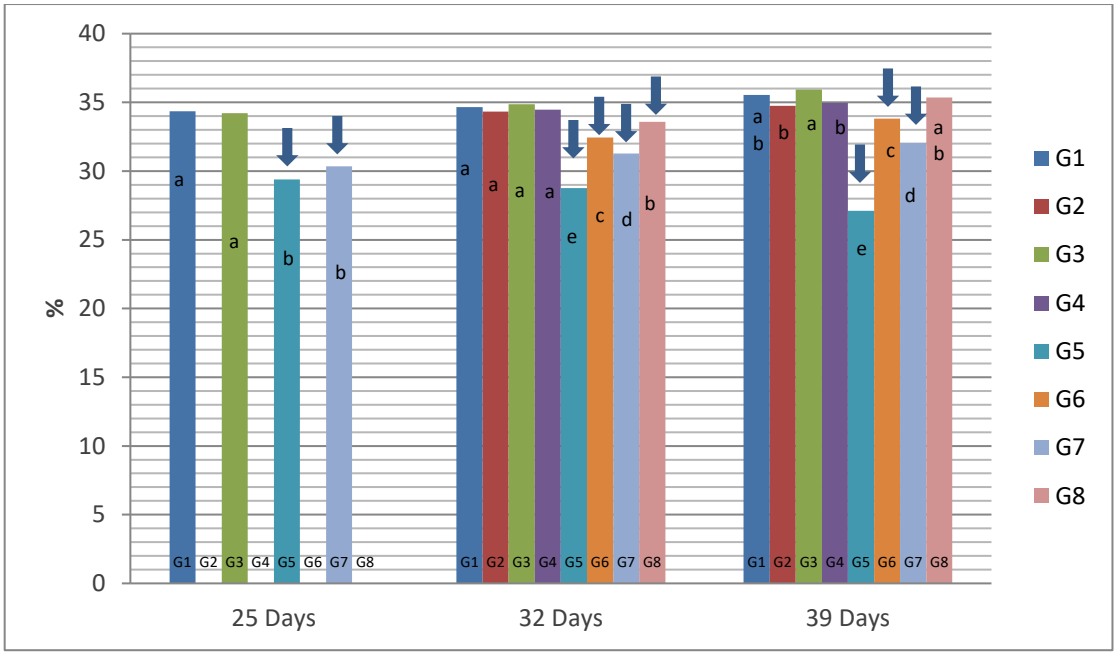


Fig. (7): Changes in **PCV** (%) among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

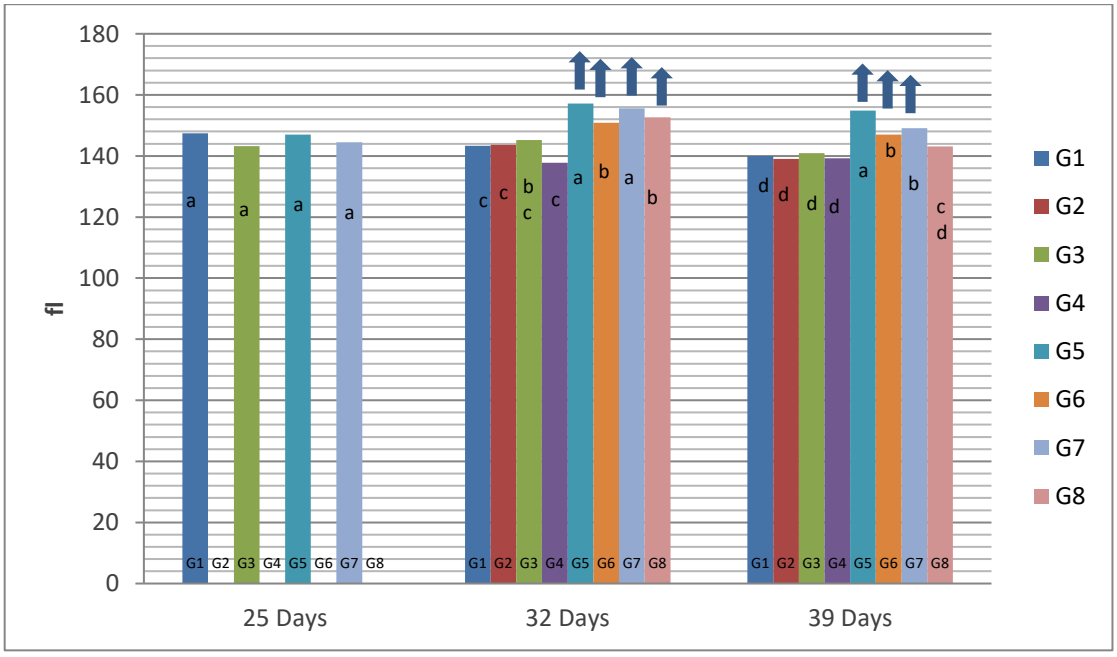


Fig. (8): Changes in **MCV** (fl) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

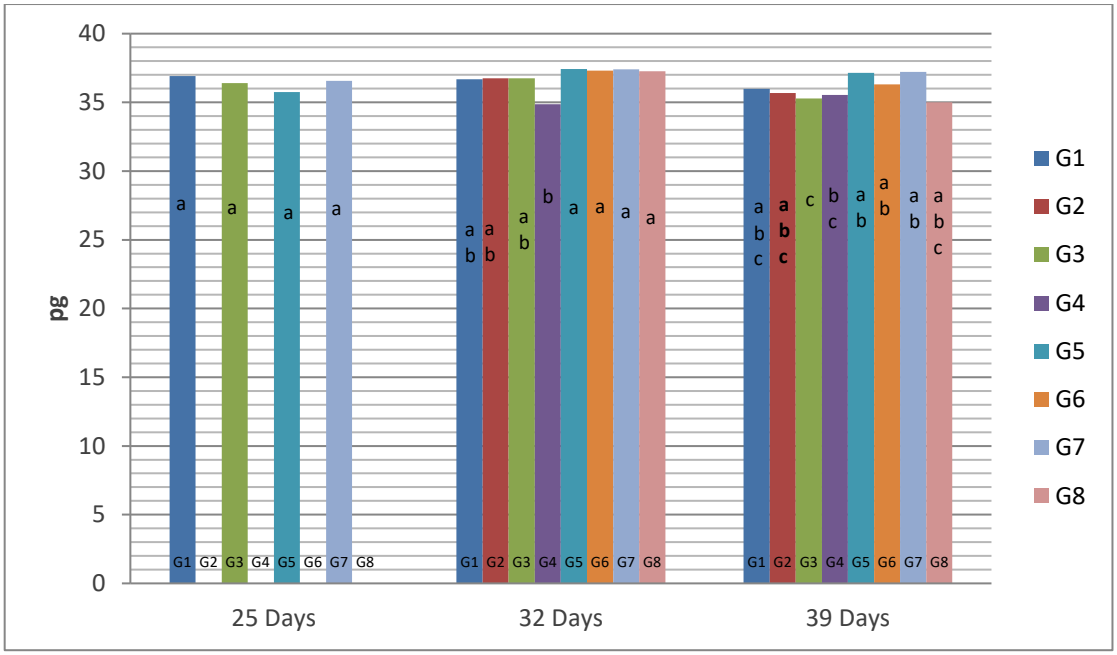


Fig. (9): Changes in **MCH** (pg) among different groups at different ages (25, 32 and 39 days old)

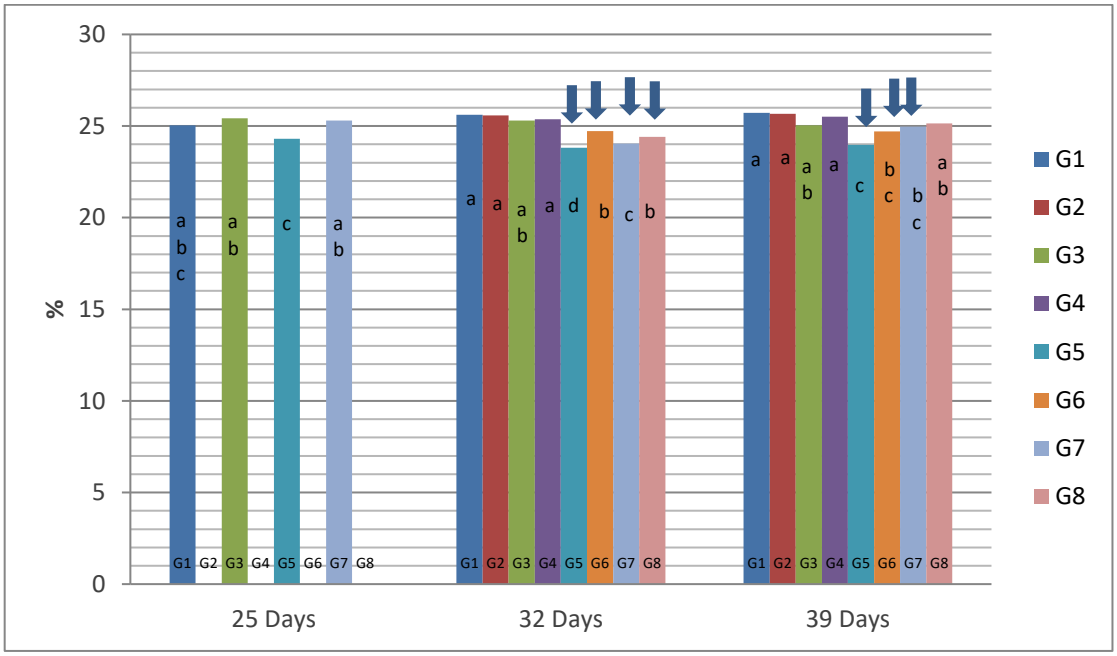


Fig. (10): Changes in **MCHC** (%) among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

Leukogram:

The leukogram revealed non-significant changes in groups (2-4) compared with normal control along the experimental periods.

At 25 days old, the results of leukogram in groups (5 and 7) revealed a significant ($P<0.05$) increase in TLC, heterophils and monocytes in addition to a significant decrease in lymphocytes. Moreover, non-significant changes in eosinophils and basophils were recorded when compared with the normal control group (**table 9 and figs. 11- 16**).

At 32 days old, groups (5-8) showed a significant ($P<0.05$) increase in TLC, heterophils and monocytes compared with normal control. On the other hand, a significant decrease in lymphocytes was recorded. Eosinophils and basophils showed non-significant changes in all groups when compared with the normal control group (**table 10 and figs. 11-16**).

At 39 days old, leukogram showed a significant ($P<0.05$) increase in TLC, heterophils and monocytes in addition to a significant decrease of lymphocytes in groups (5 - 7) when compared with normal control. Also, non-significant changes in eosinophils and basophils were reported in the same groups. The highest values of TLC, heterophils and monocytes were found in gp. (5). Moreover, group (8) showed non- significant changes in TLC, heterophils, eosinophils, lymphocytes, monocytes and basophils when compared with the normal control group (**table 11 and figs. 11-16**).

Table (9): Leukogram $x10^3/\mu l$ (mean values ± S.E) of chickens in different groups at 25 days old (N=5).

Parameters / Groups	T.L.C	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Control Gp (1)	21.09 ^{b} ± 0.07	4.43 ^{c} ± 0.04	12.79 ^{a} ± 0.05	2.20 ^{c} ± 0.01	1.08 ^{a} ± 0.01	0.59 ^{a} ± 0.01
Organic acids Gp (3)	21.15 ^{b} ± 0.43	4.35 ^{c} ± 0.24	13.00 ^{a} ± 0.22	2.21 ^{c} ± 0.01	1.09 ^{a} ± 0.01	0.60 ^{a} ± 0.01
Clostridium Gp (5)	22.25 ^{a} ± 0.43	6.98 ^{a} ± 0.22	10.80 ^{b} ± 0.38	3.21 ^{a} ± 0.01	1.08 ^{a} ± 0.01	0.58 ^{a} ± 0.01
Organic acids ➡ *Cl.* Gp (7)	22.05 ^{a} ± 0.40	6.48 ^{b} ± 0.08	10.91 ^{b} ± 0.37	2.97 ^{b} ± 0.11	1.08 ^{a} ± 0.03	0.61 ^{a} ± 0.02

Different letters in the same column indicate significant changes ($P < 0.05$).

Table (10): Leukogram $x10^3/\mu l$ (mean values ± S.E) of chickens in different groups at 32 days old (N=5).

Parameters / Groups	T.L.C	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Control Gp (1)	21.07 ^{b} ± 0.11	4.41 ^{c} ± 0.05	12.80 ^{a} ± 0.06	2.20 ^{c} ± 0.01	1.07 ^{a} ± 0.01	0.59 ^{a} ± 0.01
Amoxicillin Gp (2)	21.25 ^{b} ± 0.30	4.52 ^{c} ± 0.11	12.79 ^{a} ± 0.21	2.26 ^{c} ± 0.05	1.08 ^{a} ± 0.01	0.60 ^{a} ± 0.01
Organic acids Gp (3)	20.74 ^{b} ± 0.38	4.31 ^{c} ± 0.26	12.55 ^{a} ± 0.43	2.20 ^{c} ± 0.02	1.09 ^{a} ± 0.01	0.59 ^{a} ± 0.01
Amoxi.+Organic Gp (4)	21.15 ^{b} ± 0.14	4.43 ^{c} ± 0.06	12.82 ^{a} ± 0.08	2.21 ^{c} ± 0.01	1.09 ^{a} ± 0.02	0.60 ^{a} ± 0.02
Clostridium Gp (5)	23.25 ^{a} ± 0.55	7.10 ^{a} ± 0.20	10.54 ^{c} ± 0.36	3.95 ^{a} ± 0.10	1.08 ^{a} ± 0.02	0.58 ^{a} ± 0.02
Cl. ➡ Amoxicillin Gp (6)	23.09 ^{a} ± 0.98	6.68 ^{a} ± 0.44	11.73 ^{b} ± 0.35	3.00 ^{b} ± 0.20	1.11 ^{a} ± 0.03	0.57 ^{a} ± 0.03
Organic acids ➡ *Cl.* Gp (7)	23.15 ^{a} ± 0.80	6.92 ^{a} ± 0.36	11.68 ^{b} ± 0.32	2.85 ^{b} ± 0.11	1.10 ^{a} ± 0.02	0.60 ^{a} ± 0.01
Cl.➡ Amoxi. + Organic Gp (8)	22.55 ^{a} ± 0.19	5.86 ^{b} ± 0.05	12.25 ^{b} ± 0.11	2.77 ^{b} ± 0.08	1.08 ^{a} ± 0.01	0.59 ^{a} ± 0.01

Different letters in the same column indicate significant changes ($P < 0.05$).

Table (11): Leukogram $x10^3/\mu l$ (mean values ± S.E) of chickens in different groups at 39 days old (N=5).

Parameters / Groups	T.L.C	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Control Gp (1)	21.23 ^{c} ± 0.17	4.50 ^{c} ± 0.04	12.82 ^{a} ± 0.08	2.30 ^{c} ± 0.01	1.08 ^{a} ± 0.01	0.63 ^{ab} ± 0.02
Amoxicillin Gp (2)	21.25 ^{c} ± 0.44	4.68 ^{c} ± 0.11	12.55 ^{a} ± 0.28	2.32 ^{c} ± 0.07	1.06 ^{a} ± 0.01	0.64 ^{a} ± 0.02
Organic acids Gp (3)	21.35 ^{c} ± 0.50	4.38 ^{c} ± 0.23	12.77 ^{a} ± 0.32	2.48 ^{c} ± 0.08	1.07 ^{a} ± 0.01	0.65 ^{a} ± 0.004
Amoxi. + Organic Gp (4)	21.67 ^{c} ± 0.29	4.66 ^{c} ± 0.11	12.83 ^{a} ± 0.07	2.46 ^{c} ± 0.11	1.08 ^{a} ± 0.01	0.64 ^{a} ± 0.01
Clostridium Gp (5)	23.43 ^{a} ± 0.15	7.28 ^{a} ± 0.41	10.86 ^{c} ± 0.16	3.57 ^{a} ± 0.14	1.09 ^{a} ± 0.01	0.63 ^{ab} ± 0.01
Cl. ➡ Amoxicillin Gp (6)	22.34 ^{b} ± 0.46	5.80 ^{b} ± 0.19	11.82 ^{b} ± 0.19	3.04 ^{b} ± 0.11	1.08 ^{a} ± 0.004	0.60 ^{b} ± 0.01
Organic acids ➡ *Cl.* Gp (7)	22.89 ^{b} ± 0.23	6.40 ^{b} ± 0.35	11.74 ^{b} ± 0.26	3.06 ^{b} ± 0.04	1.09 ^{a} ± 0.01	0.60 ^{b} ± 0.01
Cl.➡ Amoxi. + Organic Gp (8)	21.45 ^{c} ± 0.43	5.00 ^{c} ± 0.26	12.45 ^{a} ± 0.20	2.31 ^{c} ± 0.07	1.09 ^{a} ± 0.01	0.60 ^{b} ± 0.01

Different letters in the same column indicate significant changes ($P < 0.05$).

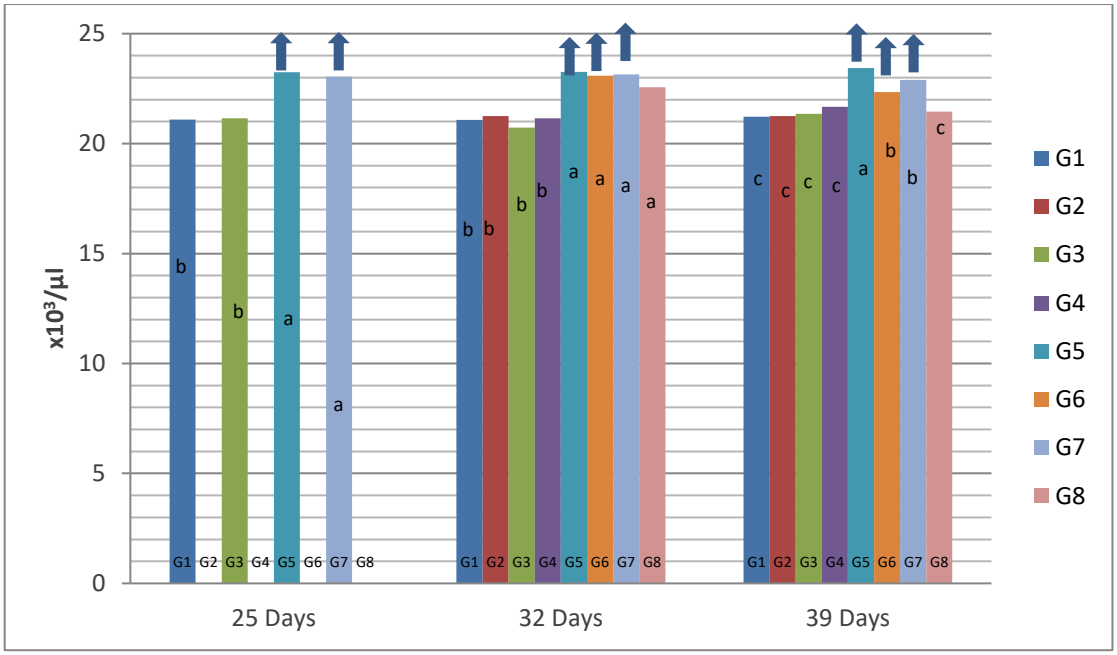


Fig. (11): Changes in **TLC** ($x10^3/\mu l$) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

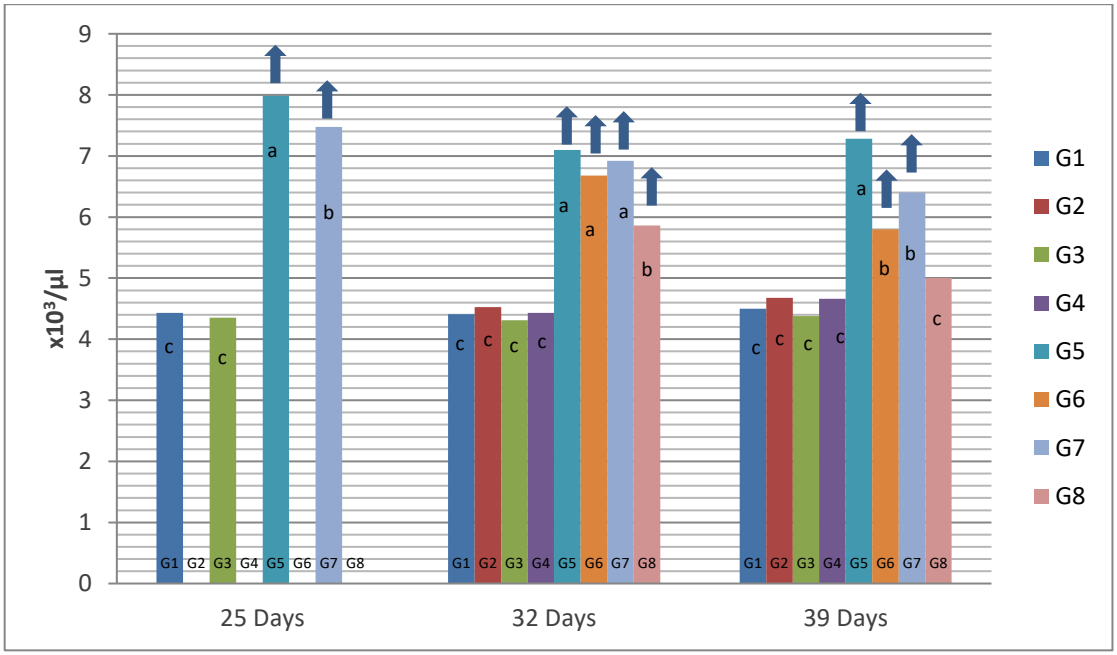


Fig. (12): Changes in **heterophils** ($x10^3/\mu l$) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

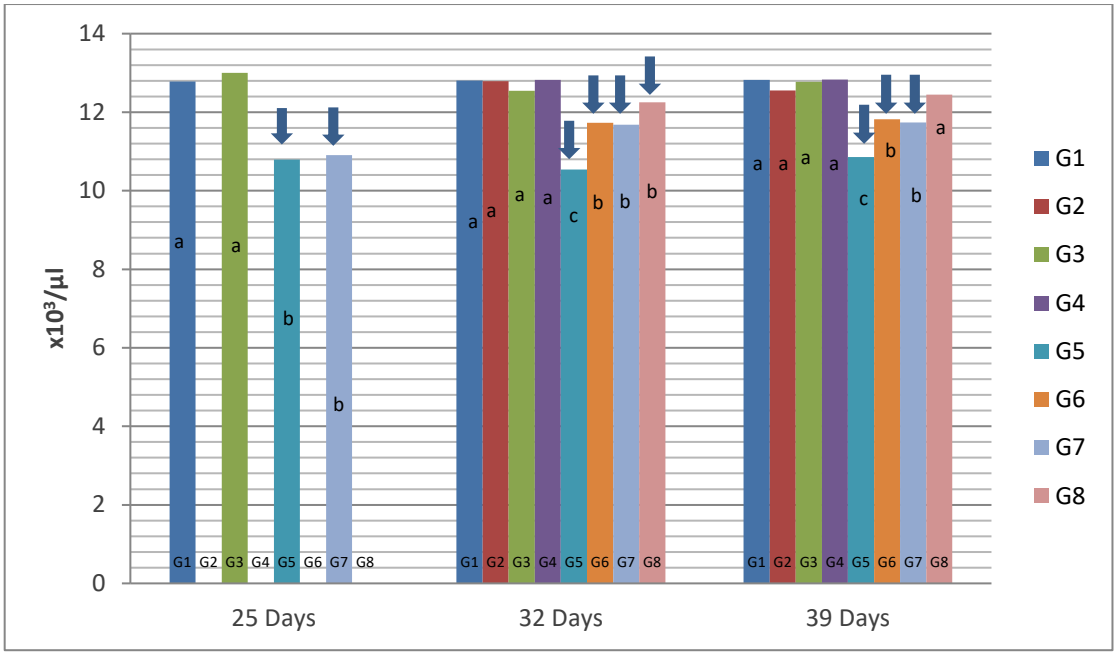


Fig. (13): Changes in **lymphocytes** ($x10^3/\mu l$) among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

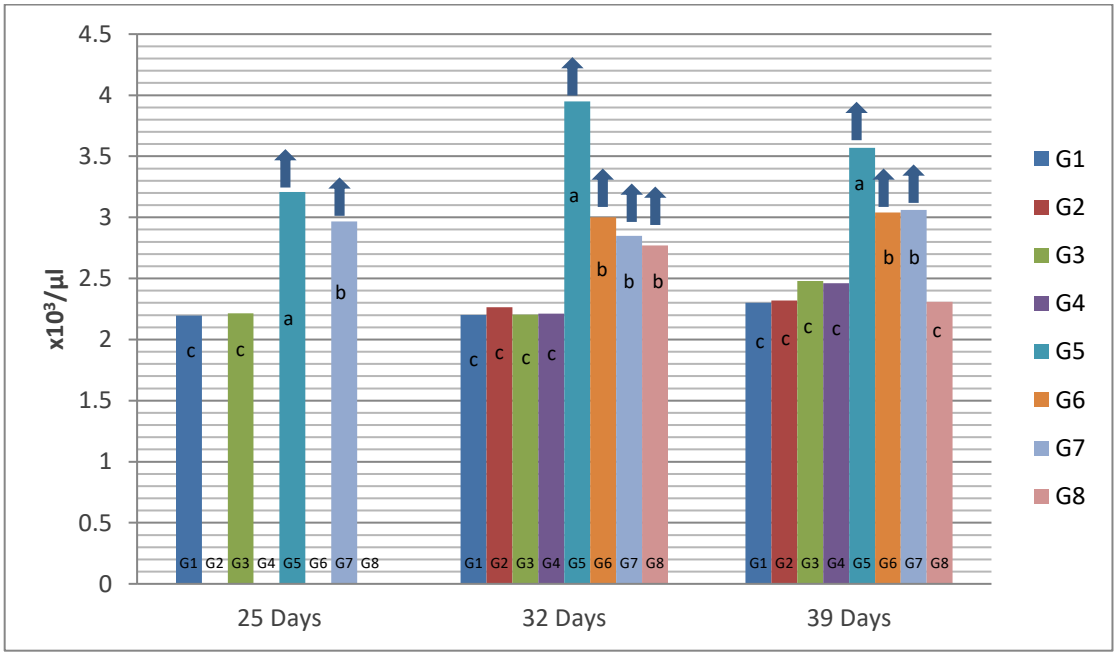


Fig. (14): Changes in **monocytes** ($x10^3/\mu l$) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

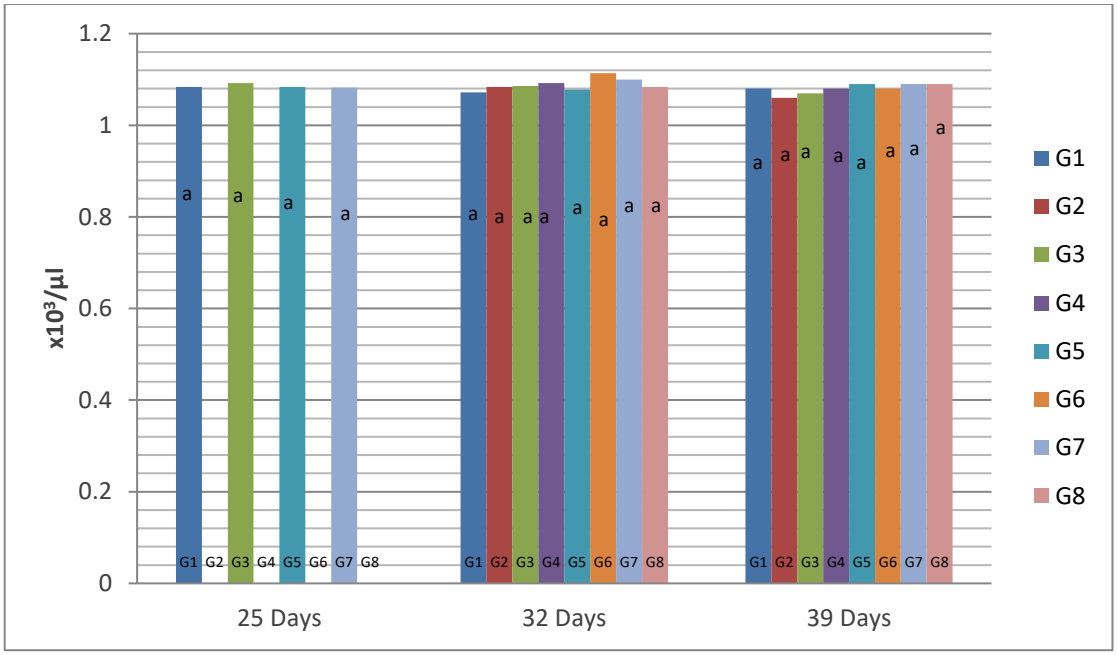


Fig. (15): Changes in **eosinophils** ($x10^3/\mu l$) among different groups at different ages (25, 32 and 39 days old)

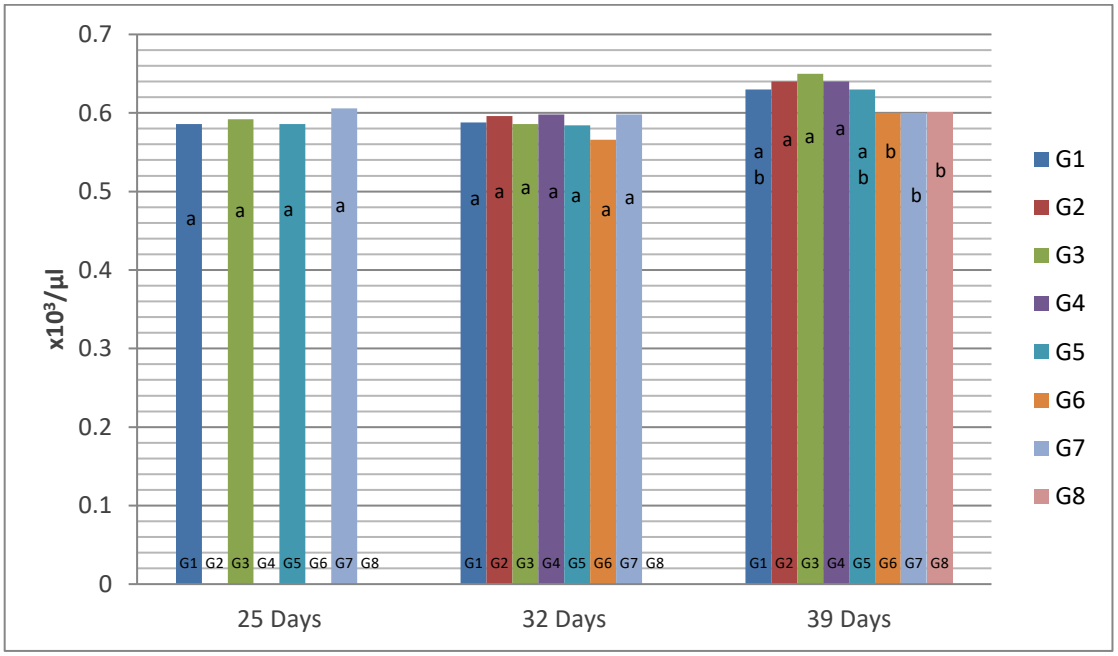


Fig. (16): Changes in **basophils** ($x10^3/\mu l$) among different groups at different ages (25, 32 and 39 days old)

IV- Biochemical results:

1) Proteinogram:

Regarding to proteinogram, groups (2-4) revealed non-significant changes when compared with normal control along the experimental periods.

At 25 days old, electrophoresis in groups (5 and 7) revealed a significant increase in serum levels of γ and total globulins with a significant decrease in total proteins and albumin when compared with normal control. Moreover, non- significant changes in serum levels of α and β-globulins was recorded (**table 12 and figs. 17- 22**).

At 32 days old, groups (5- 8) revealed a significant increase in serum levels of γ and total globulins with a significant decrease in total proteins and albumin when compared with normal control. The lowest values in total proteins and albumin were recorded in the 5^{th} group. On the other hand, non-significant changes in serum levels of α and β-globulins when compared with the normal control was noticed (**table 13 and figs. 17-22**).

At 39 days old, groups (5 - 7) showed a significant ($P<0.05$) increase in serum levels of γ-globulin and total globulins with a significant decrease in total proteins and albumin when compared with normal control. Non- significant change in serum levels of α and β-globulin was recorded. Moreover, these parameters showed non-significant changes except γ and total globulins showed a significant increase in group (8) when compared with the control group (**table 14 and figs. 17- 22**).

Table (12): Proteinogram (mean values ± S.E) of chickens in different groups at 25 days old (N=5).

Parameters / Groups	Total proteins gm/ dl	Albumin gm/ dl	Total globulins gm/ dl	α- globulin gm/ dl	β- globulin gm/ dl	γ- globulin gm/ dl
Control Gp (1)	4.29 ^{a} ± 0.02	1.45 ^{a} ± 0.01	2.84 ^{b} ± 0.02	0.84 ^{a} ± 0.01	0.83 ^{a} ± 0.02	1.17 ^{b} ± 0.01
Organic acids Gp (3)	4.33 ^{a} ± 0.04	1.49 ^{a} ± 0.01	2.84 ^{b} ± 0.04	0.83 ^{a} ± 0.02	0.87 ^{a} ± 0.01	1.14 ^{b} ± 0.01
Clostridium Gp (5)	4.07 ^{b} ± 0.02	1.10 ^{c} ± 0.01	2.97 ^{a} ± 0.03	0.87 ^{a} ± 0.03	0.84 ^{a} ± 0.01	1.26 ^{a} ± 0.03
Organic acids ➡ *Cl.* Gp (7)	4.09 ^{b} ± 0.02	1.16 ^{b} ± 0.02	2.93 ^{a} ± 0.01	0.85 ^{a} ± 0.01	0.83 ^{a} ± 0.01	1.25 ^{a} ± 0.01

Different letters in the same column indicate significant changes (P <0.05).

Table (13): Proteinogram (mean values ± S.E) of chickens in different groups at 32 days old (N=5).

Parameters / Groups	Total proteins gm/ dl	Albumin gm/ dl	Total globulins gm/ dl	α- globulin gm/ dl	β- globulin gm/ dl	γ- globulin gm/ dl
Control Gp (1)	4.26 ^{ab} ± 0.02	1.44 ^{a} ± 0.01	2.82 ^{c} ± 0.01	0.84 ^{ab} ± 0.01	0.84 ^{abc} ± 0.01	1.14 ^{b} ± 0.01
Amoxicillin Gp (2)	4.24 ^{ab} ± 0.02	1.45 ^{a} ± 0.01	2.79 ^{cd} ± 0.02	0.85 ^{ab} ± 0.01	0.82 ^{abc} ± 0.01	1.12 ^{b} ± 0.01
Organic acids Gp (3)	4.30 ^{a} ± 0.04	1.46 ^{a} ± 0.02	2.84 ^{bc} ± 0.04	0.84 ^{ab} ± 0.01	0.82 ^{abc} ± 0.01	1.18 ^{b} ± 0.04
Amoxi.+Organic Gp (4)	4.32 ^{a} ± 0.06	1.48 ^{a} ± 0.02	2.84 ^{bc} ± 0.04	0.82 ^{b} ± 0.01	0.86 ^{a} ± 0.02	1.16 ^{b} ± 0.03
Clostridium Gp (5)	3.96 ^{d} ± 0.03	1.01 ^{d} ± 0.01	2.95 ^{ab} ± 0.03	0.84 ^{ab} ± 0.01	0.81 ^{bc} ± 0.02	1.30 ^{a} ± 0.01
Cl.➡ Amoxicillin Gp (6)	4.08 ^{c} ± 0.03	1.10 ^{c} ± 0.01	2.98 ^{a} ± 0.02	0.87 ^{a} ± 0.02	0.86 ^{a} ± 0.01	1.25 ^{a} ± 0.004
Organic acids➡*Cl.* Gp (7)	4.16 ^{c} ± 0.01	1.22 ^{b} ± 0.01	2.94 ^{ab} ± 0.006	0.82 ^{b} ± 0.01	0.85 ^{ab} ± 0.02	1.27 ^{a} ± 0.01
Cl.➡ Amoxi. + Organic Gp (8)	4.19 ^{c} ± 0.01	1.24 ^{b} ± 0.01	2.95 ^{ab} ± 0.005	0.84 ^{ab} ± 0.01	0.86 ^{a} ± 0.01	1.25 ^{a} ± 0.004

Different letters in the same column indicate significant changes ($P < 0.05$).

Table (14): Proteinogram (mean values ± S.E) of chickens in different groups at 39 days old (N=5).

Parameters / Groups	Total proteins gm/ dl	Albumin gm/ dl	Total globulins gm/ dl	α- globulin gm/ dl	β- globulin gm/ dl	γ- globulin gm/ dl
Control Gp (1)	4.49 ^{a} ± 0.01	1.55 ^{a} ± 0.02	2.94 ^{c} ± 0.01	0.92 ^{a} ± 0.004	0.84 ^{abc} ± 0.01	1.18 ^{c} ± 0.01
Amoxicillin Gp (2)	4.53 ^{a} ± 0.02	1.56 ^{a} ± 0.02	2.97 ^{c} ± 0.01	0.91 ^{a} ± 0.02	0.86 ^{ab} ± 0.02	1.20 ^{c} ± 0.01
Organic acids Gp (3)	4.52 ^{a} ± 0.01	1.53 ^{a} ± 0.02	2.98 ^{c} ± 0.01	0.92 ^{a} ± 0.01	0.84 ^{abc} ± 0.01	1.22 ^{c} ± 0.02
Amoxi.+Organic Gp (4)	4.51 ^{a} ± 0.01	1.55 ^{a} ± 0.01	2.96 ^{c} ± 0.01	0.92 ^{a} ± 0.01	0.83 ^{bc} ± 0.01	1.21 ^{c} ± 0.01
Clostridium Gp (5)	4.12 ^{d} ± 0.02	0.90 ^{d} ± 0.02	3.22 ^{a} ± 0.02	0.91 ^{a} ± 0.01	0.87 ^{a} ± 0.01	1.44 ^{a} ± 0.01
Cl. ➡ Amoxicillin Gp (6)	4.41 ^{b} ± 0.01	1.40 ^{b} ± 0.01	3.01 ^{b} ± 0.01	0.92 ^{a} ± 0.003	0.82 ^{c} ± 0.01	1.27 ^{b} ± 0.003
Organic acids ➡ *Cl.* Gp (7)	4.35 ^{c} ± 0.02	1.29 ^{c} ± 0.02	3.06 ^{b} ± 0.01	0.93 ^{a} ± 0.004	0.81 ^{c} ± 0.01	1.32 ^{b} ± 0.01
Cl. ➡ Amoxi. + Organic Gp (8)	4.57 ^{a} ± 0.01	1.56 ^{a} ± 0.01	3.01 ^{b} ± 0.01	0.93 ^{a} ± 0.003	0.83 ^{bc} ± 0.01	1.25 ^{b} ± 0.01

Different letters in the same column indicate significant changes ($P < 0.05$).

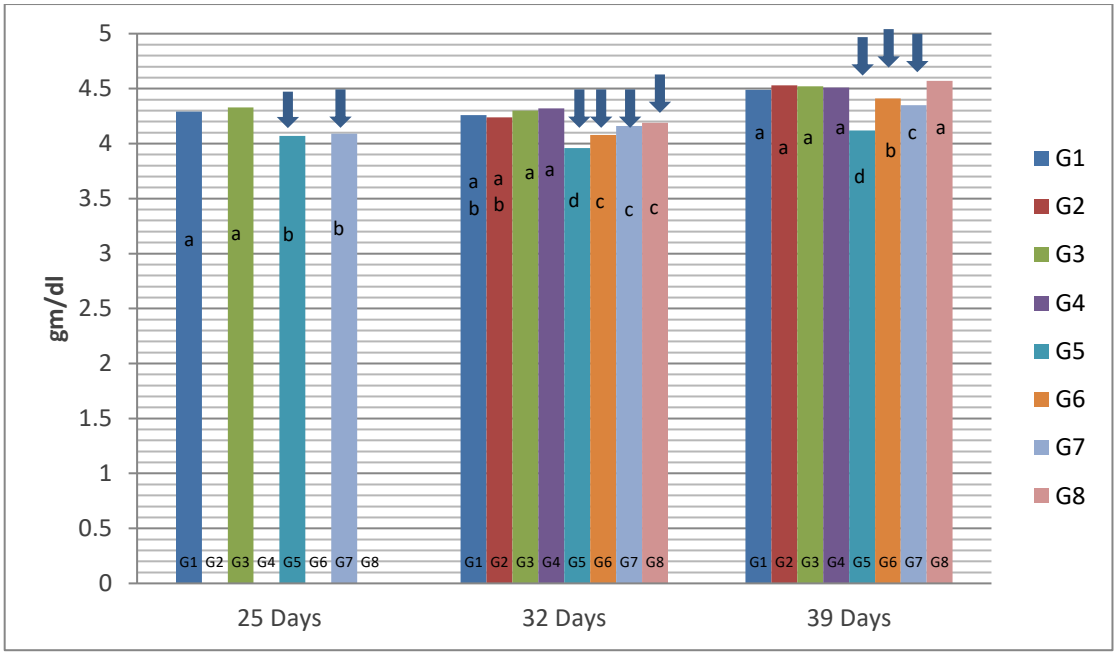


Fig. (17): Changes in **total proteins** (gm/dl) among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

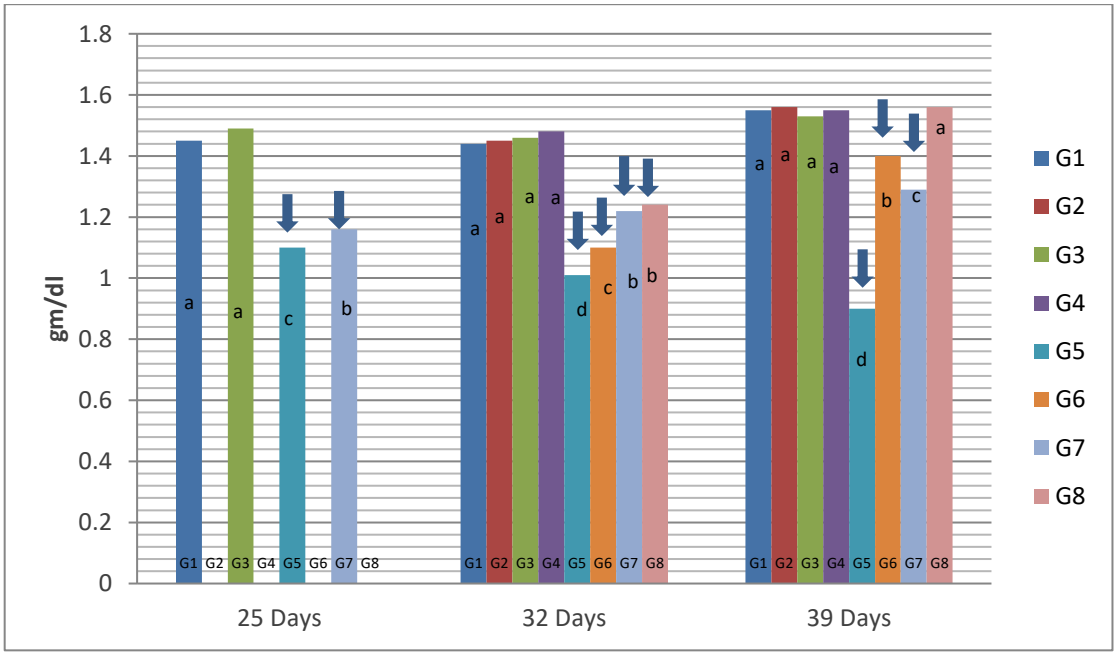


Fig. (18): Changes in **albumin levels** (gm/dl) among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

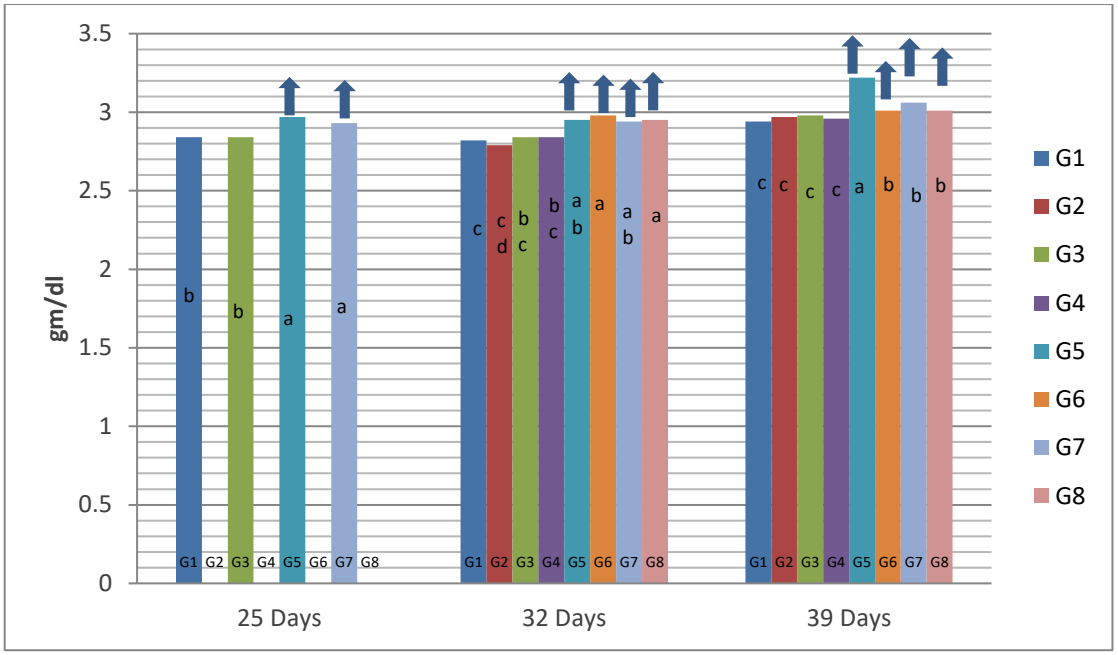


Fig. (19): Changes in **total globulins** (gm/dl) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

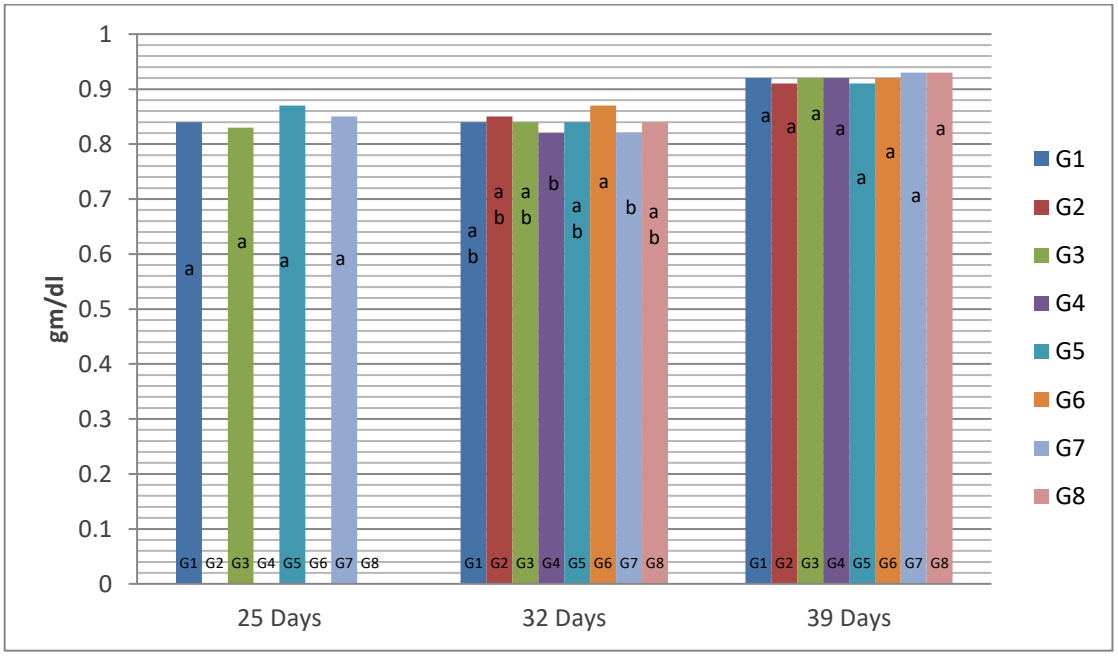


Fig. (20): Changes in ***α*- globulin** (gm/dl) among different groups at different ages (25, 32 and 39 days old)

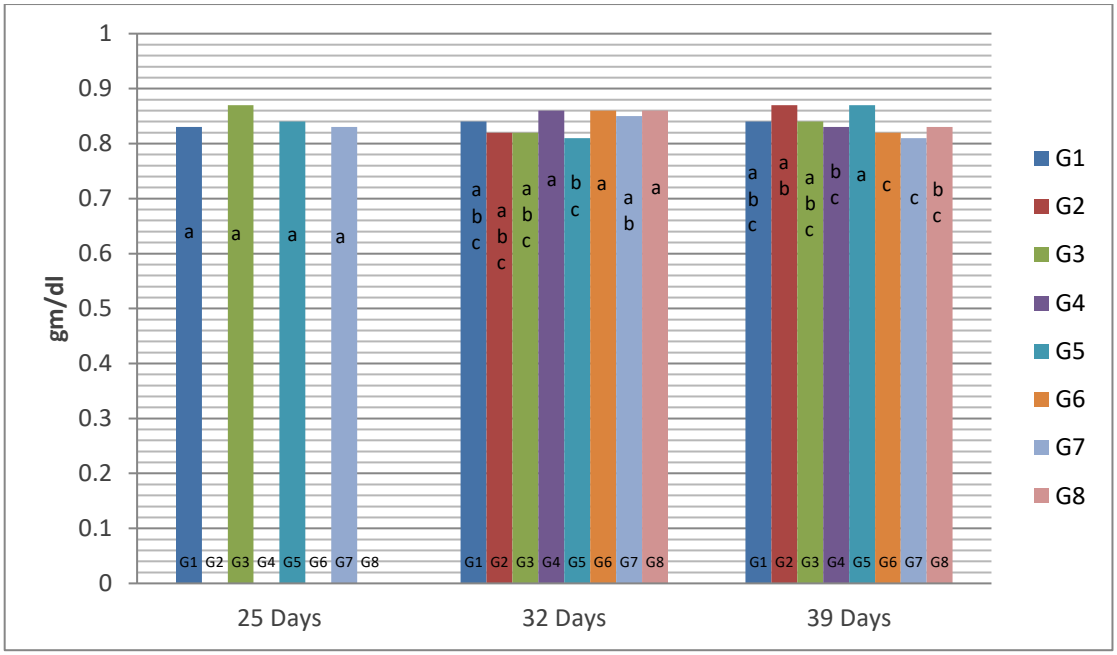


Fig. (21): Changes in **β-globulin** (gm/dl) among different groups at different ages (25, 32 and 39 days old)

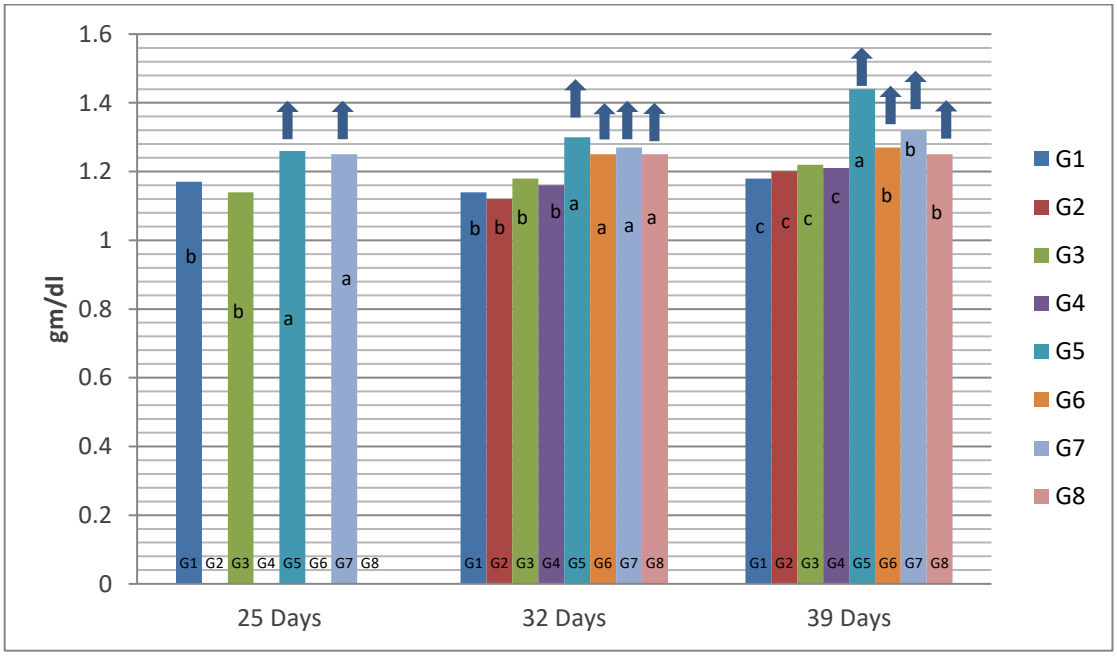


Fig. (22): Changes in **γ- globulin** (gm/dl) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

2) Some liver function tests:

Groups (2 - 4) showed non-significant changes in serum activities of AST, ALT and ALP when compared with the normal control group along the experimental periods except gp. (2) showed a significant transient increase in these enzymes activities (AST, ALT and ALP) at 32 days old only.

At 25 and 32 days old, the obtained data revealed that chickens in groups (5 - 8) had a significant ($P<0.05$) increase in the serum activities of AST, ALT and ALP when compared with the normal control group. The highest values were recorded in the 5^{th} group. On the other hand, groups (6 - 8) showed a significant decrease in the fore-mentioned parameters compared with infected non-treated group at 32 days old only (**table 15 and figs. 23- 25).**

At 39 days old, groups (5 - 7) showed a significant increase in serum activities of AST, ALT and ALP when compared with normal control. Group (8) showed non- significant changes when compared with the normal control group. Moreover, groups (6 - 8) showed a significant decrease in serum activities of these enzymes compared with infected non-treated group (**table 15 and figs. 23- 25).**

Table (15): Some liver enzymes (mean values ± S.E) of chickens in different groups along the experimental periods (N=5).

Parameters / Groups	25 days old			32 days old			39 days old		
	AST U/L	**ALT U/L**	**ALP IU/L**	**AST U/L**	**ALT U/L**	**ALP IU/L**	**AST U/L**	**ALT U/L**	**ALP IU/L**
Control Gp (1)	50.20 ^{c} ± 1.16	8.70 ^{c} ± 0.31	50.26 ^{c} ± 0.34	48.60 ^{e} ± 1.72	9.22 ^{e} ± 0.20	52.14 ^{f} ± 0.19	50.80 ^{d} ± 1.02	9.16 ^{d} ± 0.27	52.62 ^{d} ± 0.24
Amoxicillin Gp (2)	48.20 ^{c} ± 1.43	8.84 ^{c} ± 0.35	49.64 ^{c} ± 0.35	58.40 ^{d} ± 1.44	12.30 ^{d} ± 0.14	59.72 ^{e} ± 0.26	52.00 ^{d} ± 0.81	9.52 ^{d} ± 0.16	53.04 ^{d} ± 1.23
Organic acids Gp (3)	48.20 ^{c} ± 3.48	8.50 ^{c} ± 0.23	50.46 ^{c} ± 0.55	49.40 ^{e} ± 1.36	9.30 ^{e} ± 0.20	52.32 ^{f} ± 0.24	49.00 ^{d} ± 1.23	9.28 ^{d} ± 0.16	53.02 ^{d} ± 0.24
Amoxi.+Organic Gp (4)	50.60 ^{c} ± 1.44	8.66 ^{c} ± 0.17	49.10 ^{c} ± 0.82	50.00 ^{e} ± 2.72	9.76 ^{e} ± 0.29	53.24 ^{f} ± 0.32	51.20 ^{d} ± 0.58	9.60 ^{d} ± 0.14	52.36 ^{d} ± 0.28
Clostridium Gp (5)	80.40 ^{a} ± 2.08	19.42 ^{a} ± 0.23	87.86 ^{a} ± 0.45	85.00 ^{a} ± 2.00	20.16 ^{a} ± 0.46	90.76 ^{a} ± 0.22	88.00 ^{a} ± 1.84	20.68 ^{a} ± 0.30	90.48 ^{a} ± 1.54
Cl. ➡ Amoxicillin Gp (6)	80.80 ^{a} ± 3.18	19.44 ^{a} ± 0.24	88.72 ^{a} ± 0.26	70.60 ^{bc} ± 2.50	15.94 ^{b} ± 0.25	70.88 ^{c} ± 0.86	58.80 ^{c} ± 0.86	11.20 ^{c} ± 0.22	60.56 ^{c} ± 1.60
Organic acids➡*Cl.* Gp (7)	72.00 ^{b} ± 2.12	14.70 ^{b} ± 0.19	77.12 ^{b} ± 0.25	72.00 ^{b} ± 1.14	16.62 ^{b} ± 0.21	74.74 ^{b} ± 0.16	62.40 ^{b} ± 1.63	13.38 ^{b} ± 0.18	65.30 ^{b} ± 1.31
Cl. ➡ Amoxi. + Organic Gp (8)	80. 00 ^{a} ± 3.70	19.38 ^{a} ± 0.16	87.48 ^{a} ± 0.30	65.00 ^{c} ± 2.28	15.16 ^{c} ± 0.14	62.24 ^{d} ± 1.21	53.40 ^{d} ± 1.08	9.84 ^{d} ± 0.32	53.94 ^{d} ± 1.05

Different letters in the same column indicate significant changes ($P < 0.05$).

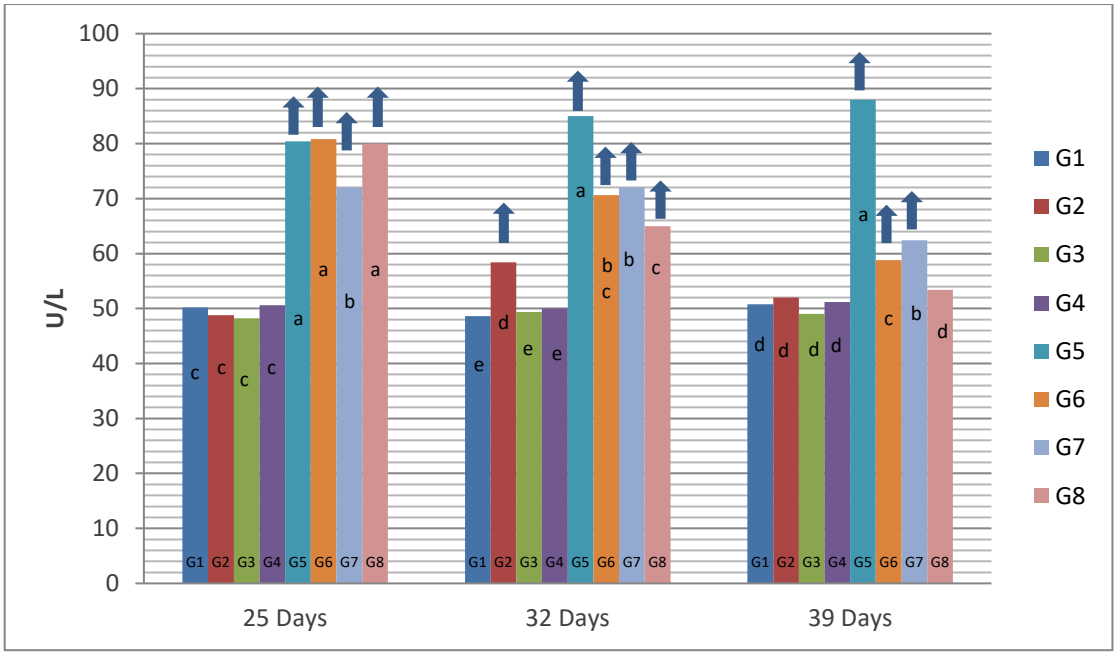


Fig. (23): Changes in serum **AST** (U/L) among different groups at different ages (25, 32 and 39 days old)

- ↑ Significant increase

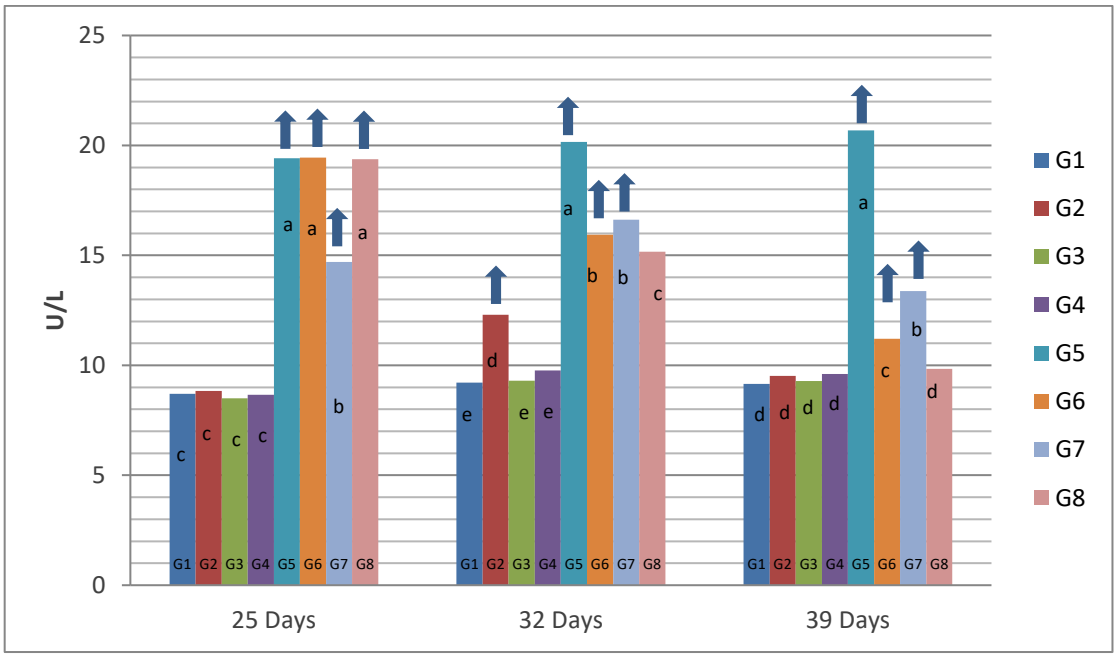


Fig. (24): Changes in serum **ALT** (U/L) among different groups at different ages (25, 32 and 39 days old)

- ↑ Significant increase

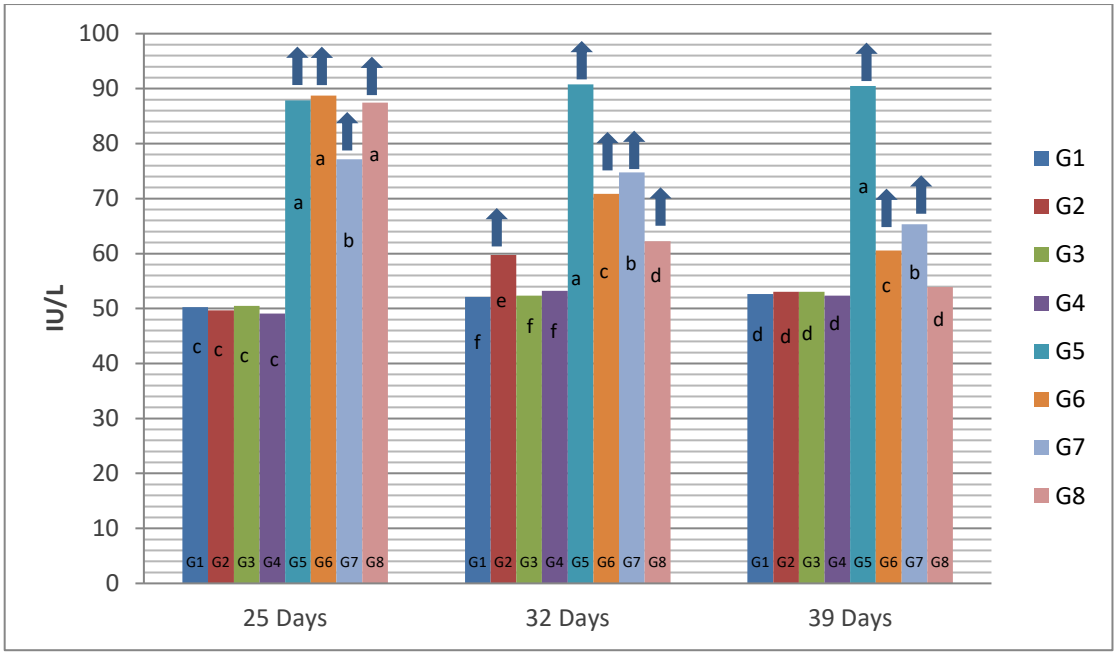


Fig. (25): Changes in serum **ALP** (IU/L) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

3) Some kidney function tests:

Groups (2 - 4) showed non-significant changes in all examined biochemical parameters when compared with the normal control group along the experimental periods.

At 25 and 32 days old, the obtained data revealed that chickens in groups (5 - 8) had a significant ($P<0.05$) increase in the serum levels of uric acid and creatinine when compared with the normal control group. The highest values were recorded in the 5th group. On the other hand, groups (6 - 8) showed a significant decrease in the fore-mentioned parameters compared with infected non-treated group at 32 days old (**table 16 and figs. 26 and 27).**

At 39 days old, groups (5 - 7) showed a significant increase in serum levels of uric acid and creatinine when compared with normal control. Group (8) showed non- significant changes when compared with the normal control group. Moreover, groups (6 - 8) showed a significant decrease in serum levels of uric acid and creatinine compared with infected non-treated group (**table 16 and figs. 26 and 27).**

Table (16): Some kidney function tests (mean values ± S.E) of chickens in different groups along the experimental periods (N=5).

Parameters / Groups	25 days old		32 days old		39 days old	
	Uric Acid mg/dl	**Creatinine mg/dl**	**Uric Acid mg/dl**	**Creatinine mg/dl**	**Uric Acid mg/dl**	**Creatinine mg/dl**
Control Gp (1)	4.50 ^{c} ± 0.39	0.79 ^{c} ± 0.02	4.80 ^{c} ± 0.26	0.80 ^{d} ± 0.02	5.20 ^{c} ± 0.18	0.83 ^{c} ± .010
Amoxicillin Gp (2)	4.30 ^{c} ± 0.25	0.81 ^{c} ± 0.02	4.70 ^{c} ± 0.36	0.82 ^{d} ± 0.03	5.60 ^{c} ± 0.34	0.89 ^{c} ± 0.04
Organic acids Gp (3)	4.40 ^{c} ± 0.35	0.78 ^{c} ± 0.01	4.90 ^{c} ± 0.28	0.79 ^{d} ± 0.02	5.10 ^{c} ± 0.32	0.82 ^{c} ± 0.02
Amoxi.+Organic Gp (4)	4.70 ^{c} ± 0.184	0. 80 ^{c} ± 0.014	4.50 ^{c} ± 0.30	0.84 ^{d} ± 0.04	5.30 ^{c} ± 0.14	0.90 ^{c} ± 0.08
Clostridium Gp (5)	8.30 ^{a} ± 0.61	1.50 ^{a} ± 0.14	10.50 ^{a} ± 0.57	1.80 ^{a} ± 0.16	11.40 ^{a} ± 0.42	1.96 ^{a} ± 0.12
Cl.➡ Amoxicillin Gp (6)	8.20 ^{a} ± 0.449	1.43 ^{a} ± 0.02	7.40 ^{b} ± 0.27	1.10 ^{c} ± 0.04	6.50 ^{b} ± 0.14	1.15 ^{b} ± 0.03
Organic acids➡*Cl.* Gp (7)	7.40 ^{b} ± 0.17	1.07 ^{b} ± 0.02	7.60 ^{b} ± 0.21	1.30 ^{b} ± 0.07	6.92 ^{b} ± 0.31	1.25 ^{b} ± 0.07
Cl.➡ Amoxi. + Organic Gp (8)	8.15 ^{a} ± 0.316	1.47 ^{a} ± 0.03	6.70 ^{b} ± 0.22	1.01 ^{c} ± 0.02	5.50 ^{c} ± 0.21	0.86 ^{c} ± 0.05

Different letters in the same column indicate significant changes ($P < 0.05$).

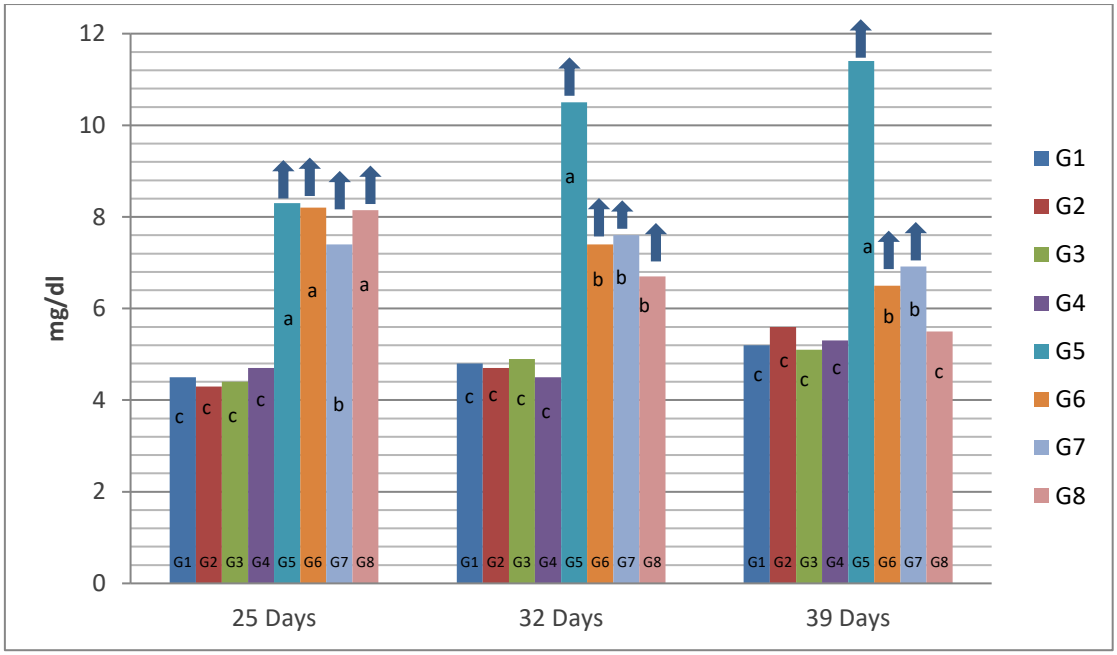


Fig. (26): Changes in serum **uric acid level** (mg/dl) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

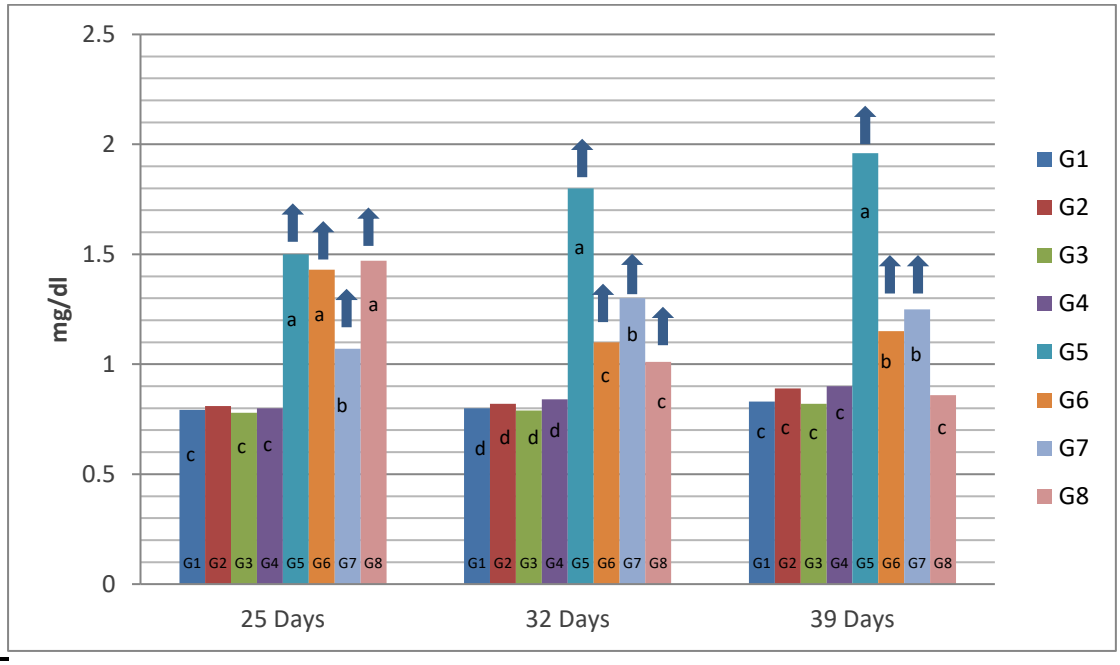


Fig. (27): Changes in serum **creatinine level** (mg/dl) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

4) Some electrolytes:

Groups (2 - 4) showed non-significant changes in all examined biochemical parameters when compared with the normal control group along the experimental periods.

At 25 and 32 days old, the obtained data revealed that chickens in groups (5 - 8) had a significant ($P<0.05$) increase in the serum level of potassium with a significant ($P<0.05$) decrease in sodium level when compared with the normal control group. The highest value of serum level of potassium and the lowest value of serum sodium were recorded in the 5^{th} group. On the other hand, groups (6 - 8) showed a significant decrease in serum level of potassium with a significant increase in serum sodium level when compared with infected non-treated group at 32 days old (**table 17 and figs. 28 and 29).**

At 39 days old, groups (5 - 7) showed a significant increase in serum level of potassium with a significant decrease in serum sodium level when compared with normal control. Group (8) showed non- significant changes when compared with the normal control group. Moreover, groups (6 - 8) showed a significant decrease in serum level of potassium with a significant increase in serum sodium level when compared with infected non-treated group (**table 17 and figs. 28 and 29).**

Table (17): Some electrolytes (mean values ± S.E) of chickens in different groups along the experimental periods (N=5).

Parameters / Groups	25 days old		32 days old		39 days old	
	Sodium m.Eq/L	**Potassium m.Eq /L**	**Sodium m.Eq/L**	**Potassium m.Eq /L**	**Sodium m.Eq/L**	**Potassium m.Eq /L**
Control Gp (1)	130.40 ^{a} ± 1.63	3.64 ^{c} ± 0.09	133.60 ^{a} ± 1.21	3.84 ^{de} ± 0.09	135.80 ^{ab} ± 1.28	3.88 ^{c} ± 0.11
Amoxicillin Gp (2)	130.20 ^{a} ± 1.356	3.68 ^{c} ± 0.11	131.00 ^{a} ± 0.95	3.92 ^{cd} ± 0.09	134.40 ^{ab} ± 0.93	3.92 ^{c} ± 0.08
Organic acids Gp (3)	131.20 ^{a} ± 1.07	3.74 ^{c} ± 0.09	133.80 ^{a} ± 1.46	3.64 ^{e} ± 0.07	135.20 ^{ab} ± 1.07	3.76 ^{cd} ± 0.10
Amoxi.+Organic Gp (4)	131.00 ^{a} ± 1.414	3.72 ^{c} ± 0.11	133.80 ^{a} ± 1.24	3.92 ^{cd} ± 0.08	137.40 ^{a} ± 1.44	4.00 ^{c} ± 0.12
Clostridium Gp (5)	112.80 ^{c} ± 1.43	4.28 ^{a} ± 0.09	110.60 ^{e} ± 1.08	4.50 ^{a} ± 0.07	105.20 ^{e} ± 1.24	4.38 ^{a} ± 0.11
Cl.➡ Amoxicillin Gp (6)	111.80 ^{c} ± 1.356	4.16 ^{a} ± 0.08	120.20 ^{c} ± 1.07	4.26 ^{b} ± 0.05	128.00 ^{c} ± 1.00	4.18 ^{b} ± 0.11
Organic acids ➡ *Cl.* Gp (7)	118.20 ^{b} ± 1.36	4.00 ^{b} ± 0.15	114.40 ^{d} ± 1.21	4.34 ^{ab} ± 0.05	123.40 ^{d} ± 1.50	4.20 ^{b} ± 0.08
Cl.➡ Amoxi. + Organic Gp (8)	111.40 ^{c} ± 1.503	4.22 ^{a} ± 0.09	124.00 ^{b} ± 1.41	4.14 ^{bc} ± 0.08	132.60 ^{b} ± 1.54	3.98 ^{c} ± 0.09

Different letters in the same column indicate significant changes ($P < 0.05$).

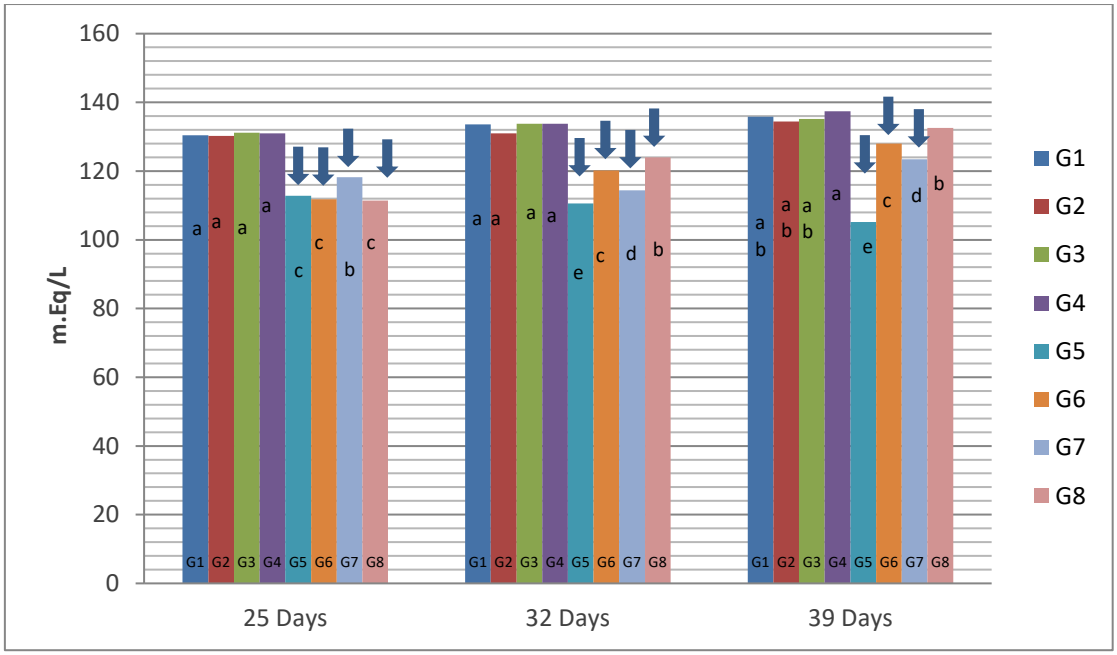


Fig. (28): Changes in serum **sodium level** (m.Eq/L) among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

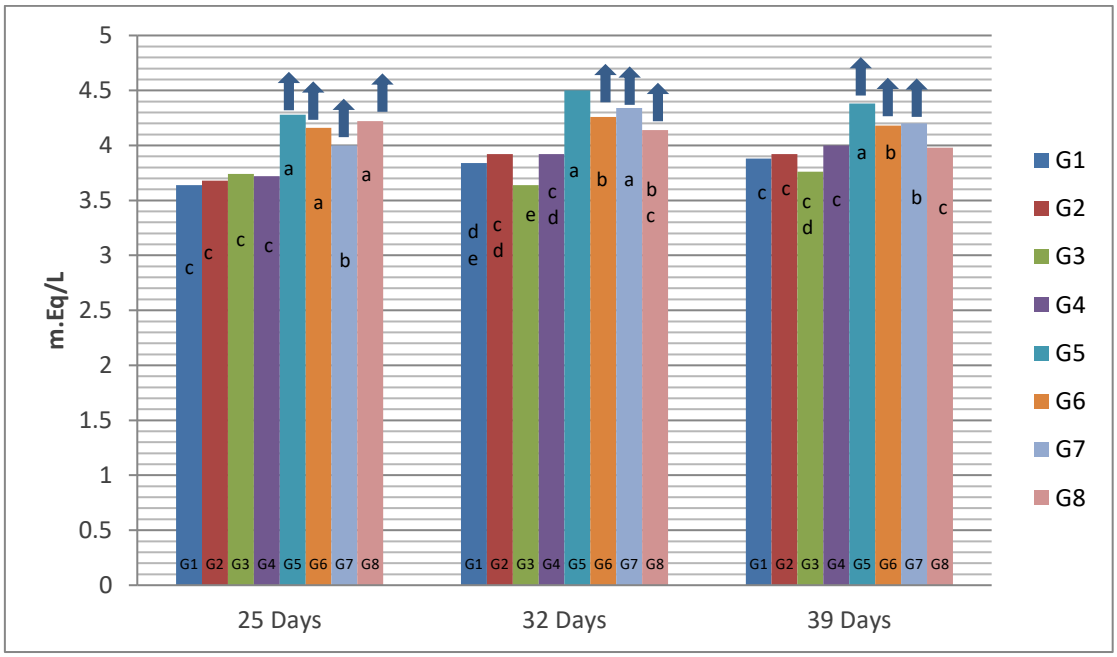


Fig. (29): Changes in serum **potassium level** (m.Eq/L) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

V- Immunological results:

1) Cellular immunity:

Groups (2 - 4) showed non-significant changes in phagocytic percentage and index when compared with the normal control group along the experimental periods.

At 25 and 32 days old, the results of chickens in groups (5 - 8) proved a significant ($P<0.05$) decrease in phagocytic percentage and index when compared with the normal control group. The lowest values of the fore-mentioned parameters were recorded in the 5^{th} group. On the other hand, groups (6- 8) showed a significant increase in phagocytic percentage and index when compared with infected non-treated group at 32 days old only **(table 18 and figs. 30 and 31).**

At 39 days old, groups (5 - 7) showed a significant decrease in phagocytic percentage and index when compared with the normal control group. Group (8) showed non-significant changes in phagocytic percentage and index when compared with the control group. Moreover, groups (6- 8) showed a significant increase in phagocytic percentage and index when compared with infected non-treated group (**table 18 and figs. 30 and 31).**

Table (18): Cellular immunity parameters (mean values ± S.E) of chickens in different groups along the experimental periods (N=5).

Parameters / Groups	25 days old		32 days old		39 days old	
	Phagocytic %	**Phagocytic index**	**Phagocytic %**	**Phagocytic index**	**Phagocytic %**	**Phagocytic index**
Control Gp (1)	74.00 ^{a} ± 1.41	4.50 ^{a} ± 0.14	76.00 ^{ab} ± 1.30	4.60 ^{a} ± 0.13	77.00 ^{ab} ± 0.949	4.70 ^{ab} ± 0.17
Amoxicillin Gp (3)	72.00 ^{a} ± 1.00	4.40 ^{a} ± 0.07	73.00 ^{b} ± 1.14	4.44 ^{a} ± 0.15	75.00 ^{b} ± 0.837	4.60 ^{b} ± 0.08
Organic acids Gp (3)	76.80 ^{a} ± 1.36	4.80 ^{a} ± 0.07	79.00 ^{a} ± 1.67	4.80 ^{a} ± 0.07	80.00 ^{a} ± 1.225	5.00 ^{a} ± 0.16
Amoxi+Organic Gp (4)	75.00 ^{a} ± 1.00	4.40 ^{a} ± 0.10	77.80 ^{a} ± 0.66	4.74 ^{a} ± 0.12	76.00 ^{b} ± 1.581	4.56 ^{b} ± 0.10
Clostridium Gp (5)	38.00 ^{c} ± 1.14	1.80 ^{c} ± 0.12	36.00 ^{f} ± 0.71	1.60 ^{d} ± 0.07	40.00 ^{d} ± 0.707	1.80 ^{d} ± 0.11
Cl. ➡ Amoxicillin Gp (6)	40.00 ^{c} ± 1.64	2.00 ^{c} ± 0.12	60.00 ^{e} ± 1.14	2.80 ^{c} ± 0.14	68.00 ^{c} ± 1.703	3.20 ^{c} ± 0.07
Organic acids ➡ *Cl.* Gp (7)	55.00 ^{b} ± 1.14	2.50 ^{b} ± 0.10	64.00 ^{d} ± 1.23	3.10 ^{bc} ± 0.12	66.00 ^{c} ± 1.225	3.00 ^{c} ± 0.17
Cl. ➡ Amoxi. + Organic Gp (8)	42.00 ^{c} ± 1.41	1.90 ^{c} ± 0.14	68.00 ^{c} ± 2.00	3.30 ^{b} ± 0.14	74.00 ^{b} ± 1.414	4.40 ^{b} ± 0.14

Different letters in the same column indicate significant changes ($P < 0.05$)

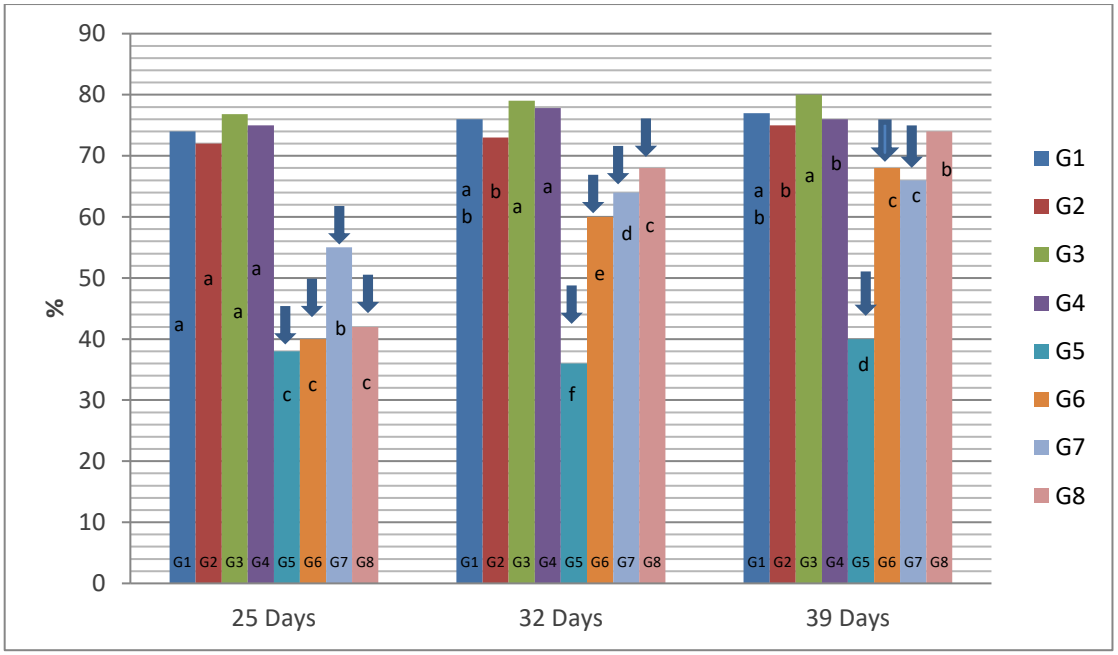


Fig. (30): Changes in **phagocytic %** among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

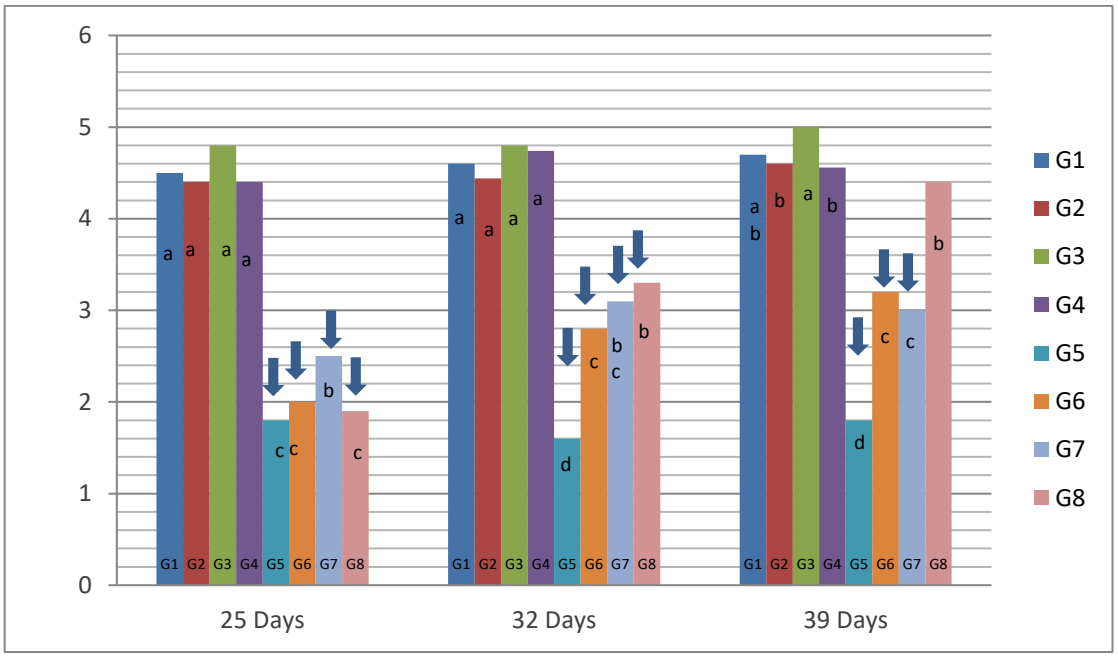


Fig. (31): Changes in **phagocytic index** among different groups at different ages (25, 32 and 39 days old)

- ⬇ Significant decrease

2) Humoral immunity:

Groups (2-4) showed non-significant changes in IgM and IgG levels when compared with the normal control group along the experimental periods.

At 25 and 32 days old, the results of chickens in groups (5 - 8) proved a significant ($P<0.05$) increase in serum levels of IgM and IgG when compared with the normal control group. The highest values of the fore-mentioned parameters were recorded in the 5^{th} group. On the other hand, groups (6 - 8) showed a significant decrease in serum levels of IgM and IgG when compared with infected non-treated group at 32 days old only **(table 19 and figs. 32 and 33).**

At 39 days old, groups (5 - 7) showed a significant increase in serum levels of IgM and IgG when compared with the normal control group. Group (8) showed non-significant changes in serum levels of IgM and IgG when compared with normal control. Moreover, groups (6 - 8) showed a significant decrease in serum levels of IgM and IgG when compared with infected non-treated group **(table 19 and figs. 32 and 33).**

Table (19): Humoral immunity parameters (mean values ± S.E) of chickens in different groups along the experimental periods (N=5).

Parameters / Groups	25 days old		32 days old		39 days old	
	Ig M mg/dl	**Ig G mg/dl**	**Ig M mg/dl**	**Ig G mg/dl**	**Ig M mg/dl**	**Ig G mg/dl**
Control Gp (1)	220.00 ^{b} ± 2.72	910.00 ^{b} ± 1.41	224.00 ^{d} ± 1.52	912.00 ^{d} ± 2.12	228.00 ^{de} ± 1.22	918.00 ^{c} ± 2.00
Amoxicillin Gp (3)	222.00 ^{b} ± 2.83	908.00 ^{b} ± 2.00	224.00 ^{d} ± 3.15	914.00 ^{d} ± 1.64	230.00 ^{cde} ± 1.76	916.00 ^{c} ± 2.12
Organic acids Gp (3)	228.00 ^{b} ± 3.96	917.00 ^{b} ± 1.41	227.00 ^{cd} ± 1.82	918.00 ^{cd} ± 1.30	232.00 ^{cd} ± 1.64	922.40 ^{c} ± 1.50
Amoxi+Organic Gp (4)	223.00 ^{b} ± 2.41	911.60 ^{b} ± 2.502	225.00 ^{d} ± 2.55	916.00 ^{d} ± 2.550	224.00 ^{e} ± 2.30	920.00 ^{c} ± 1.98
Clostridium Gp (5)	232.00 ^{a} ± 3.26	924.80 ^{a} ± 3. 77	245.00 ^{a} ± 4.30	930.00 ^{a} ± 3.21	241.00 ^{a} ± 2.61	938.40 ^{a} ± 3.67
Cl. ➡ Amoxicillin Gp (6)	234.00 ^{a} ± 3.30	926.60 ^{a} ± 2.42	238.00^{ab} ± 1.70	924.80 ^{ab} ± 1.72	235.00 ^{bc} ± 1.00	932.60 ^{ab} ± 1.218
Organic acids➡ *Cl.* Gp (7)	237.00 ^{a} ± 2.83	929.00 ^{a} ± 2.88	241.00 ^{ab} ± 1.58	926.00 ^{ab} ± 2.26	238.00 ^{ab} ± 2.12	929.60 ^{b} ± 1.57
Cl. ➡ Amoxi. + Organic Gp (8)	230.00 ^{a} ± 1.82	928.80 ^{a} ± 2.35	232.00 ^{bc} ± 2.28	923.00 ^{bc} ± 1.41	230.00 ^{cde} ± 2.35	918.20 ^{c} ± 1.99

Different letters in the same column indicate significant changes (P <0.05).

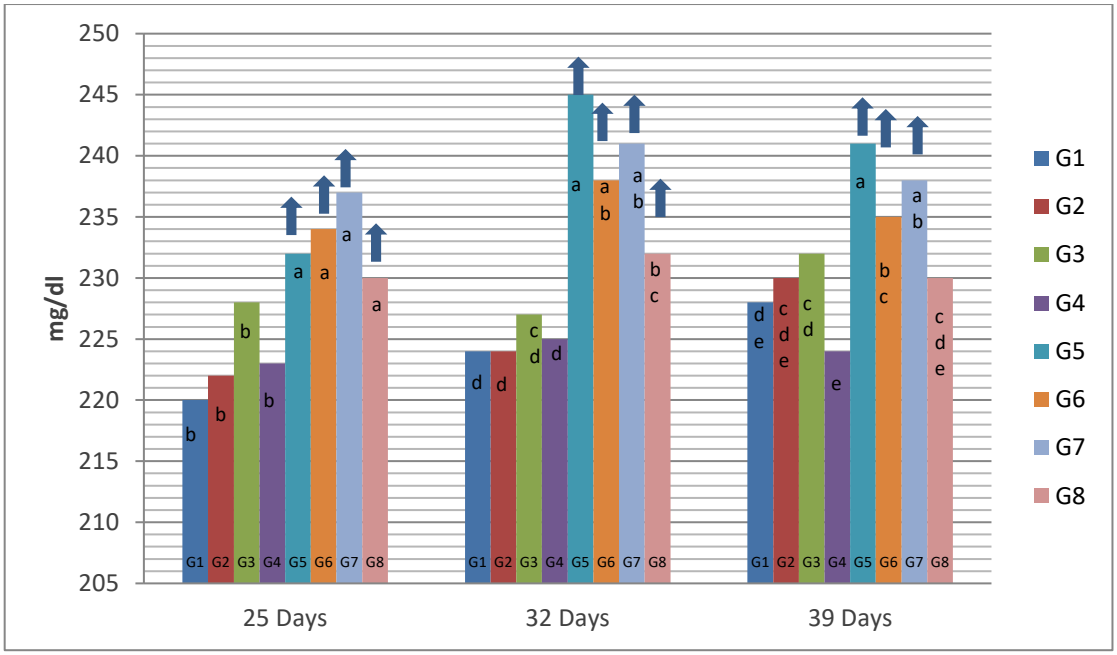


Fig. (32): Changes in serum **IgM** (mg/dl) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

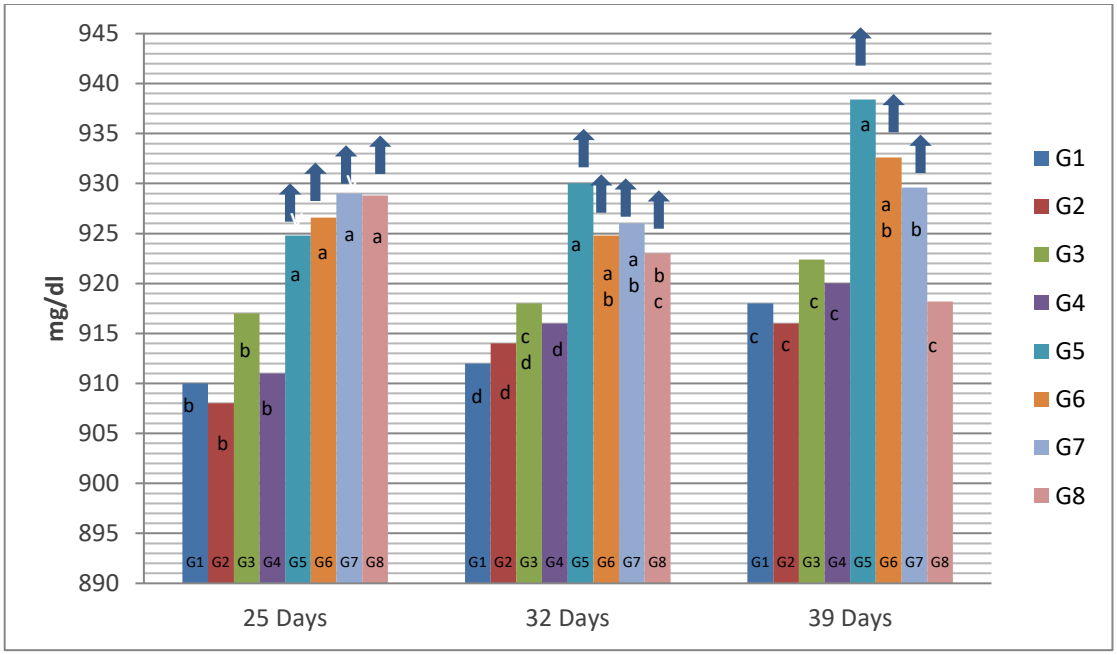


Fig. (33): Changes in serum **IgG** (mg/dl) among different groups at different ages (25, 32 and 39 days old)

- ⬆ Significant increase

HISTOPATHOLOGY RESULTS

► Group 1 (normal control):

No lesions were detected either macroscopically or microscopically along the experimental periods.

► Group 2 (amoxicillin treated group):

All organs appeared normal macroscopically and microscopically except liver, which showed lesions only at 32 days old.

- **Liver:**
  - **Macro:** Slightly enlarged.
  - **Micro:** Mild degenerative changes represented by vacuolar degeneration in the hepatic cells **(photo, 5)**.

► Group 3 (organic acids treated group):

All organs appeared normal macroscopically and microscopically except intestine and spleen.

- **Intestine:**
  - **Macro:** The intestine was apparently normal**.**
  - **Micro:** The height of the intestinal villi was increased along the experimental periods **(photo, 6)**.
- **Spleen:**
  - **Macro:** The spleen was normal in colour, size and consistency with no alterations.
  - **Micro:** Hyperplasia of the lymphoid follicle was observed in the white pulp along the experimental periods **(photo, 7)**.

► Group 4 (amoxicillin and organic acids treated group):

No lesions were detected either macroscopically or microscopically along the experimental period.

► *Cl. perfringens* infected chickens (gp. 5) :

A) At 25 days old:

The examined chickens showed severe toxaemic lesions in all organs.

- **Intestine:**
  - **Macro:** The intestine was congested, edematus and distended with gas with red necrotic focci on its surface.
  - **Micro:** The lumen contains desquamated epithelium as sheets with leukocytes and erythrocytes and undigested food particles were noted. Upper half of villi appeared partially necrotic and distorted infiltrated by leukocytes admixed with extravasated erythrocytes **(photo, 8)**. The intestinal crypts were hyperplastic and elongated. Some birds showed complete destruction of villus epithelium and the lumen contained sloughed villi and desquamated epithelium cells and undigested food particles. The intestinal glands were necrotic with partial hyalinizion and edema of the muscular coat.

- **Liver:**
  - **Macro:** The liver was enlarged, congested and friable with small necrotic focci.
  - **Micro:** Congestions in the hepatic blood vessels and portal areas contain leukocytes mainly heterophils beside mild acute cell swelling of the hepatic cells **(photo, 9)**.

- **Kidney:**
  - **Macro:** Enlargement of the kidney with some petechial hemorrhage.
  - **Micro:** Focal hemorrhage with degeneration of the renal tubules was observed **(photo, 10).**
- **Spleen:**
  - **Macro:** Enlargement and congestion of the spleen with dark red mottled appearance**.**
  - **Micro:** Necrosis, vacuolation and thickening of the wall of blood vessels were recorded in addition to a mild hemorrhage and depletion of the lymphocytes of the white pulp **(photo, 11)**.

B) At 32 days old:

- **Intestine:**
  - **Macro:** Thickening of intestinal wall and presence of pseudo-diphtheritic membrane with hemorrhage in the intestinal lumen.
  - **Micro:** Necrotic enteritis the lesions become sever manifested by complete destructions of intestinal mucosa which usually extended to involve the intestinal layers and replaced by numerous erythrocytes admixed with epithelium sheet, leukocytes and heterophiles **(photo, 12)**. The intestinal lumen contains hemorrhagic exudates and epithelial cells admixed with leukocytes. Submucosa had hyperplastic glands and inter-glandular tissue infiltrated by inflammatory cells exudate, muscular coats are necrotic and edematous.

- **Liver:**
  - **Macro:** Enlarged pale liver with rib impression on it**.**

- **Micro:** Sever acute cell swelling or necrotic changes involving single or cluster of the hepatic cells which usually infiltrated by heterophiles **(photo, 13)**. Interstitial and portal leukocytic infiltrations beside bile duct epithelium were encountered.

- **Kidney:**
  - **Macro:** Congestion and enlargement of the kidney.
  - **Micro:** Severe congestion of renal blood vessels with cystic dilation were observed **(photo, 14).**

- **Spleen:**
  - **Macro:** Congestion and enlargement of the spleen.
  - **Micro:** The splenic lesions varied from necrosis and depletions of lymphoid element which partially or completely involve the white pulps **(photo, 15)**.

C) At 39 days old:

- **Macro:** Congestion and enlargement of the internal organs.

- **Intestine:**

Severe necrosis and sloughing of some intestinal coats were seen beside edema and hyalinization of the muscular layers **(photo, 16)**. Some inflammatory cells between hyperplastic intestinal glands partially restore the destructed and sloughed layers.

- **Liver:**

Intense acute cell swelling or microsteatosis of the hepatic cells was prevalent. Moreover the portal area contain numerous bile ductules and fibrous tissue infiltrated by leukocytes mainly heterophils and mononuclear cells **(photo, 17)**.

- **Kidney:**

Congestion of renal blood vessels was recorded with WBCs infiltration of the renal medulla **(photo, 18).**

- **Spleen:**

The splenic lesions varied from necrosis, hemorrhage and depletions of lymphoid element **(photo, 19)**.

► *Cl. perfringens* infected chickens and treated with amoxicillin (gp.6) :

A) At 32 days old:

- **Macro:** Mild congestion of the intestine, liver, kidney and spleen.

- **Intestine:**

Regeneration of sloughed intestinal villi from hyperplastic mucosal intestinal crypt was detected **(photo, 20)**.

- **Liver:**

Interstitial leukocytic aggregation mainly round cells and vacuolation of the hepatic cells beside portal leukocytic infiltration and numerous bile ductules could be seen **(photo, 21)**.

- **Kidney:**

Severe congestion of some renal blood vessels with perivascular degeneration of the renal tubules was observed **(photo, 22)**.

- **Spleen:**

The splenic tissue within the normal picture and a few splenic white pulps had proliferative central arterioles and showed mild lymphoid depletion **(photo, 23)**.

B) At 39 days old:

Macro: Mild congestion of the intestine, liver, kidney and spleen.

- **Intestine:**

The intestinal mucosa restored its morphological picture and represented by regenerated villi with fused tips **(photo, 24)**.

- **Liver:**

The hepatic parenchyma was apparently normal. Portal and interstitial lymphocytic aggregations were encountered **(photo, 25)**.

- **Kidney:**

A few renal tubules exhibited mild nephrotic changes varied from cloudy swelling to hydropic degeneration with apparently normal remaining renal parenchyma **(photo, 26)**.

- **Spleen:**

The splenic tissue within the normal picture and a few splenic white pulps had proliferative central arterioles and showed mild lymphoid depletion **(photo, 23)**.

► Chickens prophylacted by organic acids before *Cl. perfringens* infection (gp. 7):

A) At 25 days old:

Macro: Mild congestion and enlargement of liver, kidney and spleen with presence of a slight pseudo-diphtheritic membrane in the intestine.

- **Intestine:**

Regeneration of villus enterocytes and the villi restore its length and structures beside hyperplastic intestinal crypts and gut associated

lymphoid follicles **(photo, 27)**. Intestinal lumen contained small amount of exudate mainly desquamated epithelium and a few leukocytes and erythrocytes.

- **Liver:**

Mild reversible changes in the hepatic cells and portal heterophilic aggregations were seen **(photo, 28)**.

- **Kidney:**

Renal blood vessels showed severe diffuse congestion with WBCs infiltration, mainly heterophils **(photo, 29).**

- **Spleen:**

Congested sinusoids and mild lymphoid depletion of white pulps were seen in all organic acid and infection group **(photo, 30)**.

B) At 32 and 39 days old:

– **Macro:** Enlarged and slightly congested intestine, liver, kidney and spleen.

- **Intestine:**

The intestinal changes post *Clostridium* infection were mild and represented by hyperplastic intestinal crypts with normal length and thickness of the intestinal villi **(photo, 31)**.

- **Liver:**

The hepatic lesions post *Clostridium* infection were similar and represented by minute leukocytic aggregation mainly lymphocytes with

mild vacuolation in hepatic cells. Proliferation of kupffer cells with still present **(photo, 32)**.

- **Kidney:**

The kidney lesions post *Clostridium* infection were similar and represented by a mild congestion of renal blood vessels with cystic dilation of the renal tubules **(photo, 33).**

- **Spleen:**

Congested sinusoids and mild lymphoid depletion of white pulps were seen in all organic acid and infection group **(photo, 34)**.

►*Cl. perfringens* infected chickens and treated with amoxicillin and organic acids (gp. 8) :

A) At 32 days old:

Macro: All internal organs appeared normal except for slight congestion in the intestine.

- **Intestine:**

Mild thickened intestinal villi and fusion of intestinal tips and the lumen contain some desquamated sheet could be seen **(photo, 35)**.

- **Liver:**

Mild leukocytes aggregations in portal area and reversible degenerative changes in the hepatic cells were noticed **(photo, 36)**.

- **Kidney:**

Glomerular congestion with interstitial polymorphnuclear infiltration was present **(photo, 37)**.

- **Spleen:**

Congestion of white pulp and endotheliosis of central arterioles were seen **(photo, 38).**

B) At 39 days old:

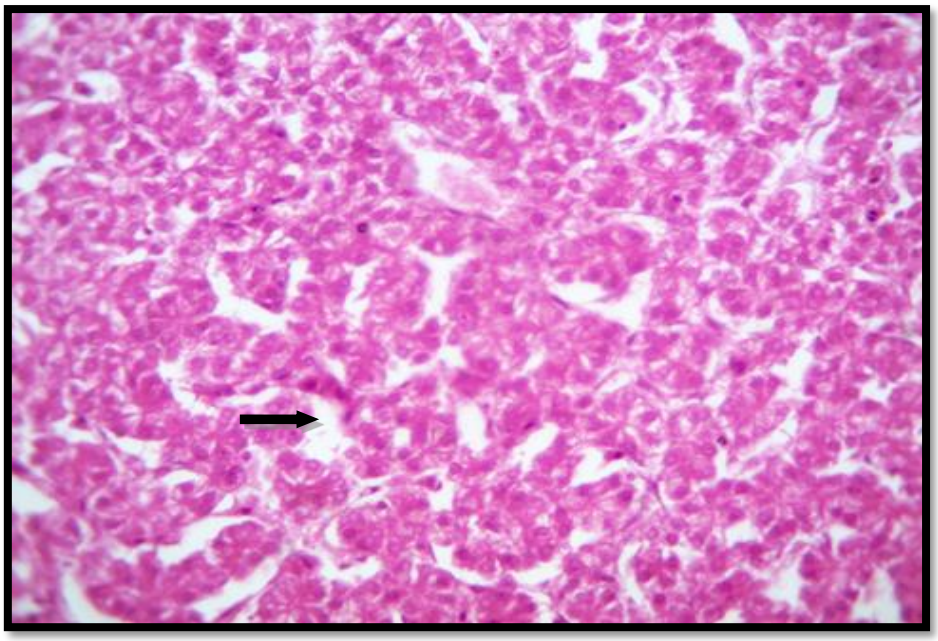
Macro: All internal organs were normal in colour and size.

- **Intestine:** Apparently normal intestinal coats with no lesions detected.

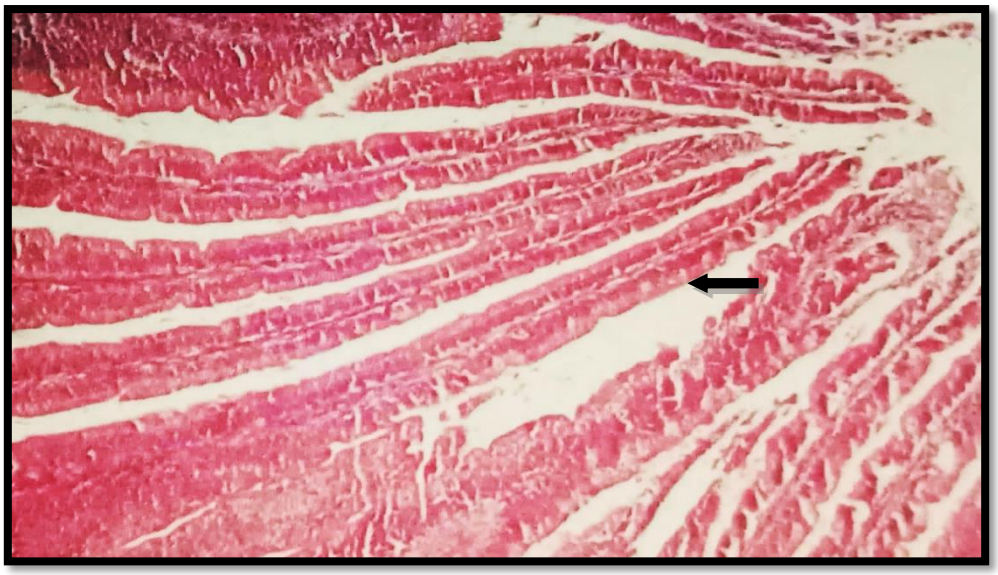
- **Liver:** All the hepatic structures had the normal morphological appearance.

- **Kidney:** No lesions were observed in the apparently normal organ.

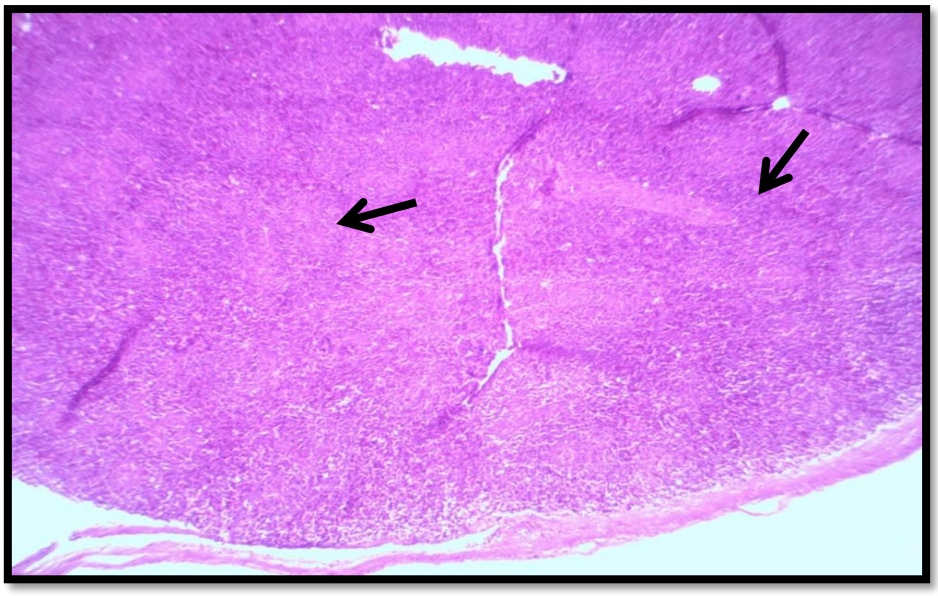
- **Spleen:** All the splenic structures had the normal morphological appearance.



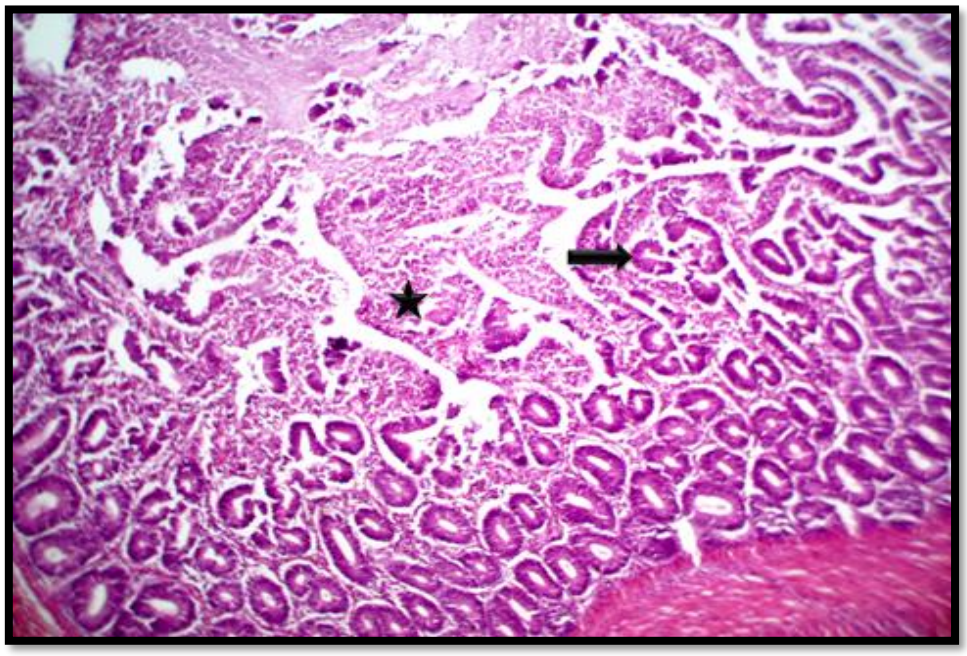
Photo, (5): Liver of chicken treated with amoxicillin (gp. 2) at 32 days old showing mild vacuolation in the hepatic cells (arrows) (H&E, X400).



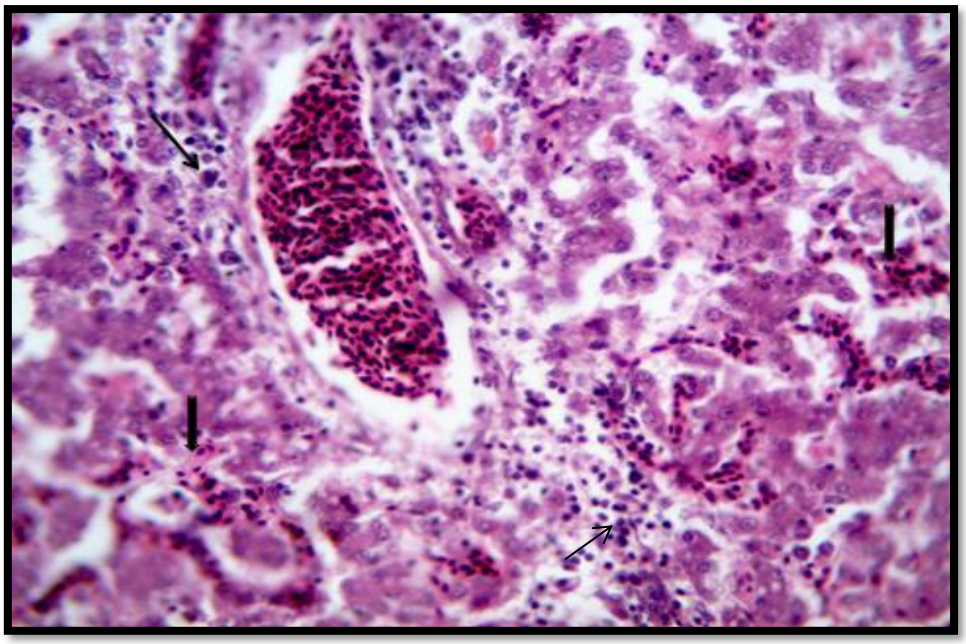
Photo, (6): Intestine of chicken administered organic acids (gp. 3) along the experimental periods showing increase in the height of intestinal villi (H&E, X300).



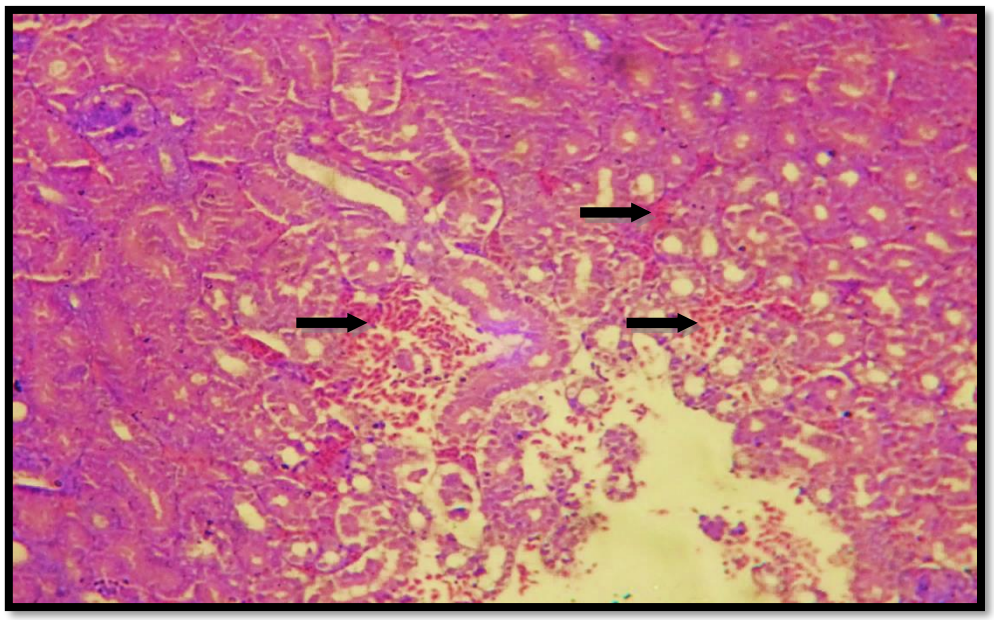
Photo, (7): Spleen of chicken administered organic acids **(**gp. 3) along the experimental periods showing hyperplasia of lymphoid tissue of the white pulp (arrows) (H&E, X200).



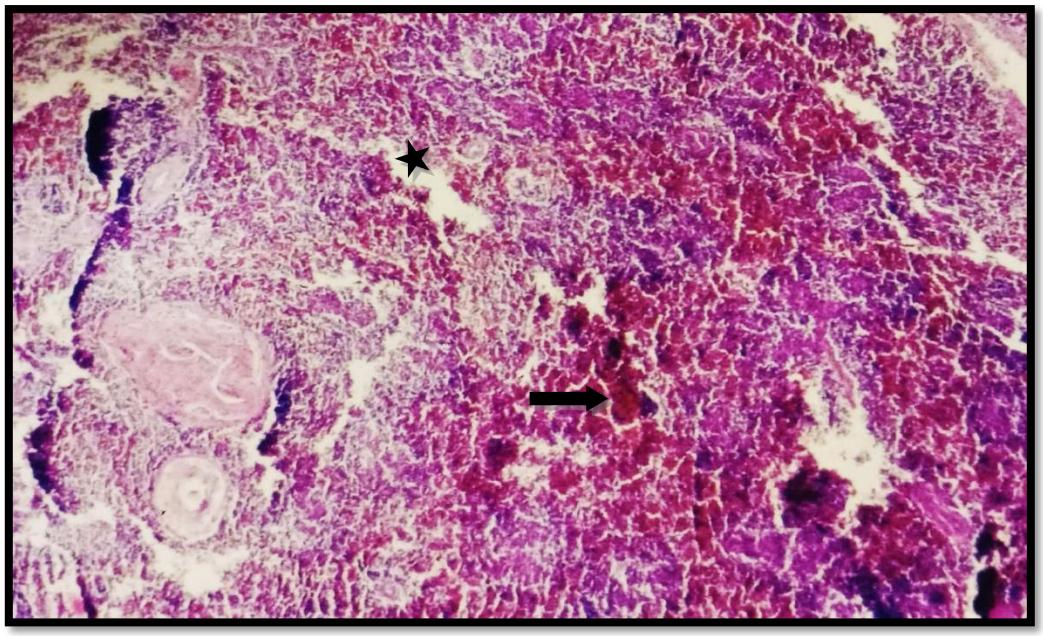
Photo, (8): Intestine of chicken infected by *Cl. perfringens* (group 5) at 25 days old showing partial necrosis of intestinal villi (arrow) with intense inflammatory reaction in sub mucosa (star) (H&E, X200).



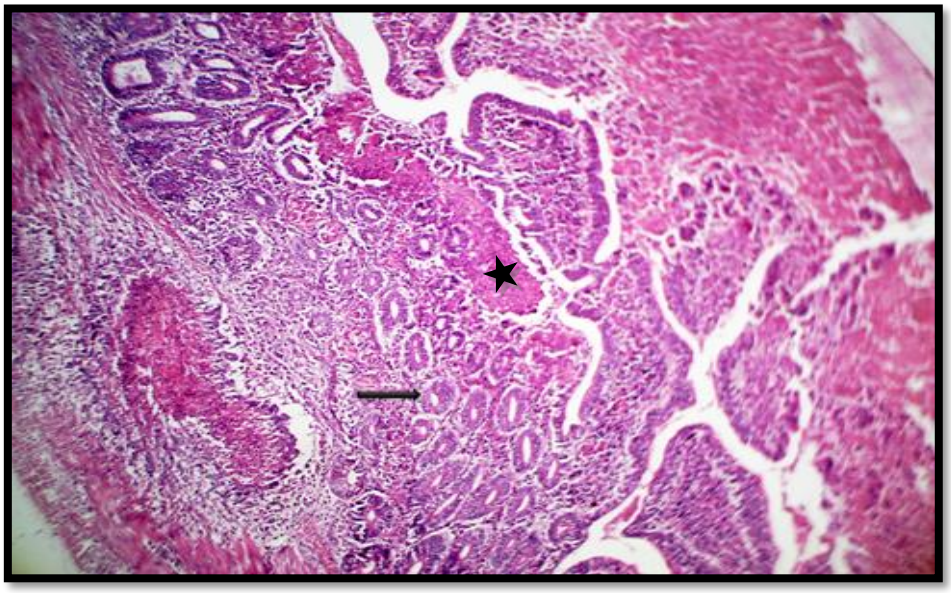
Photo, (9): Liver of chicken infected by *Cl. perfringens* (group 5) at 25 days old showing sever congestions in the hepatic blood vessels (thick arrows) and portal area contains some heterophils (thin arrow) (H&E, X400).



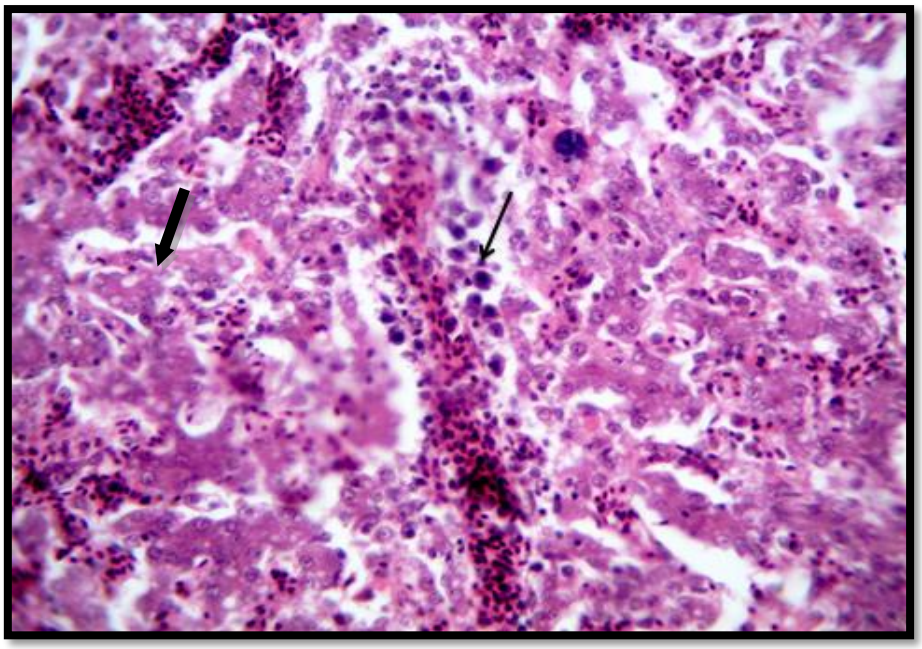
Photo, (10): Kidney of chicken infected by *Cl. perfringens* (group 5) at 25 days old showing focal hemorrhage (arrows) (H&E, X200).



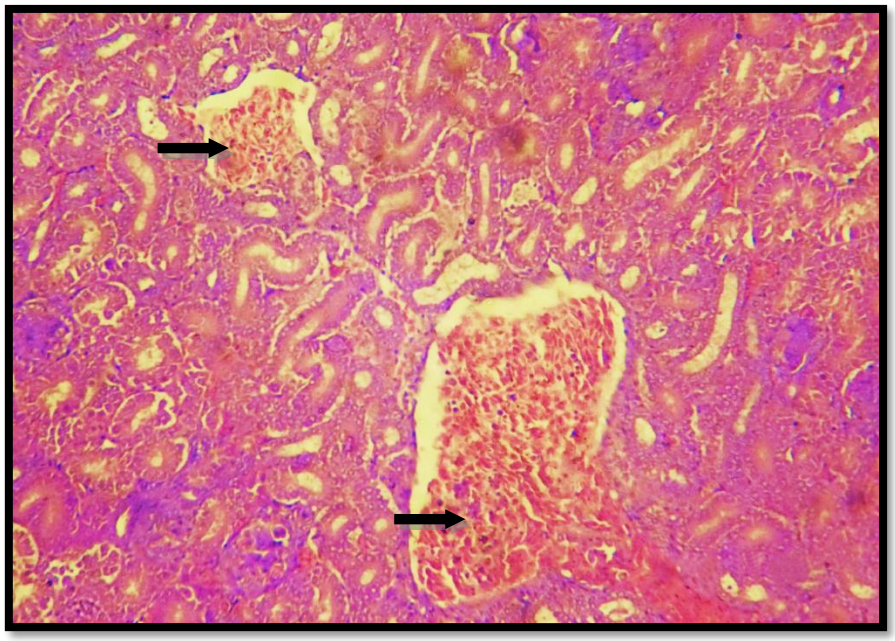
Photo, (11): Spleen of chicken infected by *Cl. perfringens* (group 5) at 25 days old showing hemorrhagic area (arrow) and lymphoid depletion from the white pulps (star) (H&E, X200).



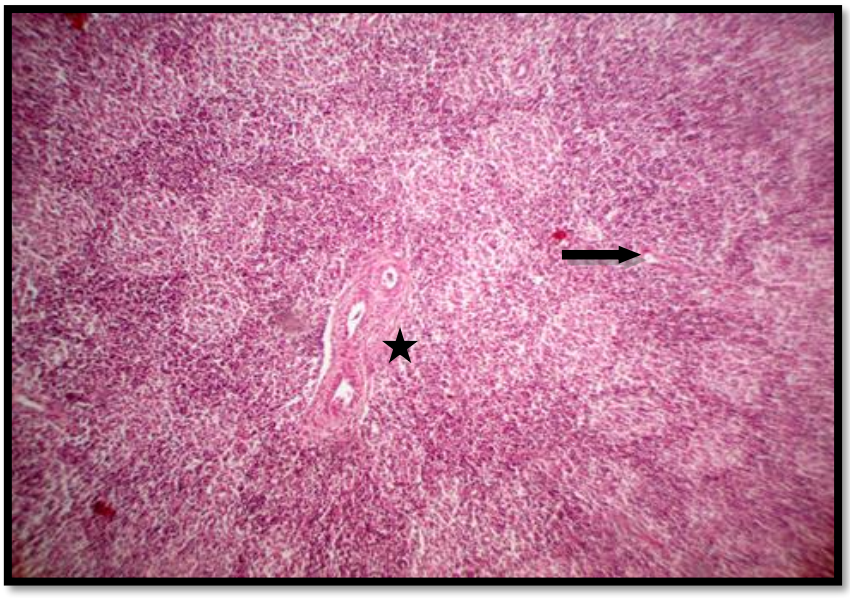
Photo, (12): Intestine of chicken infected by *Cl. perfringens* (gp. 5) at 32 days old showing necrotic mucosa extended deeper to involve the submucosa (arrow). Lumen contains intense blood exudate admixed with intestinal sheets and leukocytes (star) (H&E, X200).



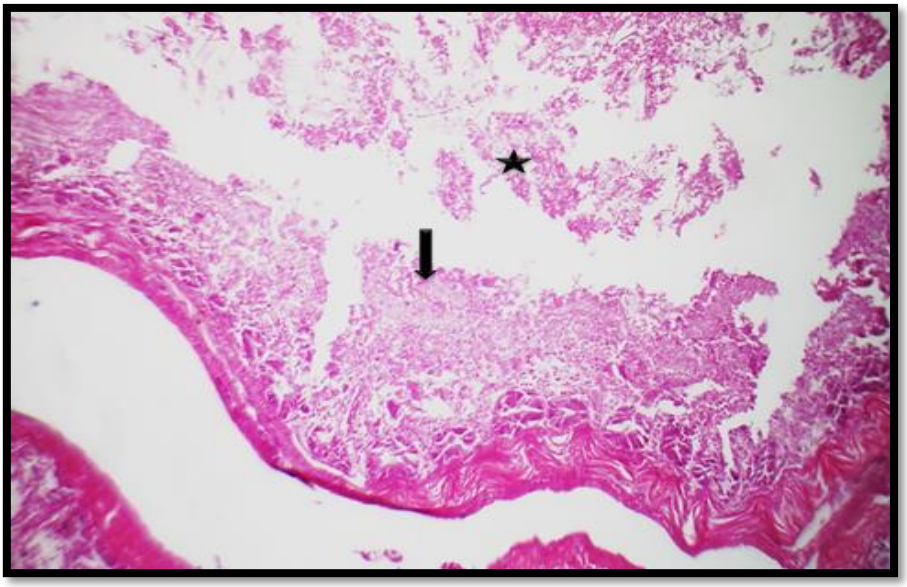
Photo, (13): Liver of chicken infected by *Cl. perfringens* (gp. 5) at 32 days old showing intense degenerative changes and congestion beside portal leukocytic infiltration (thin arrow) and individualized hepatic cell necrosis (thick arrrow) (H&E, X400).



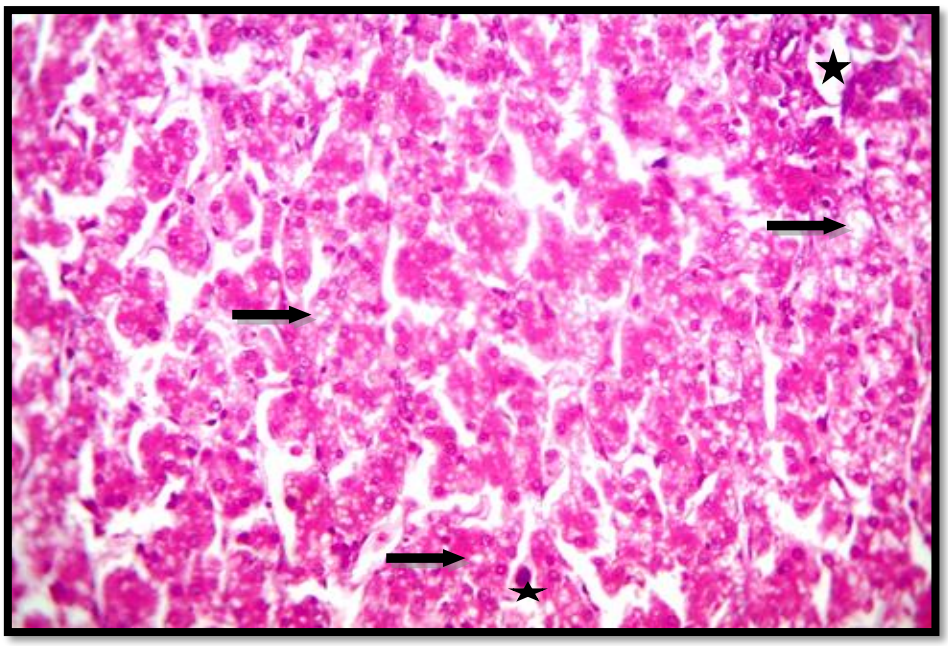
Photo, (14): Kidney of chicken infected by *Cl. perfringens* (gp. 5) at 32 days old showing severe congestion of renal blood vessels of the renal cortex (arrow) (H&E, X400).



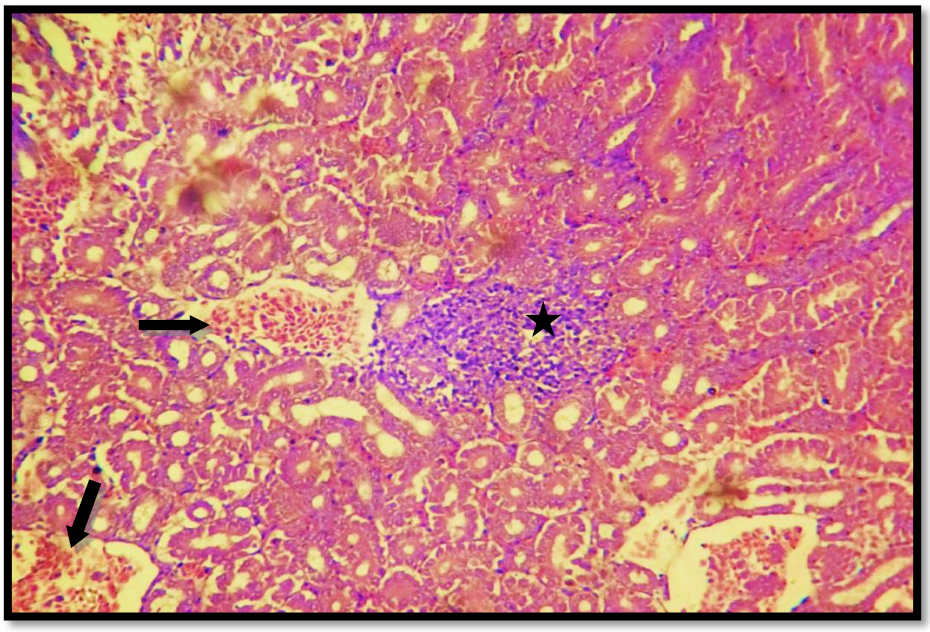
Photo, (15): Spleen of chicken infected by *Cl. perfringens* **(**gp. 5) at 32 days old showing multifocal necrosis (arrow) and depletion of splenic white pulp (star) (H&E, X300).



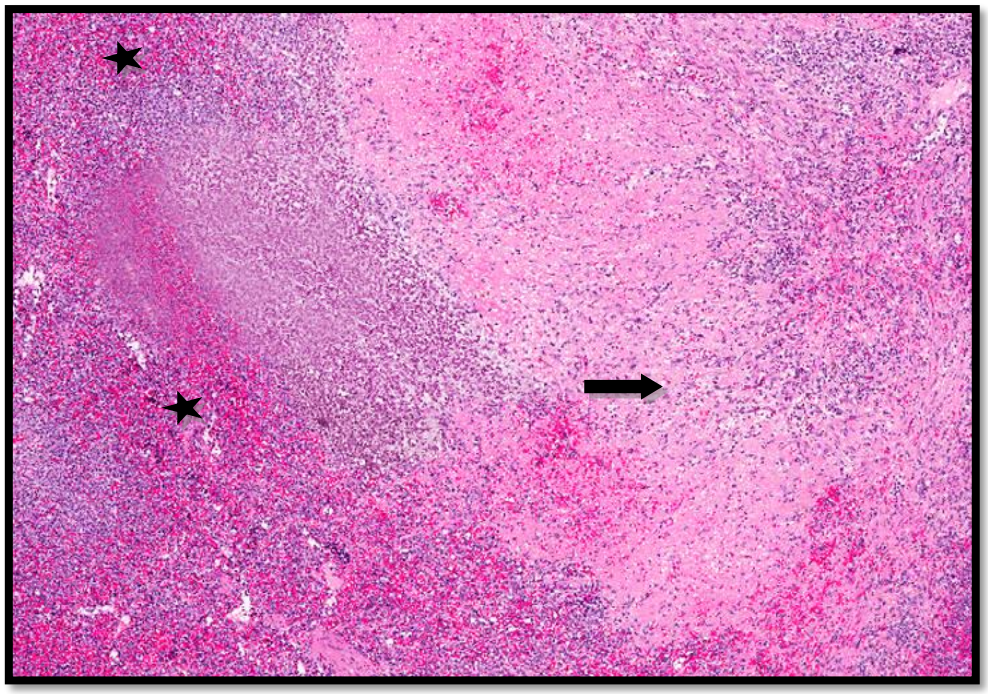
Photo, (16): Intestine of chicken infected by *Cl. perfringens* (gp. 5) at 39 days old showing severs necrotic changes (star) and sloughing in the majority of the intestinal coats (arrow) (H&E, X200).



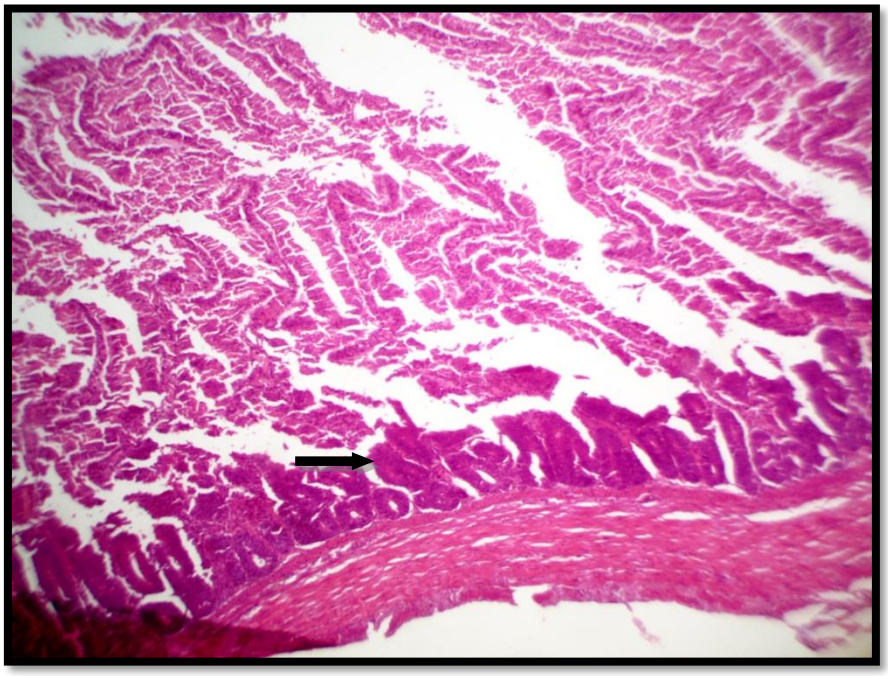
Photo, (17): Liver of chicken infected by *Cl. perfringens* (gp. 5) at 39 days old showing necrosis (stars) and microsteatosis (arrows) in the hepatic cells (H&E, X400).



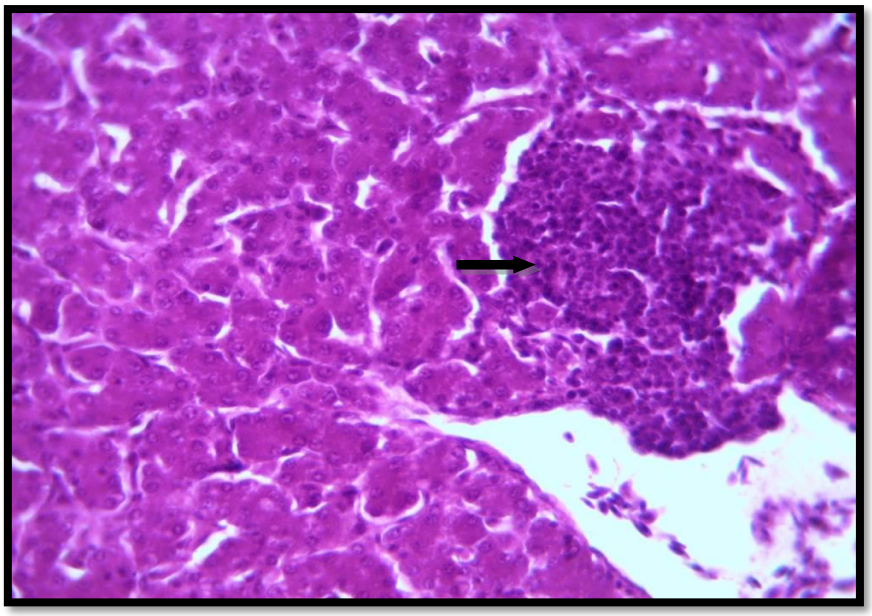
Photo, (18): Kidney of chicken infected by *Cl. perfringens* (gp. 5) at 39 days old showing congestion of renal blood vessels with WBCs infiltrating the renal medulla (star) (H&E, X 200).



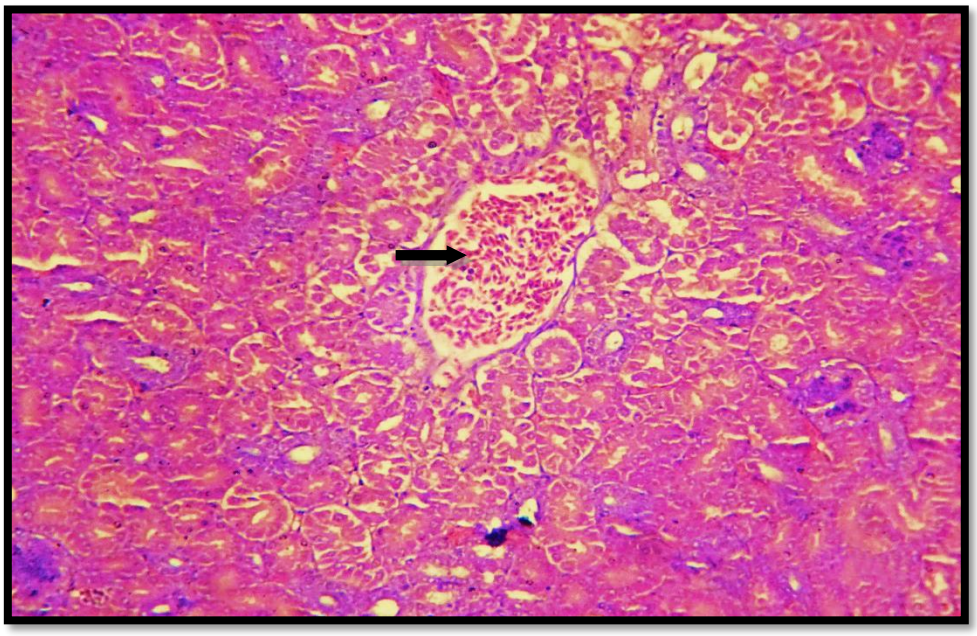
Photo, (19): Spleen of chicken infected by *Cl. perfringens* **(**gp. 5) at 39 days old showing necrosis (arrow) and hemorrhage of the white pulp (star) (H&E, X200).



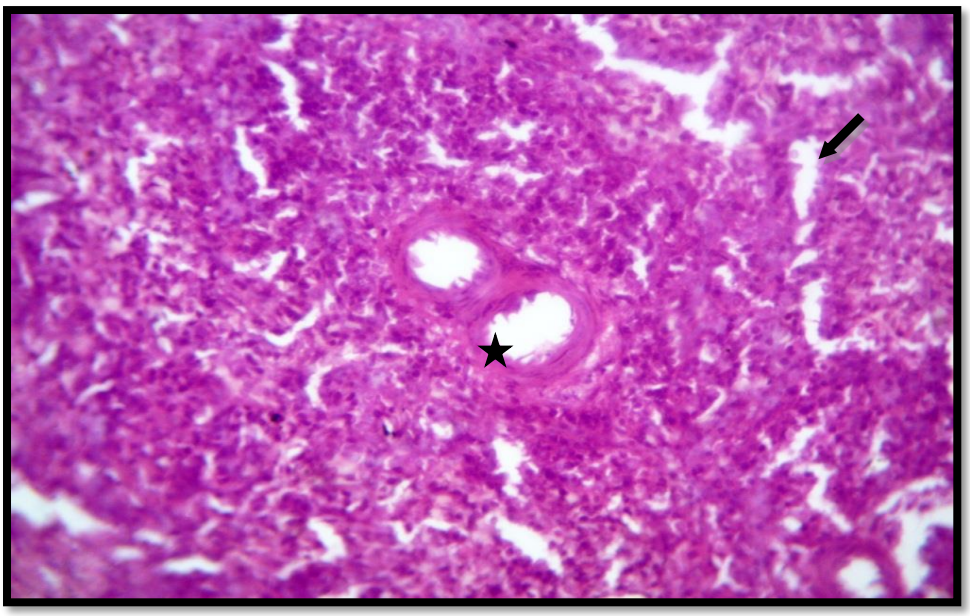
Photo, (20): Intestine of chicken infected with *Cl.perfringens* and treated with amoxicillin (gp. 6) at 32 days old showing regeneration of sloughed intestinal villi from hyperplastic intestinal crypts (arrow) (H&E, X200).



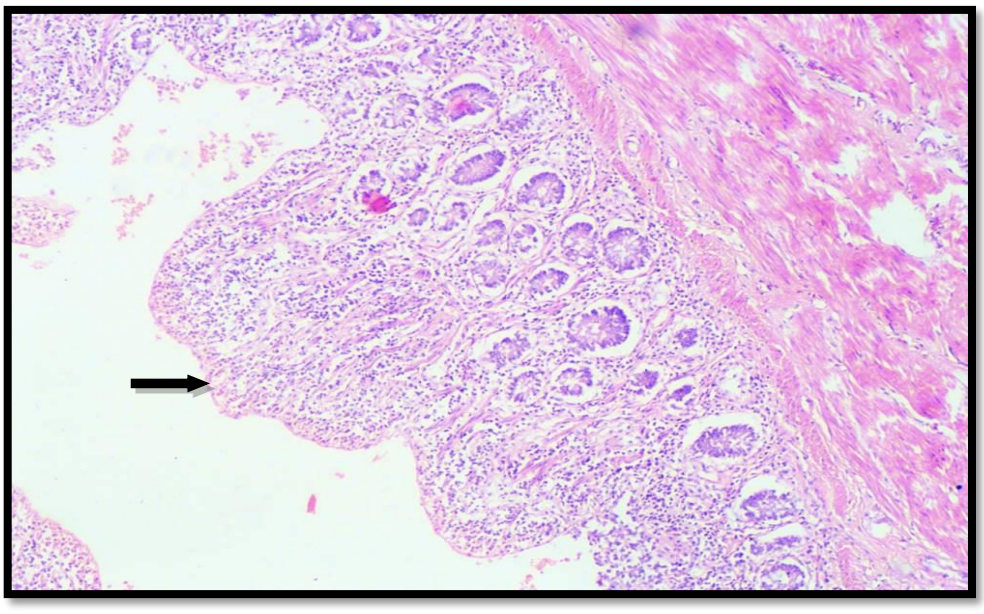
Photo, (21): Liver of chicken infected with *Cl.perfringens* and treated with amoxicillin (gp. 6) at 32 days old showing portal lymphocytic aggregation (arrow) (H&E, X400).



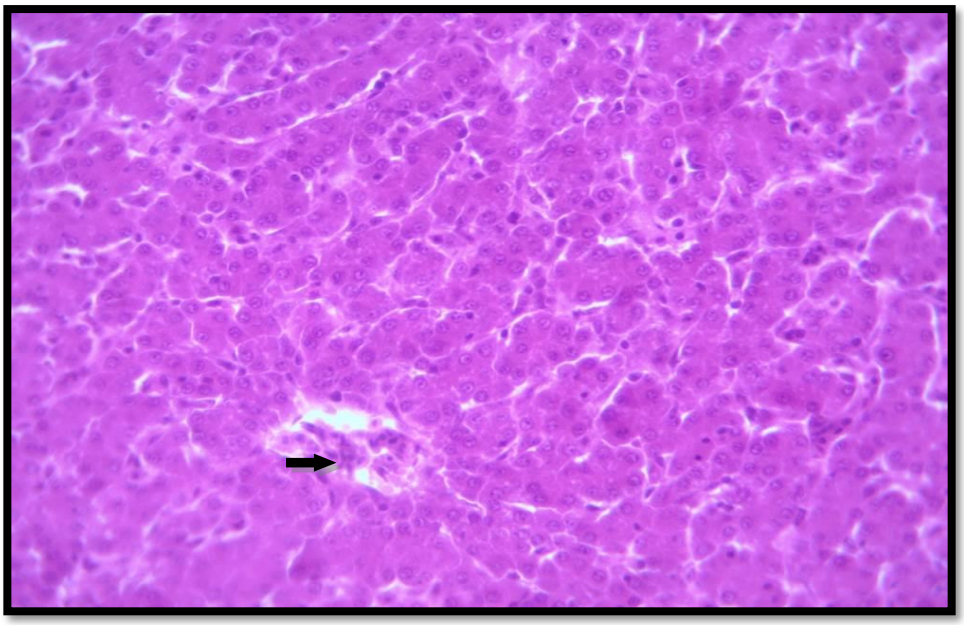
Photo, (22): Kidney of chicken infected with *Cl.perfringens* and treated with amoxicillin (gp. 6) at 32 days old showing severe congestion of some renal blood vessels (arrow) (H&E, X400).



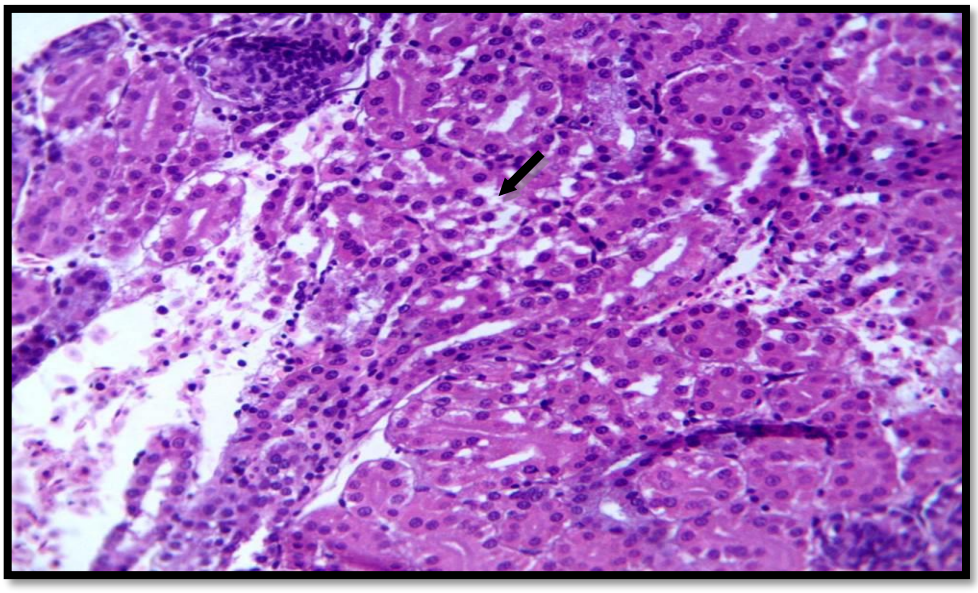
Photo, (23): Spleen of chicken infected with *Cl.perfringens* and treated with amoxicillin (gp. 6) at 32 & 39 days old showing proliferative central arterioles (star) with mild depletion of lymphoid tissue (arrow) (H&E, X300).



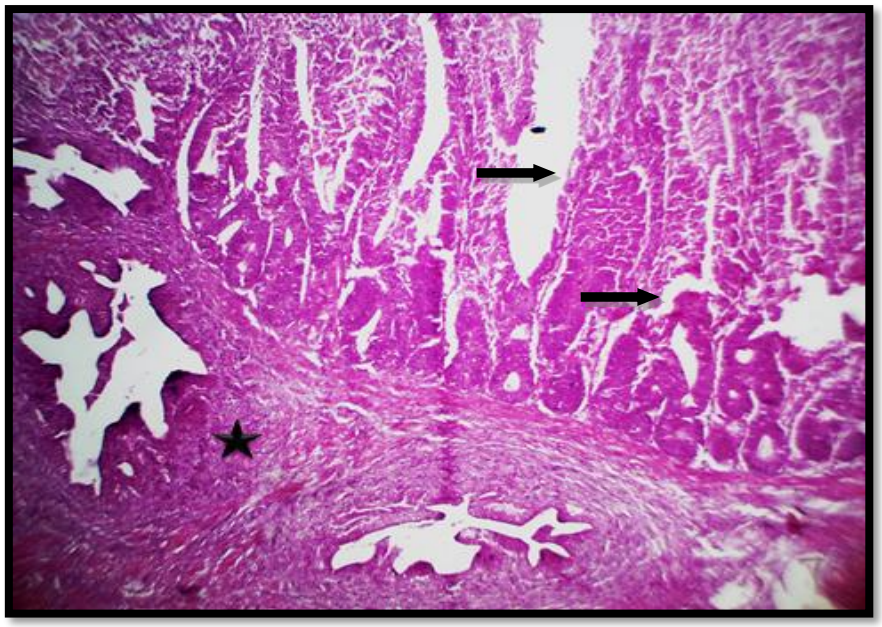
Photo, (24): Intestine of chicken infected with *Cl.perfringens* and treated with amoxicillin (gp. 6) at 39 days old showing regenerated intestinal villi with fused tips (arrow) (H&E, X400).



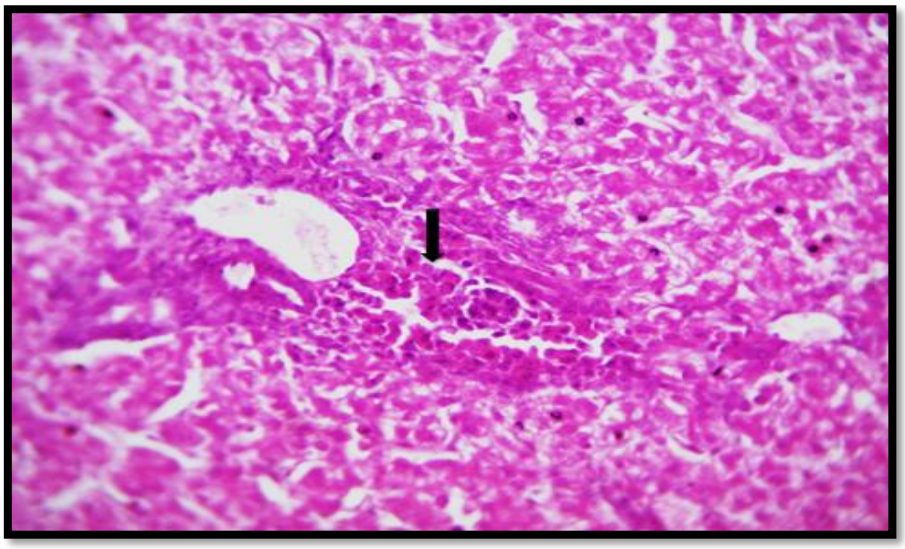
Photo, (25): Liver of chicken infected with *Cl.perfringens* and treated with amoxicillin (gp. 6) at 39 days old showing apparently normal hepatic parenchyma with portal lymphocytic aggregations (arrow) (H&E, X400).



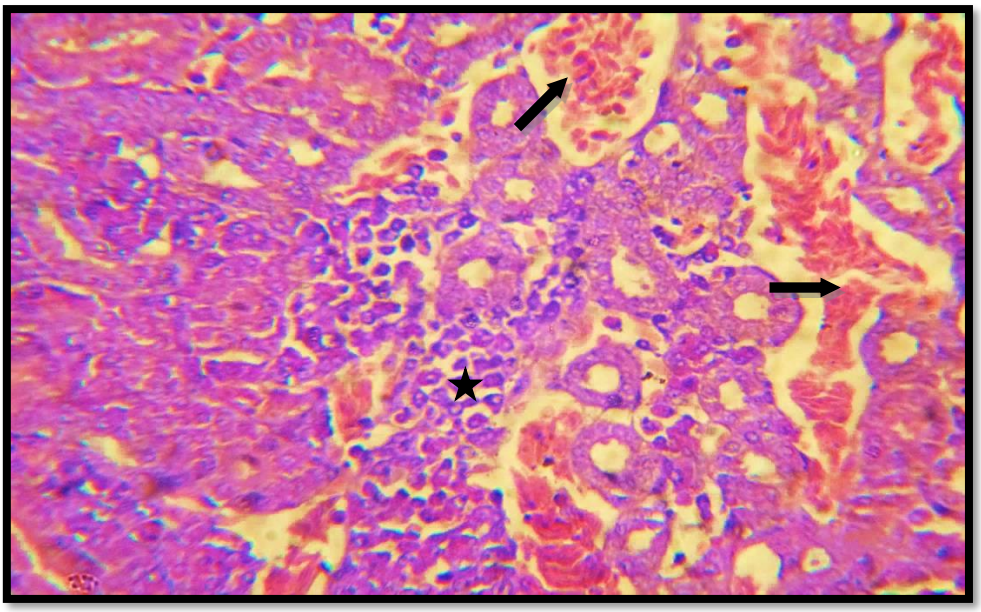
Photo, (26): Kidney of chicken infected with *Cl.perfringens* and treated with amoxicillin (gp. 6) at 39 days old showing mild nephrotic changes in a few renal tubules (arrow) within normal adjacent renal tissue (H&E, X400).



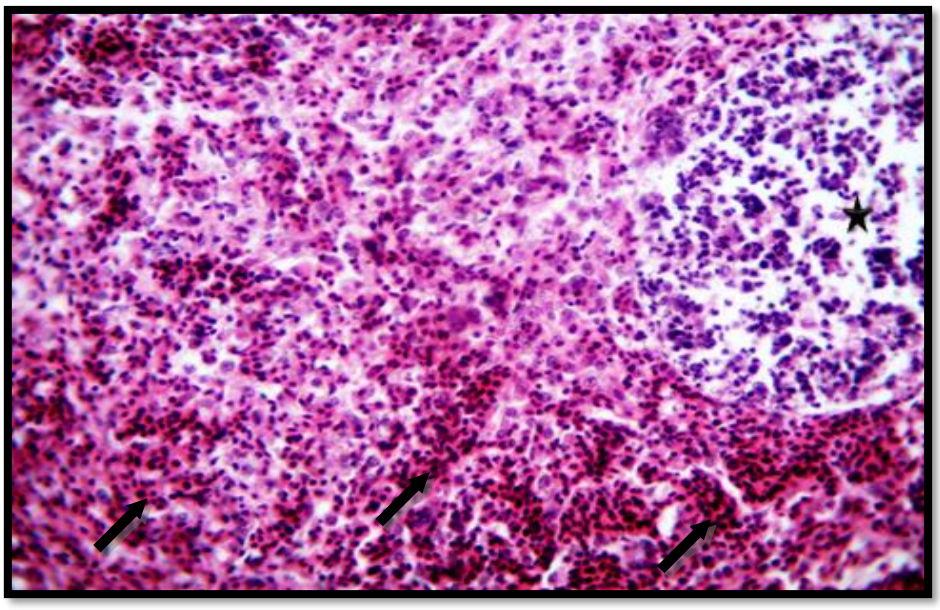
Photo, (27): Intestine of chicken prophylacted by organic acids before *Cl.perfringens* infection (gp. 7) at 25 days old showing regeneration of villus enterocytes and the villi restore its length and structures beside hyperplastic intestinal crypts (arrow) and gut associated lymphoid follicles (star) (H&E, X200).



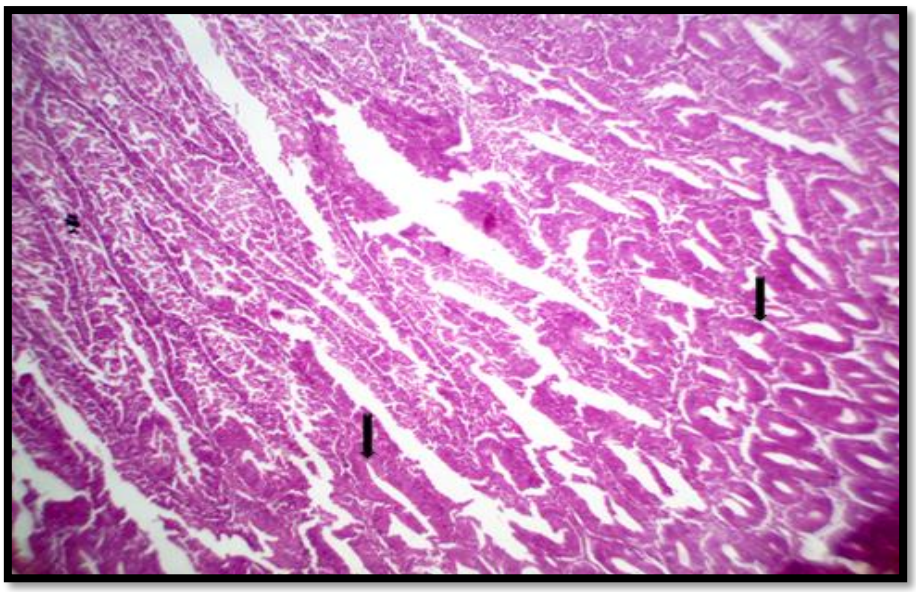
Photo, (28): Liver of chicken prophylacted by organic acids before *Cl.perfringens* infection (gp. 7) at 25 days old showing mild reversible changes in the hepatic cells and portal heterophilic aggregations (arrow) (H&E, X400).



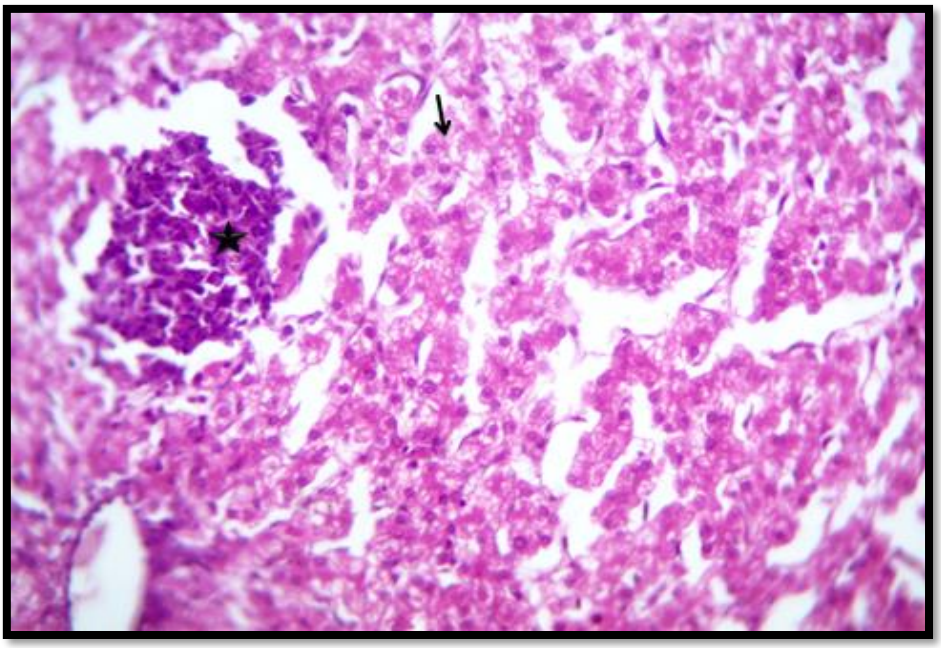
Photo, (29): Kidney of chicken prophylacted by organic acids before *Cl.perfringens* infection (gp. 7) at 25 days old showing severe diffuse congestion of renal blood vessels with WBCs infiltration, mainly heterophils (star) (H&E, X400).



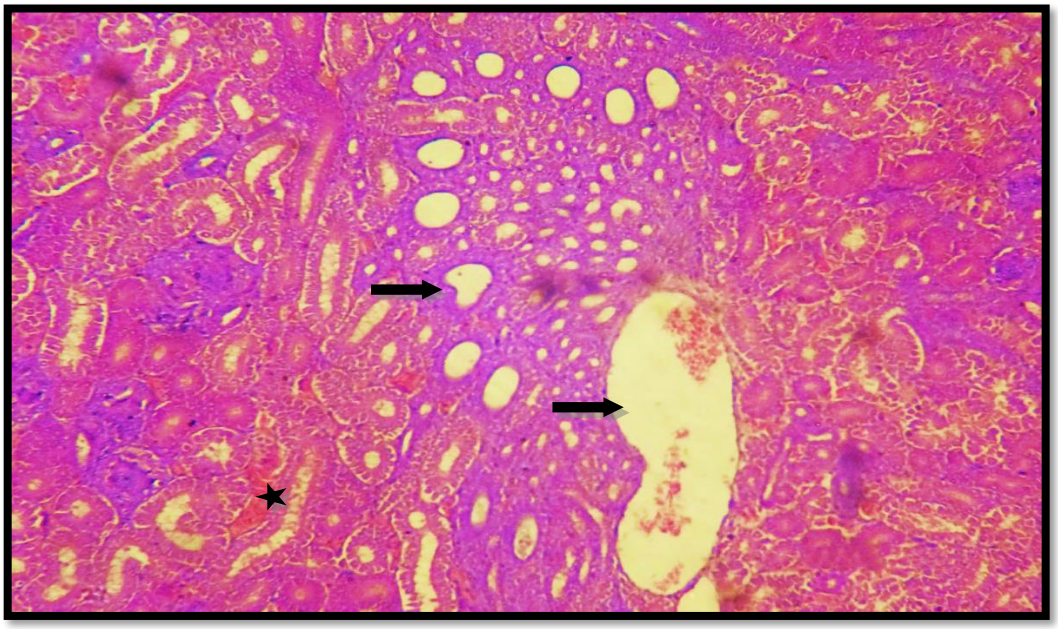
Photo, (30): Spleen of chicken prophylacted by organic acids before *Cl.perfringens* infection (gp. 7) at 25 days old showing mild congested sinusoids (arrows) and moderate proliferation of lymphocytes of white pulp (star) (H&E, X400).



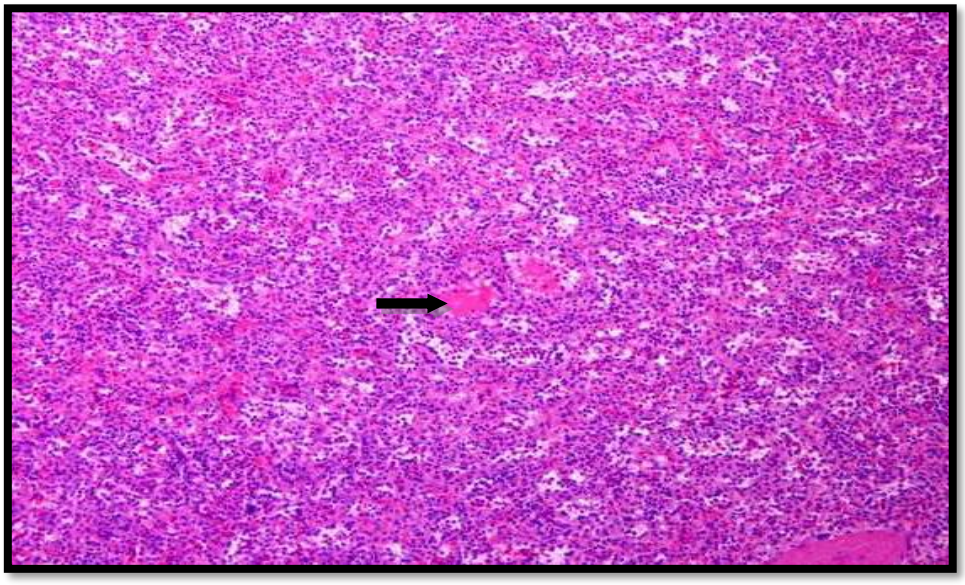
Photo, (31): Intestine of chicken prophylacted by organic acids before *Cl.perfringens* infection (gp. 7) at 32& 39 days old showing hyperplastic intestinal crypts with normal length and thickness of the intestinal villi (arrows) (H&E, X200).



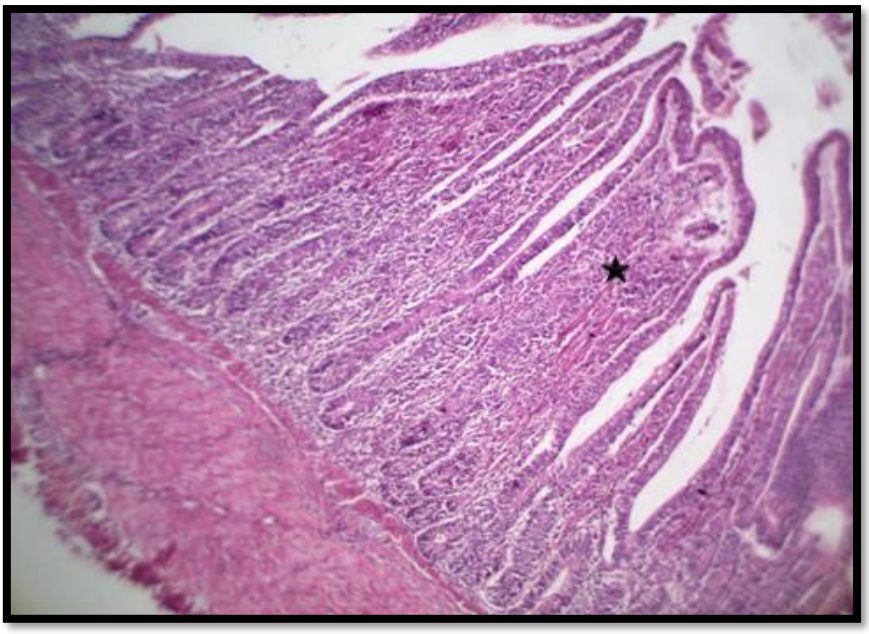
Photo, (32): Liver of chicken prophylacted by organic acids before *Cl.perfringens* infection (gp. 7) at 32& 39 days old showing minute leukocytic aggregation (star) with mild vacuolation in hepatic cells (arrow) H&E (X400).



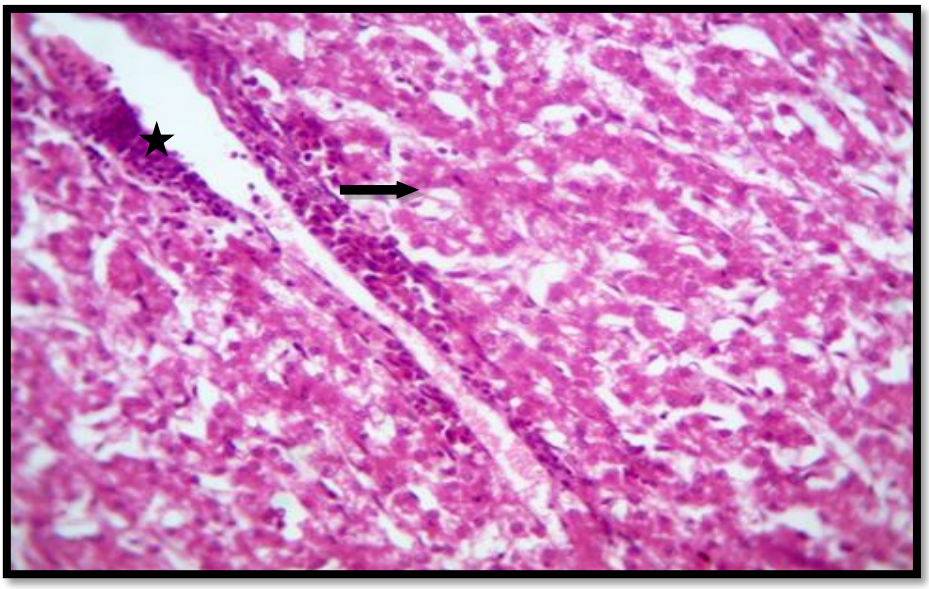
Photo, (33): Kidney of chicken prophylacted by organic acids before *Cl.perfringens* infection (gp. 7) at 32& 39 days old showing mild congestion of renal blood vessels (star) in addition to cystic dilatation of the renal tubules (arrow) (H&E, X400).



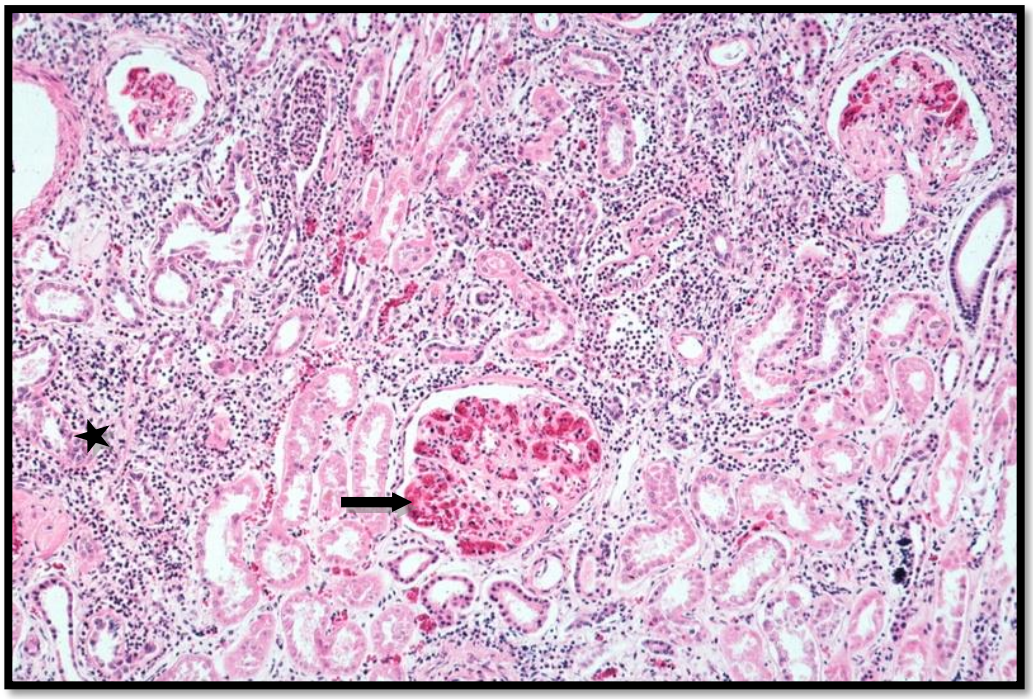
Photo, (34): Spleen of chicken prophylacted by organic acids before *Cl.perfringens* infection (gp. 7) at 32& 39 days old showing mild congested sinusoids (arrow) (H&E, X200).



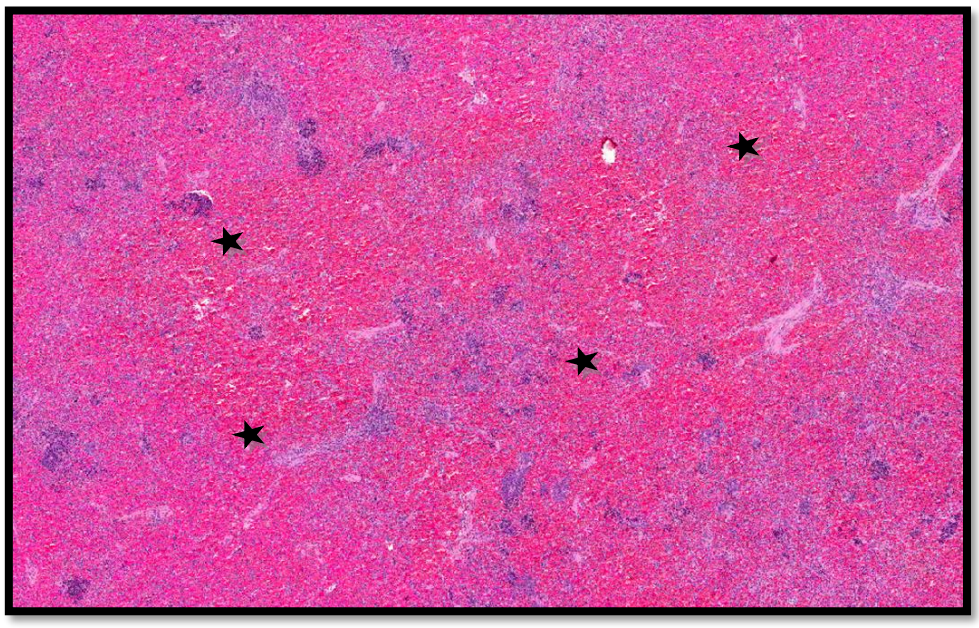
Photo, (35): Intestine of chicken infected with *Cl.perfringens* and treated with amoxicillin + organic acids (gp. 8) at 32 days old showing apparently mild thickened intestinal villi and fusion of intestinal tips (star) (H&E, X200).



Photo, (36): Liver of chicken infected with *Cl.perfringens* and treated with amoxicillin + organic acids (gp. 8) at 32 days old showing mild leukocytes in portal area (star) and reversible degenerative changes in the hepatic cells (arrow) (H&E, X400).



Photo, (37): Kidney of chicken infected with *Cl.perfringens* and treated with amoxicillin + organic acids (gp. 8) at 32 days old showing glomerular congestion (arrow) with interstitial polymorphnuclear infiltration (star) (H&E, X200).



Photo, (38): Spleen of chicken infected with *Cl.perfringens* and treated with amoxicillin + organic acids (gp. 8) at 32 days old showing congestion of the white pulp (stars) (H&E, X200).

Discussion

Clostridium perfringens is an anerobic bacterium causes necrotic enteritis (NE) in broiler chickens ***(Opengart and Songer, 2013).*** The disease causes high mortality rate and economic losses (***McDevitt et al., 2006).*** *Cl. perfringens* produces an alpha toxin resulting in enterotoxaemia in chickens ***(Timbermont et al., 2010)***. Amoxicillin is a broad spectrum antibiotic of β-lactamase group ***(Brennan et al., 2001).*** Over several years ago, necrotic enteritis in poultry has been controlled and treated by the use of antimicrobials, however, many countries banned their use in feed for human health concern. Thus alternative prevention methods such as organic acids are recommended ***(Kerry, 2007).***

In the present study, broiler chickens infected with *Cl. perfringens* (gp. 5) showed decreased appetite, depression, emaciation, ruffled feather and brownish diarrhea in addition to sudden death in some cases three days post-infection. The mortality rate was 25%. These signs may be due to direct effect of *Clostridium* and its toxins leading to lethal enterotoxaemia ***(Anders, 2006).*** As, these toxins may reach the liver and lead to disturbance of metabolic activity ***(Lovland and Kaldhusdal, 1999).*** The observed clinical signs in the present study agree with ***Asmaa (2016)*** and ***Aboubakr and Elbadawy (2017)*** as they observed loss of appetite, ruffled feathers, diarrhea, depression and emaciation with mortality rate 15% and 36.67% respectively in broiler chickens infected with *Cl. perfringens*.

Our results reported that groups (6 - 8) showed mild clinical signs with a mortality rate of 15%, 10% and 10% respectively. These results may be due to bactericidal effect of amoxicillin that acts by inhibition of

cell wall mucopeptide biosynthesis during bacterial multiplication ***(Nagaralli et al., 2002)*** which helps in reducing the clinical signs and mortality rate. Keeping with these lines, in vivo experimental studies of ***Lankriet et al., (2010)*** referred to the efficacy of amoxicillin in controling of *Cl. perfringens* infection. Moreover, in vitro studies proved efficacy of amoxicillin in the control of necrotic enteritis disease ***(Sarkar et al., 2013*** and ***Koutoulis et al., 2015).*** Chickens supplemented with organic acids followed by *Cl. perfringens* infection (gp. 7) showed depression, mild diarrhea and mortality rate was 10%. This may be due to the inhibitory effect of organic acids on microflora colonizations through lowering the pH of intestine and consequently reducing the bacterial growth. ***Mikkelsen et al., (2009)*** mentioned that growth of *Cl. perfringens* was suppressed by supplementation of organic acids. In addition, supplementation of organic acids in poultry diets affects colonization of pathogenic bacteria on the intestinal wall ***(Abudabos and Al-Mufarrej, 2014).*** Moreover, reduction of *Enterobacteriaceae* count in ileum of broilers was achieved by concurrent use of antibiotic and organic acids ***(Alp et al., 1999)***.

Regarding to body performance, broiler chickens treated with amoxicillin (gp. 2) showed a significant increase in body weight gain with decrease of FCR at 32 and 39 days old when compared with the normal control. This effect might be due to the antimicrobial effect of the drug by inhibiting the pathogenic microorganisms which damage the intestinal epithelium and the non-pathogenic commensals which compete for growth factors in the gut as mentioned by ***Aboubakr and Elbadawy (2017).*** Our results agree with ***El-Bayoumi et al., (2014)***; ***Hao et al., (2014)*** and ***Aboubakr and Elbadawy (2017)*** who recorded a significant increase in body weight and body weight gain with an improvement in

FCR of chickens administrated amoxicillin. Also, addition of organic acids to the drinking water of broiler chickens (gp. 3) produced a significant increase in body weight gain with decrease in FCR compared with normal control. These results may be due to the low pH of the intestine caused by organic acids that henders the proliferation of harmful bacteria and favors the growth of beneficial bacteria. Moreover, acidic environment of the gut stimulates the secretion of cholecystokinin, pepsin and gastrin as mentioned by ***Hayat et al., (2014)***. In addtition, acidic environment allows good absorption and digestion of nutrients and consequently improvement of general health condition ***(Dibner, 2004).*** Similar results were mentioned by ***Eman et al., (2012)*** and ***El-Bayoumi et al., (2014).*** Histopathological examination of the intestine of chickens administrated organic acids (gp. 3) revealed an increase in the height of the intestinal villi. This increase in the villus height may be due to short-chain fatty acid effect which leads to stimulation and proliferation of the normal crypt cells and enhancing of healthy tissue turnover and maintenance. Our results agree with ***Panda et al., (2009); Kum et al., (2010)*** and ***Adil et al., (2011)*** who reported that organic acids supplementation in the broiler's diet significantly improved the villus length. Administration of both amoxicillin and organic acids in the drinking water of healthy broiler chickens (gp. 4) resulted in a significant improvement in body weight gain and FCR of treated birds. This effect is based on cumulative effect of both amoxicillin and organic acids which has been discussed before.

Cl. perfringens infected chickens (gp. 5) revealed a significant decrease in feed consumption, body weight and body weight gain coupled with a significant increase in FCR along the experimental periods. Reduction of body weight gain and increased FCR could be attributed to

the deleterious effect of the pathogen and its toxins on liver and intestinal tissues where inflammation and disturbance in metabolic activity lead to reduction of absorbed nutrients from the gut. Similar discussion was mentioned by ***Miah et al., (2011)***. These results agree with ***Asmaa (2016)*** and ***El-Sheikh et al., (2018)*** who recorded a significant decrease in feed consumption, body weight and body weight gain with an increased FCR in broiler chickens infected with *Cl. perfringens*. Our results were confirmed by histopathological changes recorded in liver and intestine as destruction of intestinal mucosa and excessive damage to the villi and epithelial cells of the intestine. In addition, the intestinal lumen contained hemorrhagic exudates and epithelial cells mixed with leukocytes. Also, congestions in the hepatic blood vessels and portal areas contained leukocytes mainly heterophils were noticed. Our findings agree with ***Brennan et al., (2001)*** and ***Haesbrouk et al., (2011)*** who found congestion, necrosis and diffuse hemorrhage in intestinal villi. The latter author suggested that *Cl. perfringens* toxins and proteolytic enzymes are responsible for the initial stage of necrotic enteritis where the villi are first affected at the level of basement membrane.

Moreover, chickens infected with *Cl. perfringens* and treated with amoxicillin (gp. 6), chickens supplemented with organic acids followed by *Cl. perfringens* infection (gp. 7) and chickens in gp. (8) showed a significant increase in body weight and body weight gain with an improvement in FCR compared to the infected untreated group. These results may be due to the efficacy of amoxicillin and/or organic acids in controlling and treating of *Cl. perfringens* infection. The best result was obtained in gp. (8) by combination of amoxicillin and organic acids. Our results agree with ***El-Bayoumi et al., (2014)*** and ***El-Sheikh et al., (2018)*** who found a significant increase in B.W and B.W.G with an

improvement in feed consumption and FCR in amoxicillin and organic acids treated groups compared to the infected untreated group. Our results confirmed with histopathological examination as the degenerative lesions were subsiding in *Cl. perfringens* infected broilers and treated with amoxicillin and/or organic acids (gps. 6-8).

Regarding to erythrogram, the obtained data showed non-significant effect for amoxicillin on the hematological parameters of healthy chickens (gp. 2).This finding agree with ***Turcu et al., (2011)*** who observed non-significant changes in RBCs count, Hb content, PCV, MCV and MCHC. On the other hand, ***El-Shahat (2014)*** and ***Ayana et al., (2016)*** reported a significant decrease in RBCs count and Hb content in healthy chickens treated with amoxicillin. This discrepancy may be due to the variation in dose, duration and age of administration. Furthermore, our results revealed that healthy broilers administered organic acids (gp. 3) had no significant effect on hematological parameters. These results agree with that of ***Zaib et al., (2016)*** and ***Eugenes et al., (2018)*** who noticed that feeding of organic acids to broiler chickens had no significant effect on erythrogram.

Chickens infected with *Cl. perfringens* (gp. 5) showed a significant decrease in RBCs count, Hb concentration and PCV with development of normocytic normochromic anemia at 25 days old and macrocytic hypochromic anemia at 32 and 39 days old. From our opinion the normocytic normochromic anemia that developed at 25 days old may be due to the effect of *Cl. perfringens* on RBCs (decreased the life span) and kidney (decreased erythropoietin hormone production). The macrocytic hypochromic anemia reflected the response of bone marrow to anemia. Our results agree with ***El-Shahat (2014), Asmaa (2016), Marwa (2017)***

and ***El-Sheikh et al., (2018)*** who found a significant decrease in RBCs count, Hb content and PCV of broilers infected with *Cl. perfringens* with developmwnt of macrocytic hypochromic anemia. They attributed their results to the destruction of red blood cells by clostridial toxins. ***El-Boraay (1991)*** mentioned that hemolysis was induced by clostridial toxins through breakdown of phospholipids of erythrocytic membranes.

Moreover, *Cl. perfringens* infected broilers treated with amoxicillin (gp. 6), chickens supplemented with organic acids followed by *Cl. perfringens* infection (gp. 7) and gp. (8) displayed a significant increase in RBCs count, hemoglobin content and PCV compared to the infected untreated group. These results may be due to the bactericidal effect of amoxicillin and inhibitory effect of organic acids on pathogenic bacteria.

Regarding leukogram, administration of amoxicillin to healthy chickens (gp. 2) resulted in non-significan changes in leukogram. Our results agree with that of ***Madhuchhanda et al., (2018)*** who reported non-significant increase in TLC and heterophils accompanied by non-significant decrease of lymphocytes and eosinophils in layer hens. On the other hand, ***Turcu et al., (2011)*** and ***El-Shahat (2014)*** noted a significant leukocytosis, heterophilia and lymphocytosis after amoxicillin administration in healthy chickens. From our opinion this may be due to the difference in in dose and age of administration. Concerning the effect of organic acids on leukogram of healthy chickens (gp. 3), the present study showed non-significant changes. The obtained data agree with that of ***Ndelekwute et al., (2016) and Zaib et al., (2016)*** who recorded non-significant differences in number of WBCs and heterophils of broiler chickens supplemented with organic acids.

Infected non-treated chickens (gp. 5) showed a significant leukocytosis, heterophilia, monocytosis with a significant lymphopenia. These results may be due to the bacterial infection and inflammation that lead to leukocytosis, heterophilia and monocytosis which are responsible for phagocytosis of the infective microorganism and damaged cells. Alterations of leukogram in the present study may be due to inflammatory reactions caused by infection ***(Doxey, 1983).*** Moreover, leukocytosis is a characteristic feature of bacterial infection ***(Fraser et al., 1991).*** Also our results partially agree with ***Gheith et al., (2011)*** who detected leukocytosis, monocytosis and lymphocytosis in infected chickens. Keeping with these lines, ***El-Shahat (2014)*** and ***El-Sheikh et al., (2018)*** demonstrated a significant leukocytosis, heterophilia, monocytosis, basophilia and eosinophilia in *Cl. perfringens* infected broilers. On contrary, ***Saleh et al., (2011***) reported a significant heterophilia, leukopenia, eosinopenia, basopenia with non-significant change in monocytes in *Cl. perfringens* infected chickens. From our opinion, these variations may be related to the stage of infection or immune status of the birds.

On the other hand, treatment of *Cl. perfringens* infected broilers with amoxicillin (gp. 6) or supplementation with organic acids before infection (gp. 7) or both (gp. 8) induced an improvement in leukogram when compared with the infected non-treated chickens. Based on these findings, an improvement towards the normal level suggests the efficacy of treatment. Our results agree with ***El-Shahat (2014)*** who found a significant decrease in TLC, monocytes, heterophils and lymphocytes in broilers infected with *Cl. perfringens* and treated with amoxicillin compared with infected group. Also agree with ***El-Sheikh et al., (2018)*** who found a significant decrease in TLC, monocytes, heterophils and

lymphocytes in broilers infected with *Cl. perfringens* and treated with acidifier compared with infected group.

Regarding to the proteinogram, groups (2-4) revealed non-significant changes in proteinogram. The obtained data agree with ***Hassan (1996); El-Shahat (2014)*** *and* ***Aboubakr and Elbadawy (2017)*** who mentioned that administration of amoxicillin to healthy broiler chickens resulted in non-significant increase in serum total proteins, albumin and globulins. Also, ***Franciszek et al., (2013); Ndelekwute et al., (2016)*** and ***Eugenes et al., (2018)*** recorded non-significant changes in proteinogram of chickens supplemented with organic acids. On contrary, ***Kaya and Tuncer (2009)*** detected a significant decrease in serum total proteins level of broilers fed organic acids, however, a significant increase in serum levels of total proteins and globulins in broiler chickens supplemented with organic acids was reported by ***Hedayati et al., (2015)*** and ***Zaib et al., (2016).***

Moreover, the present study revealed that infection with *Cl. perfringens* in broiler chickens (gp. 5) resulted in a significant decrease in serum levels of total proteins and albumin coupled with a significant increase in γ and total globulins. The hypoproteinemia is due to hypoalbuminemia. The hypoalbuminemia may be due to hepatic damage, renal insufficiency, malnutrition and gastrointestinal disturbances ***(Garcia et al., 2010).*** Our histopathological findings confirm these results where intestine showed excessive damage to the villi and epithelial cells and liver showed necrosis and degenerative changes. The recorded increase in serum globulins level is due to the increase of γ globulin level which reflected the immune response against *Cl. perfringens* infection ***(Panigraphy et al., 1989)***. These findings agree with ***Sahar (2001);***

Thrall (2004); El-Shahat (2014) and ***Aboubakr and Elbadawy (2017)*** who showed the same findings in *Cl. perfringens* infected chickens. However, ***El-Sheikh et al., (2018)*** indicated a significant decrease in serum levels of α, β, γ-globulins and total globulins in *Cl. perfringens* infected chickens. Moreover, ***Saleh et al., (2011)*** recorded a significant increase in serum levels of total proteins, albumin and globulins in chickens infected with *Cl. perfringens.*

Groups (6-8) showed a significant increase in serum levels of total proteins and albumin coupled with significant decrease in γ and total globulins when compared with the infected non-treated group. The concurrent use of amoxicillin and organic acids post *Cl. perfringens* infection (gp. 8) regained its normal serum levels of total proteins and albumin at 39 days old suggesting the efficacy of this combination.

Concerning liver enzymes, healthy broiler chickens treated with amoxicillin (gp. 2) showed a transient significant increase in the serum activities of AST, ALT and ALP at 32 days old. Our results agree with ***El-Shahat (2014)*** and ***Aboubakr and Elbadawy (2017)*** who recorded a significant increase in liver enzymes in chickens administered amoxicillin in therapeutic dose. In contrast, ***Al Harthi, (2004)*** recorded non-significant changes in the serum activities of AST and ALT in amoxicillin treated chickens. On the othe hand, ***Ayana et al., (2016)*** reported a significant decrease in the serum activities of AST and ALT in amoxicillin treated chickens at sub-therapeutic dose. This discrepancy may be due to variation of dose and duration of administration. Histopathological examination of the liver of healthy broiler chickens treated with amoxicillin (gp. 2) showed mild vacuolation in the hepatic cells. This may be due to metabolism and biotransformation of the drug in

liver. Groups (3 and 4) showed non- significant changes in the serum activity of liver enzymes. This indicates that organic acids didn't have negative impact on liver (gp. 3). Moreover, organic acids corrected the negative impact of amoxicillin in gp. (4). Our results agree with ***Abudabos et al., (2015)*** who reported non- significant changes in the serum activities of AST and ALT after organic acids administration.

Infection of broiler chickens with *Cl. perfringens* (gp. 5) resulted in a significant increase in the serum activities of AST, ALT and ALP. This may be due to hepatic damage and biliary stasis caused by *Cl. perfringens* infection or its toxins. Damage of hepatic cells causes a release of cellular enzymes ***(Lovland and Kaldhusdal, 1999).*** On the same ground, a significant increase in hepatic enzymes was recorded in *Cl. perfringens* infected broilers **(*Saleh et al., 2011; Asmaa, 2016*** and ***El-Sheikh et al., 2018)***. The hepatic damage was also observed and confirmed in our histopathological study where liver showed degenerative changes, necrosis and microsteatosis.

On the other hand, treatment of *Cl. perfringens* infected broilers with amoxicillin (gp. 6) or supplementation of organic acids before infection (gp. 7) and gp. (8) induced a significant decrease in the serum activities of AST, ALT and ALP when compared with the infected chickens (gp. 5). This may be due to the bactericidal effect of amoxicillin (gp. 6) or may be due to the inhibitory effect of acidifier on bacterial growth through interrupting oxidative phosphorylation and inhibition of ATP ***(Hedayati et al, 2015)***. Treatment of *Cl. perfringens* infected chickens with amoxicillin and organic acids (gp. 8) showed normalized hepatic enzymes at 39 days old indicating their efficacy in treatment of the infection. Our results agree with ***Aboubakr and Elbadawy (2017)*** and

El-Sheikh et al., (2018) who detected a significant decrease in the serum activities of ALT, AST and ALP in *Cl. perfringens* infected broilers treated with amoxicillin or organic acids respectively compared with infected non-treated group. Our clinical results were confirmed histopathologically, where the liver showed mild reversible changes in the hepatic cells and portal heterophilic aggregations in gps. (6-8). These obtained data confirmed the positive effect of amoxicillin and organic acids in control of necrotic enteritis caused by *Cl. perfringens*. These results agree with ***El-Bayoumi et al., (2014)*** who found hydropic degeneration of the hepatocytes in chickens infected by *Cl. perfringens* and treated with amoxicillin. Also, ***Awaad et al., (2011)*** stated that liver exhibited vacuolar degeneration after using of organic acids as prophylactic against *Cl. perfringens*.

Concerning kidney function tests, administration of amoxicillin and/or organic acids to healthy broiler chickens (gps. 2- 4) had non-significant changes in the serum levels of uric acid, creatinine, sodium and potassium. This indicates that both amoxicillin and organic acids has no deleterious effect on kidneys.

Moreover, the recorded increase in serum uric acid and creatinine levels in *Cl. perfringens* infected chickens (gp. 5) may be due to the effect of clostridial toxins on kidneys. The degeneration of renal tubules prevented excretion of uric acid and creatinine leading to increase of their levels in serum of infected birds ***(Kaneko, 1980).*** These findings agree with ***Thrall (2004); Saleh et al., (2011); Aboubakr and Elbadawy (2017)*** and ***Marwa (2017)*** who noticed a significant increase of serum uric acid and creatinine in broiler chickens experimentally infected with *Cl. perfringens*. Regarding the histopathological findings in the present work,

Cl. perfringens infected chickens (gp. 5) showed congestion of renal blood vessels and degeneration of renal tubules in broiler chickens infected with *Cl. perfringens*. These results agree with ***Rahamathulla et al., (1994)*** who noticed severe degenerative changes in all parenchymatus organs of *Cl. perfringens* infected broilers. Moreover, ***Asmaa (2016)*** demonstrated congestion of renal blood vessels in infected birds. Decrease of serum sodium and increase of potassium levels in *Cl. perfringens* infected chickens (gp. 5) were noticed. This may be due to the diarrhea, loss of fluids, cellular necrosis and kidney damage under the effect of clostridial toxins. Our data agree with ***Ayana et al., (2016)*** and ***Zivcov et al., (2016)*** who reported a highly significant decrease in serum sodium level in case of diarrhea due to gastrointestinal disturbance.

Moreover, treatment of *Cl. perfringens* infected broilers with amoxicillin (gp. 6) or supplementation of organic acids before infection (gp. 7) induced a significant increase in serum level of sodium coupled with a significant decrease in serum level of potassium, uric acid and creatinine when compared with the infected group (gp. 5). This may be due to amoxicillin and organic acids ameliorated the deleterious effect of *Cl. perfringens* consequently, improved kidney lesions as observed histopathologically. Our results agree with ***Dalia et al., (2016); Aboubakr and Elbadawy (2017); Omnia (2017)*** and ***El-Sheikh et al., (2018)*** who detected a significant decrease in serum levels of uric acid and creatinine in *Cl. perfringens* infected broilers treated with amoxicillin or organic acids compared with the infected group. Serum levels of sodium, potassium, uric acid and creatinine returned to its normal levels at 39 days old in broilers infected with *Cl. perfringens* and treated with amoxicillin and organic acids (gp. 8) due to efficacy of combination of amoxicillin with organic acids in treatment of infection.

Poultry health is associated with the efficacy of the immune system ***(Sarker et al., 2000).*** Pathogenic infection stimulates monocytes to be functional macrophages ***(Qureshi, 2003).*** Phagocytosis of monocytes-macrophages reflects the extent of resistance of chickens to various diseases ***(Beal et al., 2005).*** IgM and IgG isotypes are indicators for resistance of disease in poultry ***(Star et al., 2007).*** Restriction of severity of infection and inhibition of inflammatory responses are conducted by direct interaction of IgM antibodies with pathogen ***(Ehrenstein and Notley, 2010).***

Healthy chickens administrated amoxicillin and/or organic acids groups (2-4) showed non-significant changes in the serum levels in phagocytic % and index, serum levels of IgM and IgG. This may be due to amoxicillin and organic acids have no deleterious effect on immune system of birds.

Broiler chickens infected with *Cl. perfringens* (gp. 5) showed a significant decrease in phagocytic % and index coupled with a significant increase in serum levels of IgM and IgG when compared with normal control group. The reduction in phagocytic activity may be due to the negative effect of infection on the immune system of chicken. Recorded increase in serum levels of globulins reflected the immune response against *Cl. perfringens* infection ***(Panigraphy et al., 1989)***. Bacteria stimulates the immune system resulting in release of immunoglobulins ***(Kabir et al., 2004)***. These results agree with that of ***Hala et al., (2015); Chake et al., (2017)*** and ***El-Sheikh et al., (2018)*** who proved that *Cl. perfringens* infection in chicken resulted in a decrease in phagocytic index and phagocytic %. Histopathological examination of spleen of *Cl.*

perfringens infected chickens (gp. 5) showed necrosis, hemorrhage, vacuolation and depletion of the lymphocytes. This may be due to the deleterious effect of *Cl. perfringens* and its toxins. Our results agree with ***Alaqaby (2009)*** who reported hemorrhagic areas in the red pulp and lymphoid depletion from the white pulp.

Treatment of infected chickens with amoxicillin (gp. 6) or organic acids (gp. 7) or by both (gp. 8) showed an improvement of the examined immunological parameters and the best results were obtained by combination of both amoxicillin with organic acids. This may be due to the curative effect of both amoxicillin and organic acids against *Cl. perfringens*. Our results agree with ***Dalia et al., (2016)*** who stated that phagocytic % and index significantly increased in infected chickens treated with acidifiers compared with the infected non-treated group. Our clinical results are confirmed histopathologically, where the spleen showed mild congested sinusoids in addition to mild lymphoid depletion of the white pulp.

Summary

Clostridium perfringens is a bacterial pathogen causing severe economic losses through mortality and weight loss in broiler chickens. The present work aims to study the effect of *Cl. perfringens* infection on broiler chickens and compares between the effect of amoxicillin and / or organic acids on *Cl. perfringens* infection through the evaluation of hemogram, blood chemistry and immune response.

One hundred and sixty one-day old chicks were divided into eight equal groups. Group (1): normal control, Group (2): chickens administrated amoxicillin (15 mg/kg.b.wt.) from 25-29 days old, Group (3): chickens administrated organic acids (1 ml /l water) from 1st day old till the end of the experiment , Group (4): chickens administrated amoxicillin and organic acids from 25-29 days old, Group (5): *Clostridium perfringens* type A (1.9×10^{9} organism/ml) infected chickens at 19th days old 3 times orally day after day, Group (6): *Clostridium perfringens* infected chickens and treated with amoxicillin (15 mg/kg.b.wt.) for 5 successive days, Group (7): chickens administrated organic acids (1 ml /l water) then infected with *Cl. perfringens* and Group (8): chickens infected with *Cl. perfringens* and treated with both amoxicillin and organic acids.

Clinical signs were observed. Body weight and feed consumption were determined weekly. Blood and serum samples were collected at 25, 32 and 39 days old.

Cl. perfringens infected chickens (gp. 5) showed a significant decrease in appetite, depression, emaciation and ruffled feather and in some cases brownish diarrhea and sudden death. The mortality rate was 25%. Groups (6-8) showed mild clinical signs and the mortality rate was 15%, 10% and 10% respectively.

A significant increase in body gain coupled with a significant decrease in FCR were reported in groups (2- 4) along the experimental periods except groups (2 and 4) at 25 days old showed non-significant changes. The best feed conversion rate was recorded in organic acids administrated chickens (gp. 3). Moreover, a significant ($P<0.05$) decrease in feed consumption, body weight and body weight gain coupled with an increase in FCR of chickens infected with *Cl. perfringens* (gps. 5- 8) was noticed along experimental periods except gp. (8) which was treated with amoxicillin and organic acids showed non-significant changes in feed consumption, body weight gain and FCR at 39 days old when compared with the normal control group.

The erythrogram and leukogram of groups (2- 4) showed non-significant changes in all examined parameters along the experimental periods. *Cl. perfringens* infected chickens (gps. 5- 8) showed a significant decrease in RBCs count, Hb concentration and PCV of with development of normocytic normochromic anemia at 25 days old and macrocytic hypochromic anemia at 32 and 39 days old except for (gp. 8) at 39 days old which regained near its normal values. While, a significant increase in total leukocytic count, heterophils, monocytes with a significant decrease in lymphocytes of groups (5-8) was detected along the experimental periods except for (gp. 8) at 39 days old which regained near its normal values.

Concerning the proteinogram, groups (2- 4) showed non-significant changes in proteinogram along the experimental periods. Chickens infected with *Cl. perfringens* (gps. 5- 8) showed a significant decrease in serum levels of total proteins and albumin with a significant increase in serum levels of γ and total globulins along the experimental periods except for (gp. 8) which nearly regained its normal levels of serum total proteins and albumin at 39 days old when compared with the normal control group.

Amoxicillin administrated group (2) showed a significant transient increase in the serum activities of AST, ALT and ALP at 32 days old only coupled with normal serum levels of uric acid, creatinine, sodium and potassium. Groups (3 and 4) showed non-significant changes in all examined parameters of liver and kidney function tests along the experimental periods. While *Cl. perfringens* infected broilers (gps. 5- 8) showed a significant increase in serum activities of AST, ALT, ALP and levels of uric acid, creatinine and potassium with a significant decrease in serum sodium level along the experimental periods except birds in (gp. 8) showed non-significant changes at 39 days old.

Concerning cellular and humoral immunity parameters, groups (2-4) showed non-significant changes in all examined parameters along the experimental periods. The current work showed that infection of broilers with *Cl. perfringens* (groups 5- 8) resulted in a significant decrease phagocytic % and index with a significant increase of IgM and IgG along the experimental periods except for (gp. 8) which nearly regained its normal levels at 39 days only.

Conclusion

From the present study, it could be concluded that:

- *Clostridium perfringens* infection negatively affects body weight gain, FCR, hematological, biochemical and immunological parameters in broiler chickens.

- Amoxicillin was effective in treatment of *Cl. perfringens* infection and improving of measured parameters towards its normal levels.

- Administration of organic acids provided a protective effect against *Cl. perfringens* infection and reduced clinical signs of the disease beside its positive effect on body weight gain and FCR.

- Treatment of *Cl. perfringens* infection with both of amoxicillin and organic acids was the best treatment and the best obtained results. Using both treatments at the same time resulted in a positive effect in treatment, ameliorating the severity of infection and a significant improvement in some immunological and biochemical parameters.

➢ Recommendation:

Using of amoxicillin and organic acids in treatment of *Cl. perfringens* infection of broiler chickens.

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الملخص العربى

الكلوستريديم بيرفرنجنز هو مرض بكتيري يصيب دجاج التسمين و يسبب خسائر اقتصادية ضخمة من خلال الوفيات و نقص الوزن الحي للطائر و ارتفاع معدل التحويل الغذائي.

يهدف هذا العمل إلى دراسة تأثير العدوي بالكلوستريديم بيرفرنجنز علي دجاج التسمين و مقارنة تأثير الأموكسي سيلين و/ أو الأحماض العضوية علي العدوي بالكلوستريديم بيرفرنجنز من خلال تقييم معدل التحويل الغذائي و صورة الدم و كيمياء الدم و الاستجابة المناعية.

تم تقسيم مائة و ستون كتكوت عمر يوم واحد الي ثمان مجموعات متساوية :

- مجموعة (1): مجموعة ضابطة.
- مجموعة (2): تم اعطائها الاموكسي سيللين (15 مج/ كجم وزن حي) يوميا من عمر 25-29 يوم في مياه الشرب.
- مجموعة (3): تم اعطائها أحماض عضوية (1 مل/ لتر ماء شرب) منذ اليوم الأول و حتي انتهاء التجربة.
- مجموعة (4): تم اعطائها الاموكسي سيللين (15 مج/ كجم وزن حي) و الاحماض العضوية من عمر 25-29 يوم.
- مجموعة (5): معداة معمليا بالكلوستريديم بيرفرنجنز نوع (أ) بجرعة 1.9 10^9x ميكروب لكل مل عند عمر 19 يوم 3 مرات يوم بعد يوم.
- مجموعة (6): معداة معمليا بالكلوستريديم بيرفرنجنز نوع (أ) و معالجة بالاموكسي سيللين (15 مجم/ كجم وزن حي) لمدة 5 أيام متتالية في ماء الشرب.
- مجموعة (7): تم اعطائها أحماض عضوية (1 مل/ لتر ماء شرب) منذ اليوم الأول و حتي انتهاء التجربة و تمت اصابتها معمليا بعدوى الكلوستريديم بيرفرنجنز نوع (أ).
- مجموعة (8): معداة معمليا بالكلوستريديم بيرفرنجنز نوع (أ) و معالجة بالاموكسي سيللين و الأحماض العضوية لنفس المدة.

تم ملاحظة الأعراض الظاهرية و تم تسجيل الوزن الحي للطائر و معدل استهلاك العليقة أسبوعيا. تم تجميع عينات دم و مصل عند عمر 25و 32 و 39 يوم.

نتج عن الإصابة بالكلوستريديم بيرفرنجنز اسهال بني في بعض الحالات و نقص في الشهية و عدم حيوية الريش و حدوث نفوق مفاجئ بمعدل 25% في الطيور المصابة مجموعة (5). بينما في الطيور المصابة و المعالجة بالأموكسي سيللين أو/مع الأحماض العضوية مجموعات (6-8) كان هناك أعراض ظاهرية طفيفة و معدل نفوق بلغ 15% و 10% و 10% علي التوالي.

أظهرت الدراسة حدوث زيادة معنوية في وزن الجسم المكتسب و نقص معدل التحويل الغذائي في الطيور السليمة المعالجة بالأموكسي سيللين أو المعطاة الأحماض العضوية مجموعات (2-4) علي جميع فترات التجربة ما عدا عند عمر 25 يوم مجموعات (2و4) لم يكن بها تغيير معنوي مقارنة بالمجموعة الضابطة بينما حدث نقص معنوي في وزن الجسم المكتسب للدجاج المصاب بعدوي الكلوستريديم بيرفرنجنز مع ارتفاع معدل التحويل الغذائي مجموعات (5 - 8) علي جميع فترات التجربة فيما عدا مجموعة (8) عند عمر 39 يوم لم يكن بها تغيير معنوي في وزن الجسم المكتسب و معدل التحويل الغذائي عند مقارنتها بالمجموعة السليمة الضابطة. أفضل معدل تحويل غذائي كان في مجموعة (3) المعطاة الأحماض العضوية.

مجموعات الدجاج (2-4) لم يحدث بها أي تغيير في القيم الخاصة بفحص كرات الدم الحمراء و كرات الدم البيضاء بينما اتضح حدوث نقص معنوي في عدد كرات الدم الحمراء و نسبة الهيموجلوبين و قيم الهيماتوكريت و الخلايا الليمفاوية و زيادة معنوية في العدد الكلي لكرات الدم البيضاء ووحيدات النواة و متغايرة الحبيبات في الدجاج المعدي بالكلوستريديم بيرفرنجنز مجموعات (5 - 8) مع تطور الأنيميا علي مدار الفترات التجريبية ما عدا مجموعة رقم (8) التي اقتربت من العودة الي معدلاتها الطبيعية عند عمر 39 يوم.

مجموعات الدجاج (2-4) لم يحدث بها أي تغيير في القيم الخاصة بمستويات البروتينات. حدث نقص معنوي في البروتين الكلي لمصل الدجاج المعداه بالكلوستريديم بيرفرنجنز مجموعات (5 - 8) علي مدار الفترات التجريبية نتيجة نقص الالبيومين و زيادة في جلوبيولينات الجاما والجلوبيولينات الكلية مقارنة بالمجموعة السليمة الضابطة ما عدا مجموعة رقم (8) التي اقترب مستوي مصل البروتين الكلي و الألبيومين من العودة الي معدلاته الطبيعية عند عمر 39 يوم.

اتضح من الدراسة أن الأموكسي سيلين مجموعة (2) أحدث زيادة معنوية مؤقتة فقط عند عمر 32 يوم في مستوي انزيم الاسبرتيت امينو ترانسفيريز و انزيم الالانين امينو ترانسفيريز و انزيم الفوسفاتيز القاعدي في مصل الدجاج مقارنة بالمجموعة السليمة الضابطة. مجموعات الدجاج (3و4) لم يحدث بها أي تغيير في كل المعاملات التي تم فحصها. كما حدث ارتفاع في نشاط انزيم الاسبرتيت امينو ترانسفيريز و انزيم الالانين امينو ترانسفيريز و انزيم الفوسفاتيز القاعدي و مستوي حمض البوليك و الكرياتنين و مستوي البوتاسيوم بالاضافة الي انخفاض مستوي الصوديوم في مصل الدجاج المعدي بالكلوستريديم بيرفرنجنز مجموعات من (5- 8) علي طول فترات التجربة ما عدا مجموعة (8) التي اقتربت من العودة الي معدلاتها الطبيعية عند عمر 39 يوم .

مجموعات الدجاج (2-4) لم يحدث بها أي تغيير في القيم الخاصة بالمعاملات المناعية التي تم فحصها. نتج عن الإصابة بالكلوستريديم بيرفرنجنز مجموعات من (5 – 8) حدوث نقص معنوي في نسبة معامل الخلايا المتبلعمة و مؤشر البلعمة و زيادة معنوية في الجلوبيولينات المناعية مقارنة بالمجموعة السليمة الضابطة علي طول فترات التجربة ما عدا مجموعة (8) التي اقتربت من العودة الي معدلاتها الطبيعية عند عمر 39 يوم .

الخـلاصـــة

أظهرت الدراسة أن الاصابة بعدوى الكلوستريديم بيرفرنجنز يؤثر سلبيا علي الوزن الحى للدجاج التسمين و معدل التحويل الغذائى و صورة الدم و القيم البيوكيميائية و المقاييس المناعية.

بالاضافة الى أن الأموكسي سيلين كان له تأثير واضح في علاج العدوي بالكلوستريديم بيرفرنجنز و عودة الخلل الموجود في صورة الدم و بعض القيم البيوكيميائية إلي اتجاه المعدل الطبيعي في الطيور المعالجة.

كما أن اضافة الأحماض العضوية قبل العدوي بالكلوستريديم بيرفرنجنز قد قدم درجة من الحماية ضد العدوي ظهر في انخفاض الأعراض الخاصة بالمرض بجانب تأثيره الايجابي علي الوزن الحي للطائر و معامل التحويل الغذائي.

علاج عدوي الكلوستريديم بيرفرنجنز بالأموكسي سيلين و الأحماض العضوية معا كان أفضل علاج و أفضل نتائج تم الحصول عليها. استخدام كلا من العلاجين معا في نفس الوقت كان له تأثير ايجابي في العلاج و التخفيف من شدة العدوي بالاضافة الي التحسن الملحوظ في بعض القيم و المقاييس المناعية و البيوكيميائية.

➢ التوصية:

استخدام كلا من الأموكسي سيلين و الأحماض العضوية في علاج العدوي بالكلوستريديم بيرفرنجنز في دجاج التسمين.

جامعة الزقازيق
كلية الطب البيطري
قسم الباثولوجيا الإكلينيكية

دراسات باثولوجية إكلينيكيــة علي تأثير العدوي بالكــولستريديـم في الدجــاج

رسالة مقدمة من

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معهد بحوث الصحة الحيوانية – الزقازيق

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