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EFFECT OF TYLOSIN AND SALINOMYCIN ON SOME BIOCHEMICAL AND HEAMATOLOGICAL PARAMETERS IN BROILER CHICKENS CHALLENGED WITH *CLOSTRIDIUM PERFRINGENS.*

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ABSTRACT

The aim of this study was to determine the effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed on liver and kidney function as well as on some heamatological parameters in broiler chicken challenged with necrotic enteritis caused by *Clostridium perfringens*. Two hundred fifty one-day old chick were divided into 5 groups: non-infected non-treated group, group infected with isolate *Clostridium perfringens* and non-treated, group infected and administrated with 100 ppm tylosin in feed, group infected and administrated with 60 ppm salinomycin in feed, and group infected and administrated with 100 ppm tylosin and 60 ppm salinomycin in combination. Blood samples were collected at one, 6, 15 days post treatment to investigate the liver and kidney function as well as on some heamatological parameters. The results showed administration of tylosin and salinomycin alone or in combination induced significant increase in liver enzyme activities and significant increase in uric acid and creatinine levels compared to non-infected non-treated group (control group). And induced significant improvement in liver enzymes, protein pictures, serum uric acid and creatinine levels when compared with infected non-treated chickens. And induced significant improvement in both erythrogram and leukogram parameters compared with infected non-treated groups.

KEYWORDS: Tylosin, Salinomycin, Necrotic enteritis, biochemical, heamatological.

1-INTRODUCTION

Clostridium perfringens is a Gram-positive, rod-shaped, anaerobic, spore-forming bacterium that is commonly found in soil, sewage and in the gastro-intestinal tract of animals and humans as a member of the normal gut microbiota. According to the current classification, *Clostridium perfringens* isolates are divided into five types (A, B, C, D and E) on the basis of the production of four major toxins (alpha, beta, epsilon and iota) **(Songer, 1996).** Certain strains of *Clostridium perfringens* type A cause necrotic enteritis in poultry **(Al-Sheikhly and Truscott 1977).**

Control of this condition is dependent upon a variety of elements including reducing exposure to potential dietary risk factors, reduction of concurrent enteric infections, particularly coccidiosis, and the use of feed additives with activity against *Clostridium perfringens* **(Ficken and Wages, 1997).**

Tylosin, is a macrolide antibiotic having bacteriostatic action against anaerobic bacteria, Gram-positive bacteria and mycoplasma **(Giguere 2006).**

Tylosin is considered as a bacteriostatic time-dependent antibacterial agent that inhibits bacterial protein synthesis through reversible binding to the 50S subunit of the ribosome **(Vannuffel and Cocito, 1996).**

The macrolide antibiotic tylosin has been shown to have in vitro activity against *Clostridium perfringens* **Stutz and Lawton (1984), Kondo (1988) and Watkins et al., (1997)** and to reduce the incidence of mortality associated with *Clostridium perfringens* enterotoxaemia in an intraduodenal *Clostridium perfringens* inoculation model **(Vissiennon et al., 2000).**

Salinomycin is an ionophorous antimicrobial and also act coccidiostatic and will therefore also decrease the intestinal damage caused by Eimeria infections **(McDougald et al., 1996),** thus reducing the most important predisposing factors for *Clostridium perfringens* associated necrotic enteritis. Ionophorous antibiotics, such as salinomycin, has a very low minimal inhibitory concentrations against *Clostridium perfringens* **Watkins et al., (1997).**

The objective of this study was to evaluate the efficacy of tylosin phosphate and salinomycin administered in the

feed for the treatment of necrotic enteritis in broiler chickens by evaluating the biochemical parameters (liver function and kidney function), and the hematological parameters.

2-MATERIAL AND METHODS

2.1 Materials

2.1.1. Drug

Tylosin phosphate (PROTYLO feed additive) obtained from Atco pharm.
Salinomycin (SALICOX feed additive) from Atco pharm.

2.1.2. Microorganisms

Clostridium perfringens type A was obtained from poultry diseases and management. Faculty of veterinary medicine. Benha university, Qalyobia, Egypt.

2.1.3. Experimental Chicken

Two hundred fifty-one-day old chick were divided into 5 groups:

Group (1)
Fifty chicks were served as non-infected non-treated group.

Group (2)
Fifty chicks of 16 days age were infected with isolate *Clostridium perfringens* type A in feed and served as infected non-treated group.

Group (3)
Fifty chicks were infected with isolate of *Clostridium perfringens* type A and administrated with 100 ppm tylosin in feed for 7 consecutive days.

Group (4)
Fifty chicks were infected with isolate of *Clostridium perfringens* type A and administrated with 60 ppm salinomycin in feed for 7 consecutive days.

Group (5)
Fifty chicks were infected with isolate of *Clostridium perfringens* type A and administrated with100 ppm tylosin and 60 ppm salinomycin in feed for 7 consecutive days.

2.2. METHODS

2.2.1. Blood Samples

Blood samples were taken at the end of one day, 6^{th} day and 15^{th} post- treatment in all groups.

Ten birds of each group were sacrificed for collection of blood samples. Two blood samples were taken from each group for hematological and biochemical studies.

The first blood sample was collected without anticoagulant for separation of clear serum for biochemical analysis. These serum samples were used for biochemical analysis to determine serum transminases activities (AST and ALT), ALP, total protein, albumin, uric acid and creatinine.

The second sample of blood was collected in test tube mixed with heparin as anticoagulant. The sample was shacked several times to ensure mixing of blood with anticoagulant. These blood sample were used for hematological studies to determine erythrocytic count, total leucocytic count, hemoglobin concentration and packed cell volume.

2.2.2 Serum biochemical analysis

A. Liver function tests

1. Determination of serum aspartate aminotransferase activity (AST)
Serum AST was determined colorimeterically using spectrophotometer using specific kits according to **(Reitman and frankel (1957).**

2. Determination of serum alanine aminotransferase activity (ALT)
Determination of ALT was carried out using a spectrophotometer of using specific kits according to **Retman and frankel (1957).**

3. Determination of serum alkaline phosphatase (ALP)
Serum alakaline phosphatase was determined according to **Chariman (1983**).

4. Determination of serum total protein
Colorimetric determination of total protein level in the serum of chickens was carried out using spectrophotometer, using specific kits according to **Doumas (1975).**

5. Determination of serum albumin level
Colorimetric determination of serum albumin was carried out according to **Bauuer (1982)** using spectrophotometers and specific kits.

B. Kidney function testes

1. Determination of serum uric acid
Determination of serum uric acid was carried out by spectrophotometer, using specific kits according **White et al., (1970).**

2. Determination of serum creatinine
Colorimetric determination of serum creatinine was carried out using spectrophotometer according to **Folin (1934)** using specific kit.

2.2.3. Hematological studies

1. Blood cell count
Total erythrocytes and leucocytes were counted using the improved Neubauer chamber and natt and herrick; solution as diluting fluid according to the method described by **Natt and Herrick, (1952).**

2. Haemoglobin determination
Hemoglobin was determined colorimeterically, according to the method described by **Wintrobe (1967**).

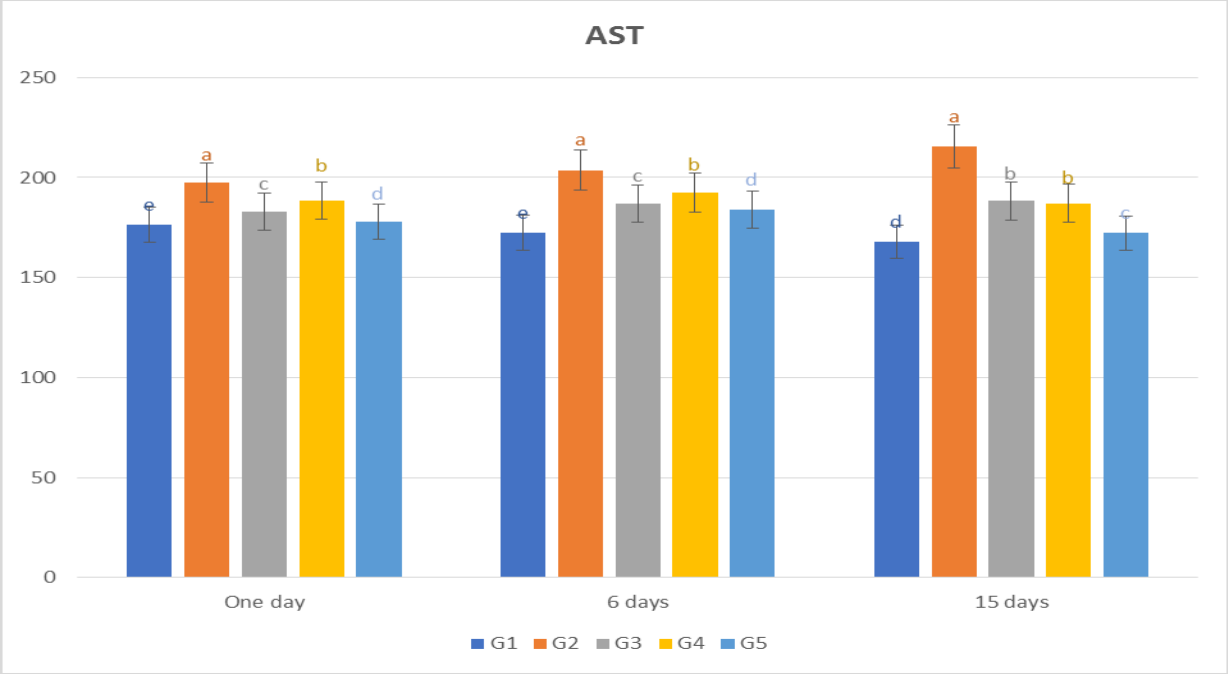
3. Paced cell volume (PCV) %
The packed cell volume was determined using the microhematocrit method according to **(Cohen 1967)**.

2.2.4. Statistical analysis
Statistical analysis was conducted with the Statistical Package for Social Science **(SPSS Inc. Released, (2009)** to determine if variables differed between groups, according to **Snedecor and Cochran (1989)**. The Shapiro-Willk test was used to test the normal distribution of the data before statistical analysis was performed. Compare between means were conducted by one-way ANOVA and subsequent Duncan's multiple range test **(Duncan, 1955).** Probability values of less than 5% ($P < 0.05$) were considered significant.

3- RESULTS

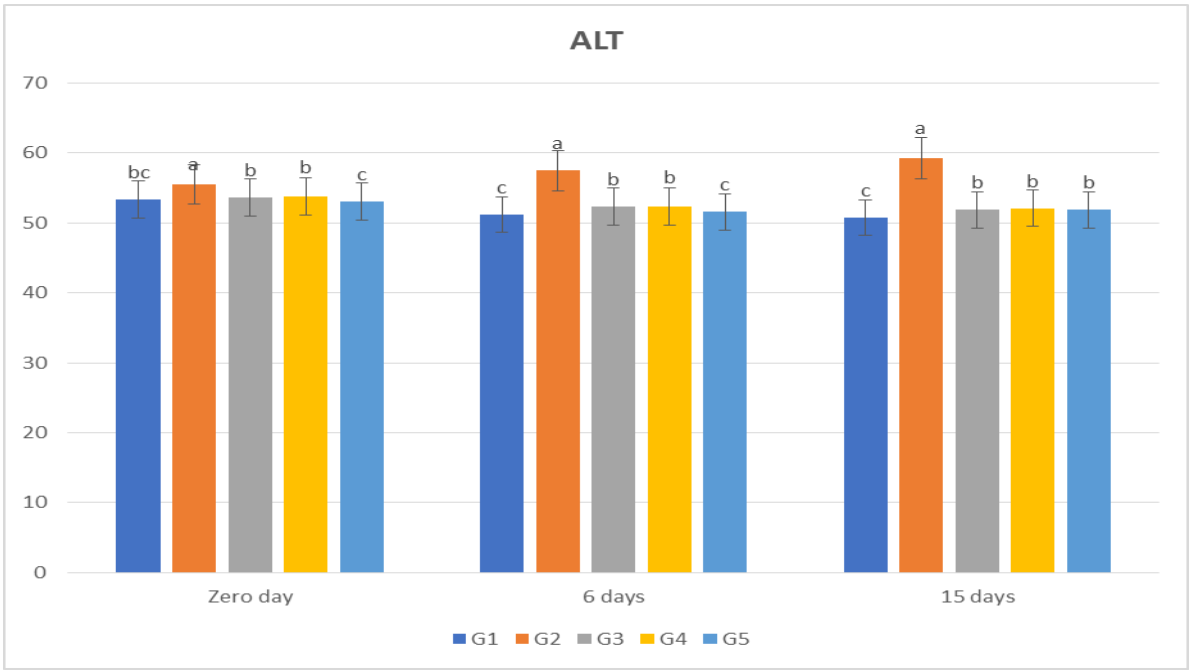
3.1. Effect on liver function
Table (1) demonstrated the effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed on AST, ALT, ALP, Total protein and Albumin level of healthy and experimentally infected chickens with *Clostridium perfringens.*

The data showed a significant increase in AST, ALT and ALP in all infected groups either treated or non-treated at (one, 6 day and 15 day) post treatment when compared with non-infected non-treated group (control group), while the infected treated groups with tylosin and salinomycin either alone or in combination showed a significant decrease in AST, ALT and ALP when compared with infected non-treated group. And showed significant decrease in Total protein and Albumin levels in infected groups either treated or non-treated at (one ,6 day and 15 day) post treatment when compared with non-infected non-treated group (control group), while the infected treated groups with tylosin and salinomycin either alone or in combination showed a significant improvement when compared with infected non-treated group.



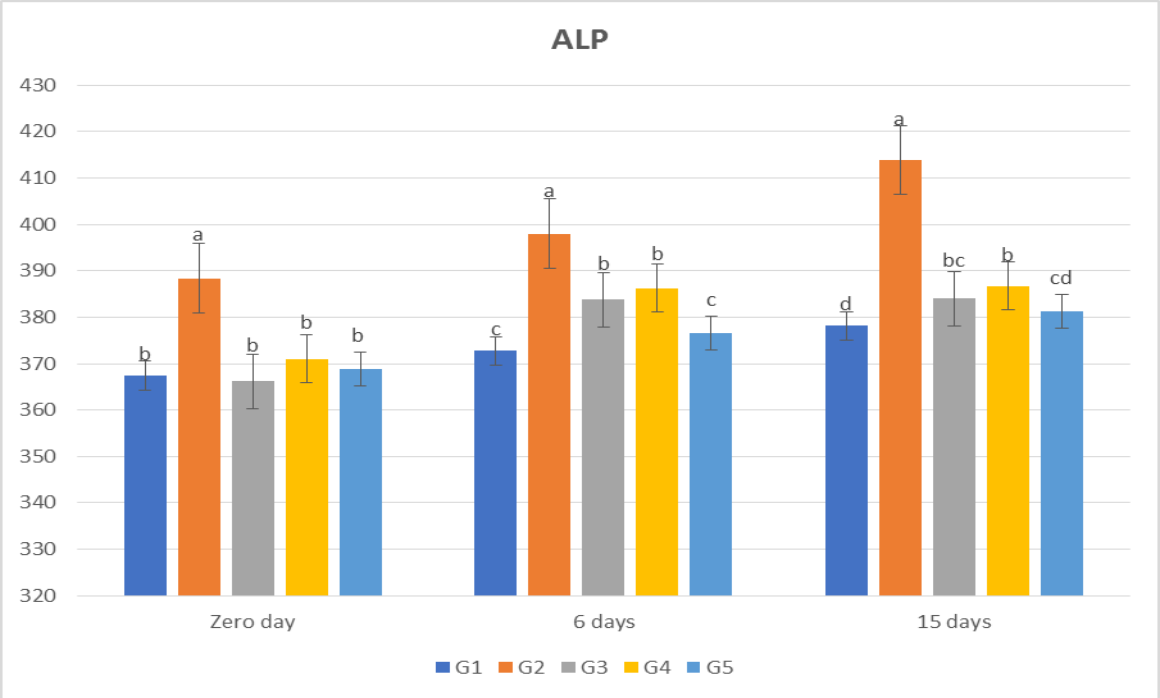
G (1): non-infected and non-treated chickens
G (2): infected and non-treated chickens
G (3): infected and treated tylosin
G (4): infected and treated salinomycin
G (5): infected and treated tylosin and salinomycin.

Figure (1): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on AST level of healthy and experimentally infected chickens with Clostridium perfringens. (n=10). *(U/L)* (Mean ±SEM).



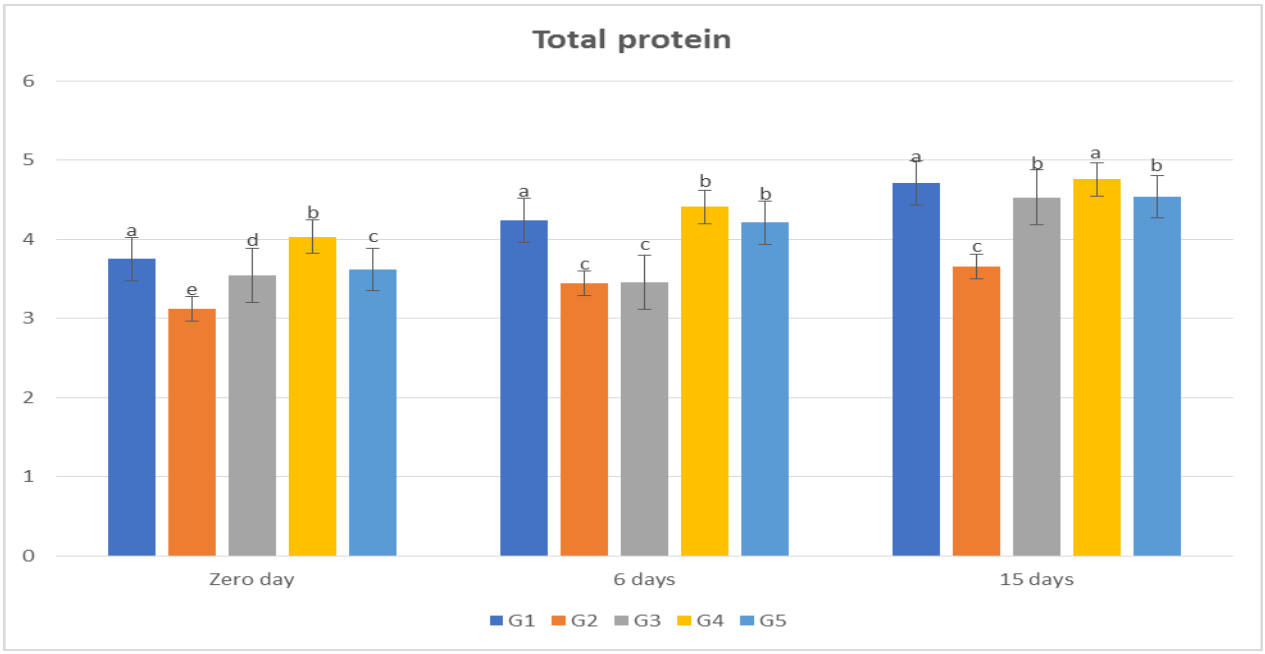
G (1): non-infected and non-treated chickens
G (2): infected and non-treated chickens
G (3): infected and treated tylosin
G (4): infected and treated salinomycin
G (5): infected and treated tylosin and salinomycin.

Figure (2): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on ALT level of healthy and experimentally infected chickens with *Clostridium perfringens.* ***(U/L)*** **(n=10). (Mean ±SEM).**



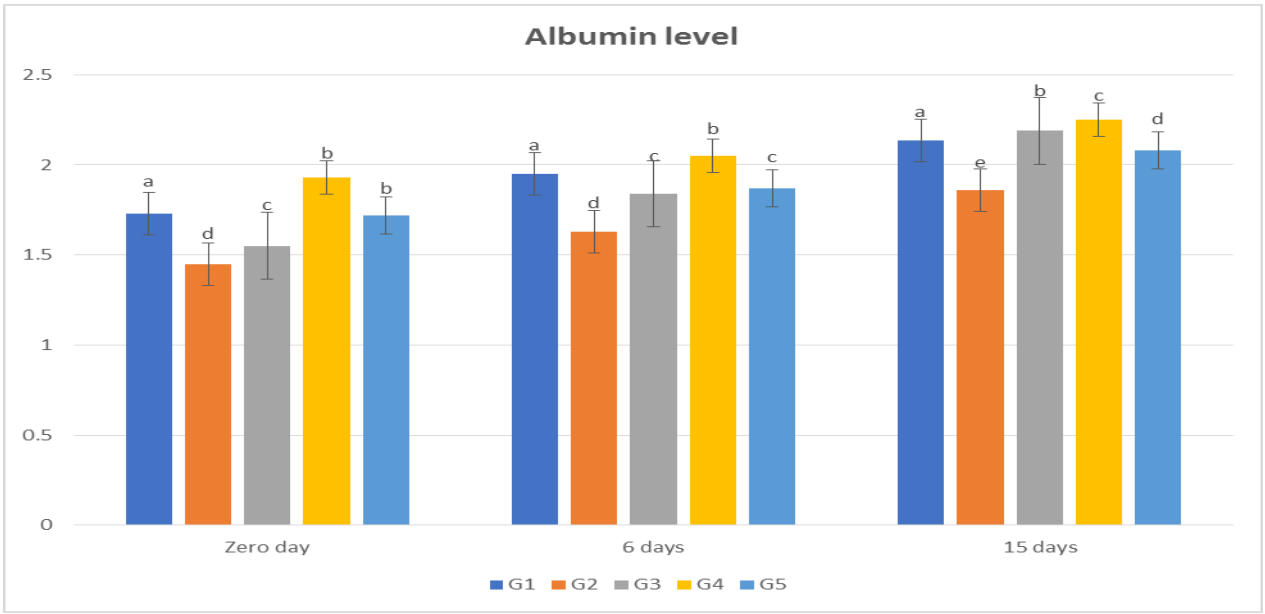
G (1): non-infected and non-treated chickens.
G (2): infected and non-treated chickens.
G (3): infected and treated tylosin.
G (4): infected and treated salinomycin.
G (5): infected and treated tylosin and salinomycin.

Figure (3): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on ALP level of healthy and experimentally infected chickens with *Clostridium perfringens.* ***(U/L)*** **(n=10). (Mean ±SEM).**



G (1): non-infected and non-treated chickens
G (2): infected and non-treated chickens
G (3): infected and treated tylosin
G (4): infected and treated salinomycin
G (5): infected and treated tylosin and salinomycin.

Figure (4): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on Total protein level of healthy and experimentally infected chickens with *Clostridium perfringens.* **(g/dl). (n=10). (Mean ±SEM).**



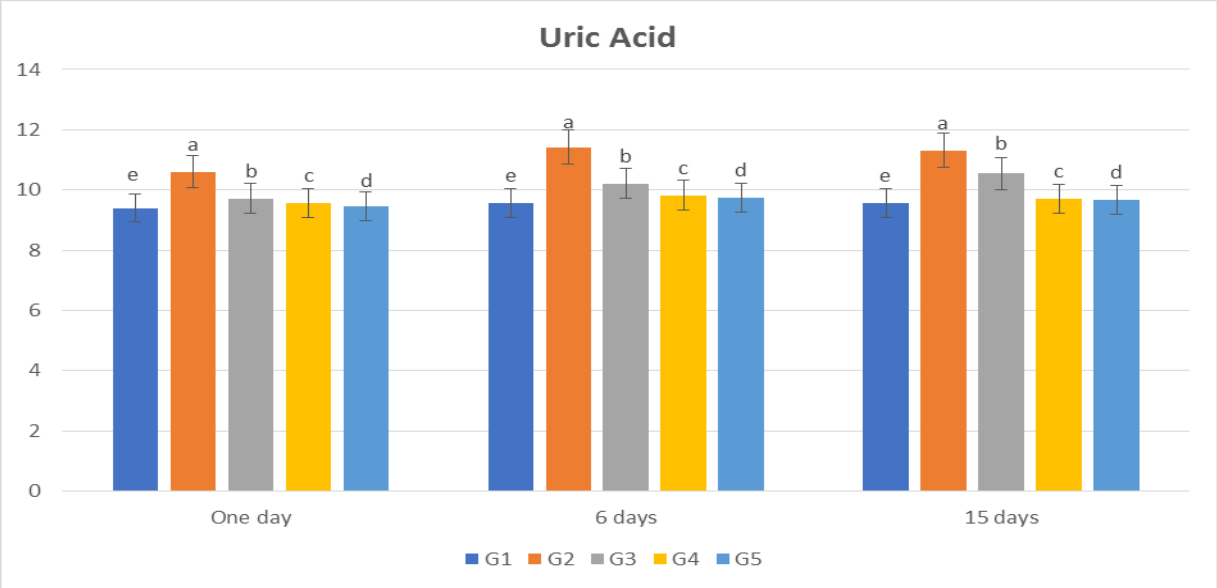
G (1): non-infected and non-treated chickens
G (2): infected and non-treated chickens
G (3): infected and treated tylosin
G (4): infected and treated salinomycin
G (5): infected and treated tylosin and salinomycin.

Figure (5): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on Albumin level of healthy and experimentally infected chickens with *Clostridium perfringens.* **(g/dl). (n=10). (Mean ±SEM).**

3.1. Effect on kidney function

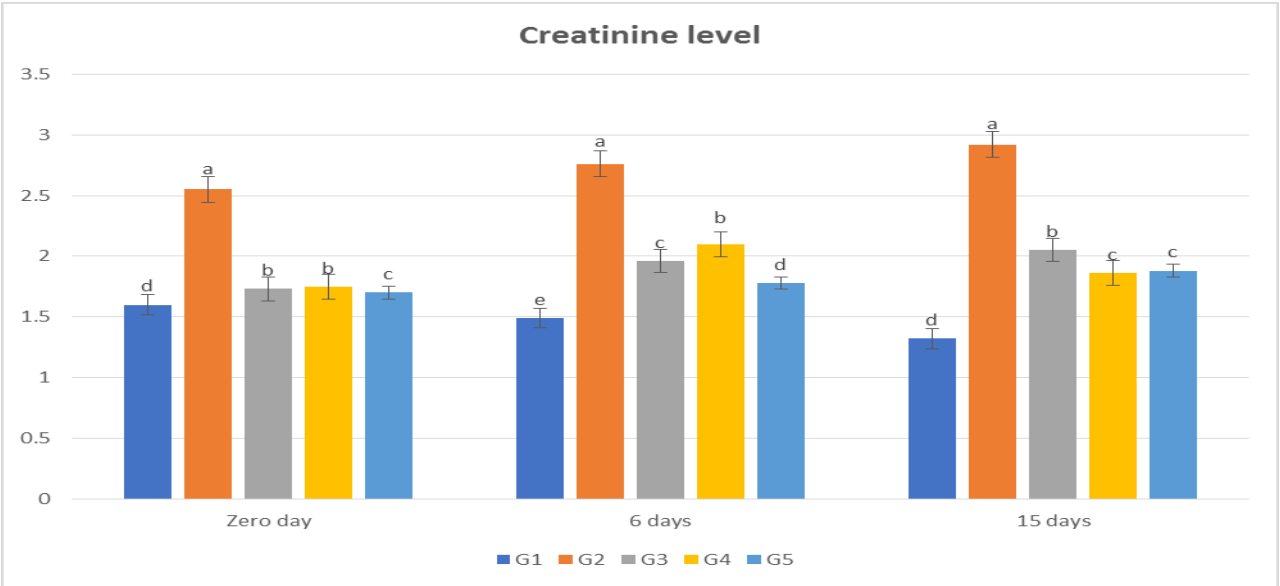
The effect tylosin (100 ppm) and salinomycin (60 ppm) in feed on uric acid and creatinine levels in chickens infected with *Clostridium perfringen.* were illustrated in **table (2).**

The result of presented study illustrated that at 0, 6th, 15th days post treatment there was a significant increase in serum uric acid and creatinine in all infected groups (treated or non-treated) when compared with control group. However, infected groups treated with tylosin and with salinomycin and group treated with tylosin and salinomycin in combination showed a significant decrease in serum uric acid and creatinine levels when compared with infected non-treated group.



G (1): non-infected and non-treated chickens.
G (2): infected and non-treated chickens.
G (3): infected and treated tylosin.
G (4): infected and treated salinomycin.
G (5): infected and treated tylosin and salinomycin.

Figure (6): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on Uric Acid level of healthy and experimentally infected chickens with *Clostridium perfringens.* **(mg/dl) (n=10). (Mean ±SEM).**



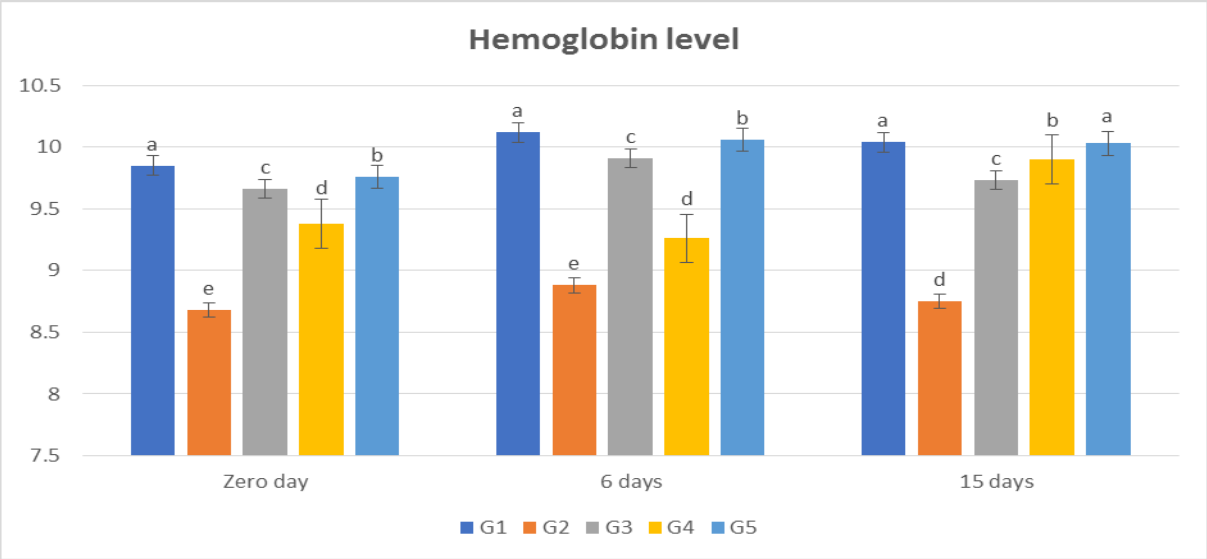
G (1): non-infected and non-treated chickens.
G (2): infected and non-treated chickens.
G (3): infected and treated tylosin.
G (4): infected and treated salinomycin.
G (5): infected and treated tylosin and salinomycin.

Figure (7): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on creatinine level of healthy and experimentally infected chickens with *Clostridium perfringens.* ***(U/L)*****. (n=10). (Mean ±SEM).**

4.3. Effect on haematological picture

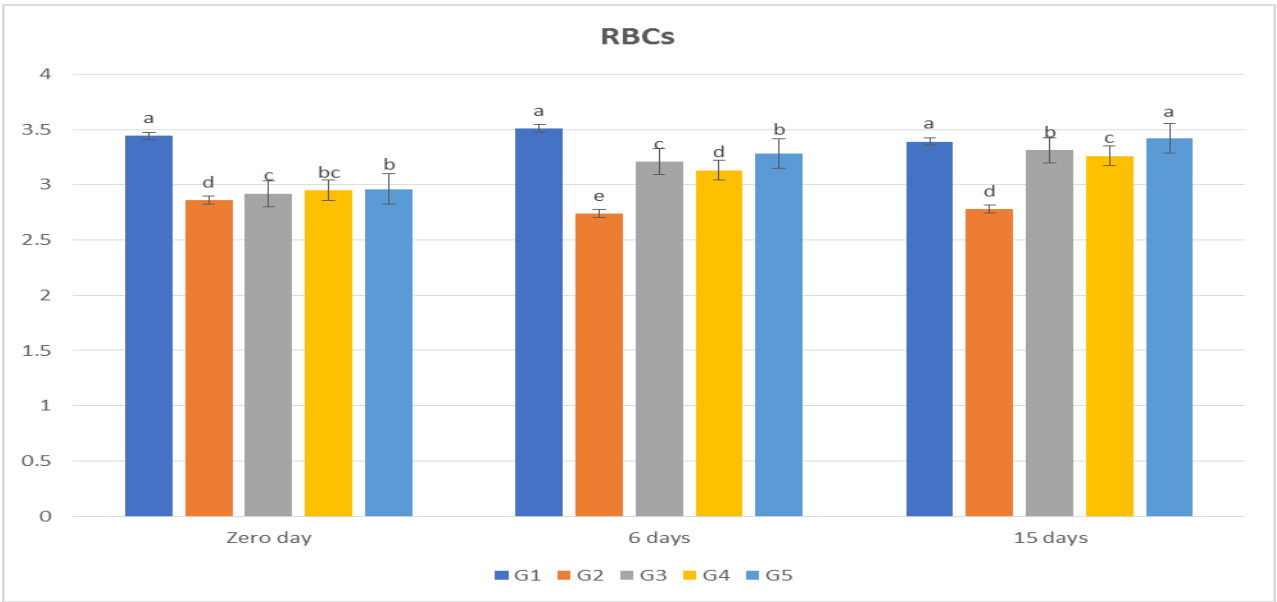
The effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed on blood hemoglobin level of healthy and experimentally infected chickens with *Clostridium perfringens, at* (one ,6th, 15th) days post treatment were illustrated in **tables (12) and figure (14).**

The data showed infected chickens with *Clostridium perfringens* and medicated by tylosin and salinomycin either alone or in combination showed significant improvement in both erythrogram and leukogram all over of the course of the study compared with infected non-treated groups.



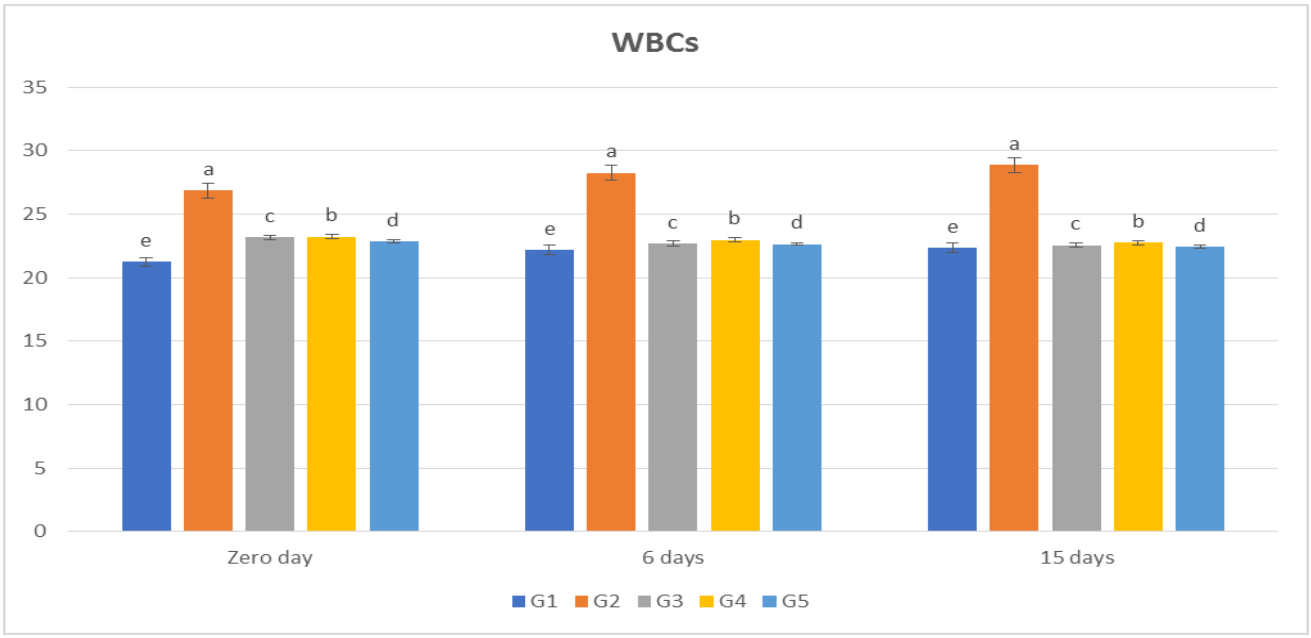
G (1): non-infected and non-treated chickens.
G (2): infected and non-treated chickens.
G (3): infected and treated tylosin.
G (4): infected and treated salinomycin.
G (5): infected and treated tylosin and salinomycin.

Figure (8): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on hemoglobin level of healthy and experimentally infected chickens with *Clostridium perfringens*. (n=10). (Mean ±SEM).



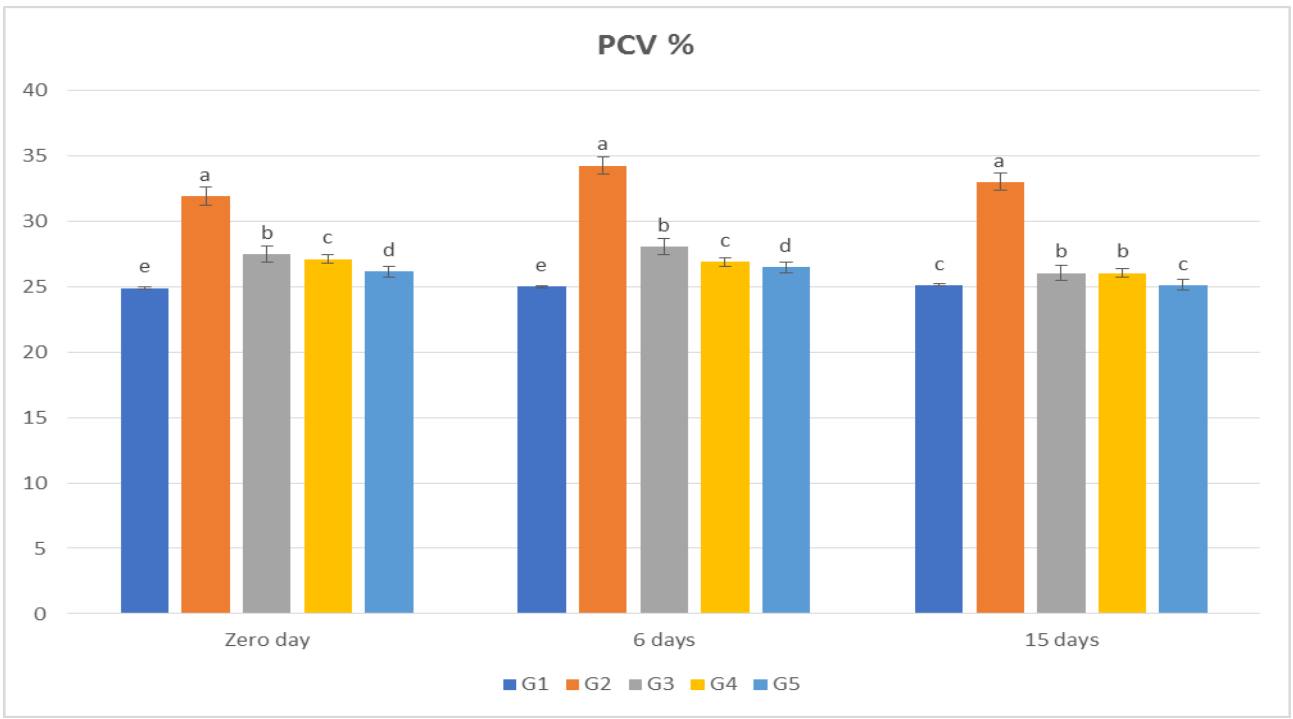
G (1): non-infected and non-treated chickens.
G (2): infected and non-treated chickens.
G (3): infected and treated tylosin.
G (4): infected and treated salinomycin.
G (5): infected and treated tylosin and salinomycin.

Figure (9): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on blood RBCs level of healthy and experimentally infected chickens with *Clostridium perfringens*. ($\times 10^6 mm^3$).



G (1): non-infected and non-treated chickens.
G (2): infected and non-treated chickens.
G (3): infected and treated tylosin.
G (4): infected and treated salinomycin.
G (5): infected and treated tylosin and salinomycin.

Figure (10): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on blood WBCs level of healthy and experimentally infected chickens with *Clostridium perfringens*. ($\times 10^3 mm^{3)}$.



G (1): non-infected and non-treated chickens.
G (2): infected and non-treated chickens.
G (3): infected and treated tylosin.
G (4): infected and treated salinomycin.
G (5): infected and treated tylosin and salinomycin.

Figure (11): Effect of tylosin (100 ppm) and salinomycin (60 ppm) in feed for 7 consecutive days on blood PCV % level of healthy and experimentally infected chickens with *Clostridium perfringens*.

4. DISCUSSION

4.1. Effect on liver function

The results of present study showed significant increase in liver enzymes (AST, ALT, and ALP), and significant decrease in total protein and serum albumin levels in chickens infected with *Clostridium perfringens* when compared with non-infected chickens.

This elevation in liver enzymes (AST, ALT, and ALP) in broiler chickens infected with *Clostridium perfringens* might be due to liver and kidney damage by closterdial toxins **Doxy (1983).** *Clostridium perfringens*-induced necrotic enteritis is usually associated with liver lesions **Løvland, and Kaldhusdal, (1999).** During subclinical infection, the intestinal damage can allow the bacteria to reach the bile duct and portal blood stream. Colonization in liver by large numbers of *Clostridium perfringens* resulted in cholangiohepatitis. Diseased livers were enlarged and have a pale appearance with red or white foci. **Sasaki et al., (2000).**

Reduction in proteinogram in infected chickens might be attributed to a state of anorexia and mal absorption of nutrients from inflamed intestine leading to inability of liver to synthesis proteins **Kaneko (1989)**.

These findings are agreed with **Mabrouk (2016)** stated that broiler chicken infected with *C. perfringens* showed significant decrease in serum total protein, serum albumin associated with significant increase in liver enzymes (AST, ALT and ALP). The obtained data coincides with the results of **Campell and Coles (1986),** they stated that ALT is mostly of hepatic origin and so their high level in the serum was indicative to liver damage. Also, the elevation of serum AST level post infection was mainly due to damage in liver cells **Halliwell, (1981).**

These findings were supported by those previously obtained by **Allam et al., (2013**) who reported that *Clostridium perfringens* infection in duckling induce significant increase in liver enzymes. This could be due to the destructive effect of *Clostridium perfringens* and Clostridial toxins on the liver cells and these results were in accordance with **Oda (2012).** Elevation in liver enzymes also were reported previously by **Kadry et al., (2009)** in chickens infected with *Clostridium Perfringens.*

Medication of *Clostridium perfringens* infected broiler chickens with tylosin and salinomycin in their tested doses either alone or in combination induced significant increase in liver enzyme activities compared to non-infected non-treated group (control group). These data agreed with **Oda (2012)** who found that, broiler chicken infected with *Clostridium perfringens* and treated with metronidazole showed elevation in serum levels of liver enzymes. Also, broiler chicken infected with *E. coli* treated with therapeutic doses of ciprofloxacin induced significant elevation in serum liver enzymes (AST, ALT and ALP) activities **EL-Kadeem (2005).**

On the other hand, chickens infected and treated with tylosin and salinomycin either alone or in combination showed significant improvement in liver enzymes and protein pictures when compared with infected non treated chickens, that might due to the antibacterial action against *C. perfringens* which lead to increasing the integrity of the intestinal walls, promoting the absorption of more nutrients and secretion of digestive enzymes which enhance the nutrient digestibility leading to improved protein profile. These results supported by **Atta et al., (2014)** who found that treatment of infected chickens with amoxicillin (25mg/kg b. wt) significantly decreased the elevated serum levels of AST and ALT enzymes as compared to the infected group. And also, supported by **Lesson et al., (2005)** who stated that, sodium butyrate improved liver enzymes and protein picture in broiler suffering from necrotic enteritis.

4.2. Effect on kidney function

The results of our study showed significant increase in uric acid and serum creatinine levels in all infected groups with *Clostridium perfringen* either treated or non-treated when compared with control group.

The obtained results revealed that experimental infection of broiler chickens with *Clostridium perfringens* induced a significant increase in uric acid and creatinine levels all over the experimental period. Elevation in uric acid could be attributed to the degenerative change in renal tubules preventing excretion of uric acid and creatinine leading to an increase in their levels in serum **Kaneko, (1980).** These results are confirmed by the pathological changes as the kidneys showed degenerative changes in some tubules (cloudy swelling and hydropic degeneration).

Our results were supported by the results obtained by **Halliwell (1981),** who reported that *Clostridium perfringens* infection in broiler chickens induced significant increase in uric acid and creatinine attributing this elevation to kidney damage induced by bacterial toxins. Increase in uric acid and creatinine were recorded by **Kadry et al., (2009)** in broiler chickens infected with *Clostridium perfringens*.

In the current work, the administration of tylosin and/or salinomycin either alone or in combination to *Clostridium. Perfringens* infected broiler chickens produced a significant increase in serum uric acid and creatinine level compared with normal control group. This result was in accordance with that obtained by **Sameh et al., (2005),** who stated that uric acid and creatinine concentration was significantly increased only at 1st day post treatment in broiler chicken infected with *Clostridium perfringens* and treated with metronidazole. On the same ground**, Kadry et al., (2009)** and **Allam et al., (2013**) reported that uric acid and creatinine

concentrations were non-significantly increased post treatment of broiler chickens infected with *Clostridium perfringens* using metronidazole. Compared with normal control group and a significant decrease compared with infected non-treated groups.

4.3. Effect on hematological picture

In present study, the infected chicken with *Clostridium perfringens* showed significant decrease in RBCs count, Hb and PCV % associated with significant increase in WBCs all over the experimental period. Reduction in erythrogram parameters caused by *Clostridium. perfringens* infection might be due to bacterial toxin **Doxy (1983).** *Clostridium perfringens* can produce up to 17 toxic or potentially toxic exoproteins **Songer (1996).** Alpha-toxin has been indicated as the main virulence mediator for Necrotic enteritis in poultry. Clostridial toxins caused breakdown of phospholipids of erythrocytes membrane and cause hemolysis by damaging circulating erythrocytes **Allam et al., (2013).** These results are compatible **with Kadry et al., (2009), Sayed et al., (2016)** and **Sawsan et al., (2018).** This decline in the blood components also may be due to the severe bleeding and tissue damage in the intestinal mucosa.

Total WBC increased significantly in blood of infected non-treated group in comparison with control group, this elevation of total white blood cells due to increases in the polymorph nuclei number (neutrophil and eosinophil), the neutrophil infiltration increases immediately after any infection as a first defense line followed by increases in eosinophil concentration as a response to parasitic infestation. **Patra et al., (2010).**

Infected chickens with *Clostridium perfringens* and medicated by tylosin and salinomycin either alone or in combination showed significant improvement in both erythrogram and leukogram all over of the course of the study compared with infected non-treated groups. This improvement in hematological parameters might be due to antimicrobials effects which suppress the invasion of *Clostridium perfringens* so improved absorption of essential substance for erythropoiesis **Kamel (2004).**

These findings agreed with **Odunsi and Onifade (1998)** and **Odunsi et al., (1999)** stated that the hematological values in case of virginiamycin and zinc bacitracin supplementation in the broiler's diets are analogus with the normal ranges which suggests adequate and healthy nutrition, and the preponderant values in the antibiotic supplemented groups justifies the established positive relationship between hematological parameters and performance of the birds.

These results are in contrast with **Burke and Cunha (2003)** who stated that various antibiotics exert diverse effects on different elements of blood, some agents produce anemia or leukopenia, and some may cause pancytopenia.

Hematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. Blood parameters are good indicators of physiological, pathological and nutritional status of an animal concerned.

6- CONCLUSION

The present study concluded that

- Using tylosin (100 ppm) and salinomycin (60 ppm) in combination in feed has a beneficial effect against necrotic enteritis in broiler chickens.
- Special attention should be taken with tylosin and salinomycin due to the adverse effect on liver and kidney in broiler chickens.

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