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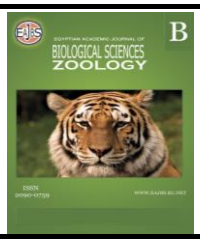

Evaluation of Some Treatments for Coliform Infection in Chicken

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ABSTRACT

This work was designed to investigate the effect of yeast*(Saccharomyces cerevisiae)* and Sangrovit as natural feed additives on chicken production performance, some hematological parameters, some biochemical parameters, histopathological examination of (liver and kidney) and total coliform count of intestinal content in normal and experimentally infected chickens with *E.coli* O78. A total of 120 unsexed one-day-old, broiler chickens (Cobb) were randomly assigned to 6 treatment groups each of 20 as follows: G1 as the control; G2 received 0.3% Yeast supplement; G3 received 0.1% Sangrovit supplement; G4 infected by *E.coli* only; G5 infected by *E.coli* and treated by 0.3% Yeast and G6 infected by *E.coli* and treated by 0.1% Sangrovit. Our work revealed that Yeast and Sangrovit as feed additives have marked impacts on growth-promoting, immunomodulating and antimicrobial effects with no adverse effects on haematological and serum biochemical parameters and can contribute in controlling and prevention of *E.coli* induced experimental infection in broiler chicken.

INTRODUCTION

Phytobiotics may be considered as plant-derived products added to the feed in order to improve the performance of agricultural livestock. Phytobiotics represent a wide range of bioactive compounds that can be extracted from various plant sources. In recent years, some interesting and novel applications of phytobiotics in the production and well– being of monogastric animals have emerged (Vidanarachchi *et al*., 2009).

Hassan *et al.* (2018) stated that using phytobiotics (Sangrovit) in broilers diets as feed additive appeared to be superior compared to antibiotic growth promoter, also it significantly improved growth performance and carcass traits more efficiently and safely than antibiotic growth promoter and it can be a good alternative to antibiotic growth promoter in broiler diets.

Yakhkeshi *et al.* (2011) demonstrated that sangrovit can reduce the pathogenic bacteria in the digestive tract of broilers which can help to improve intestinal health of them, and so it can be used as an alternative for antibiotics in broilers feed.

Probiotics are live microbial feed supplements which have a beneficial effect on the

host animal by improving its microbial balance (Fuller, 1989). Probiotics have been found to increase feed intake, growth, and immune responses(Isolauri *et al*., 1995).

Many results suggested that *Saccharomyces cerevisiae (SC)* could act as a Growth promoter; because of its natural improvement of digestibility and absorption of nutrients and controlling infections by enteric pathogens Cruickshank, (2002) and Miazzo *et al.,*(2005).

E.coli infection in poultry usually associated with high economical losses due to high morbidity and mortality rates, decreased food conversion rates, loss of body weight, condemnation of whole affected carcass, or affected organs after slaughter (Gross 1990).

The present work aimed to investigate the effect of incorporation of Yeast and Sangrovit in the feed of broiler chicken challenged with *E.coli* infection on growth performance, a total count of coliform in the small intestine, and some serum biochemical parameters of each additive.

MATERIALS AND METHODS

One hundred and twenty, one day old, Cobb broiler chicks with an average body weight 43-45 gm were obtained from Ismailia / Misr Poultry Company, Ismailia city, Egypt. The chicks were housed in floor pens and randomly allocated into 6 groups of 20 birds each and reared for 6week. Feed and water were provided *ad libitum*, the temperature of the room was maintained at 33°C initially, and reduced by 3°C / wk. until reached 21°C, at which the room temperature was maintained for the end of the experiment.

Experimental Design:

One hundred and twenty apparent healthy chicks were grouped at one day old randomly into six equal groups, each group contain 20 chicks reared for 6 weeks as in the following G1received a basal diet and kept as a control without any treatment, G2 received a basal diet supplemented with 0.3% Yeast, G3 received a basal diet supplemented with 0.1% Sangrovit, G4 received a basal diet and then infected with 0.5 ml saline suspension containing 2×10^7 CFU of *E.coli* (O78) by an intranasal route at 21 days of age, G5 received a basal diet supplemented with 0.3% Yeast and infected with 0.5 ml saline suspension containing 2×10^7 CFU of *E.coli* (O78) by an intranasal route at 21 days of age, G6 received a basal diet containing 0.1% Sangrovit and infected with 0.5 ml saline suspension containing 2×10^7 CFU of *E.coli* (O78) by an intranasal route at 21 days of age according to the method described by Peighambari *et al.*, (2000)..

Treatments, and Growth Performance Parameters:

Probiotic (Instant dried yeast) *Saccharomyces cerevisiae*, AKMAYA, Turkey, and Phytobiotic (Sangrovit), Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany. Growth performance parameters were estimated as follow: mean live body weight (gm), body weight gain(gm), feed intake (gm), feed conversion ratio (FCR) was determined according to (Brady, 1968).

Total Coliform Count:

At 28 days and 42 days of age two birds per group, were randomly selected and slaughtered (Abdel-Raheem *et al.*,2012**).** All viscera were removed carefully by hand and one gram of the intestinal content from the ileocecal junction portion of the intestinal tract was collected and weighted in clean previously sterilized Petri dishes. One gram of the intestinal contents was transferred to a series
of sterile test tubes containing nine ml of 0.1% sterile buffered peptone water and mixed well to prepare decimal serial dilutions of sample homogenate up to 10^{-8}. The total coliform count was carried out by pouring plate method.

Blood samples were obtained from the wing vein of each bird. 2 ml of blood was collected in a clean tube containing sodium salt of EDTA; this sample was used for evaluation of the hematological parameters and 3 ml of blood was collected in a plain centrifuge tube and used for the preparation of serum for determination of biochemical parameters.

Determination of Hematological Parameters:

Total erythrocyte count was determined by Neubauer Hemocytometer with Natt and Herrick solution as diluting fluid according to the method described by Natt and Herrick (1952). Determination of hemoglobin (Hb) was performed as described by Lamberg and Rothstein (1977).Packed cell volume (PCV) was measured by microhematocrit centrifuge according to Coles (1986). Leukocytic counts were performed using an improved Neubaur Hemocytometer and Natt and Herick solution.

Total white blood cells and differential leukocyte count were calculated according to the standard technique described by (Jain, 1986 andTerry ,1988). For differential leukocytic count, blood films were made on clean slides, dried on-air, fixed with absolute methyl alcohol and stained with Giemsa stain, the percentage, and absolute value for each type of white cells were calculated according to Feldman *et al.*, (2000).

Determination of Biochemical Parameters:

They were performed using commercial diagnostic kits for estimation of serum level of ALT, AST, creatinine, and uric acid using (SPINREACT kits, Spain), Suez canal university hospital laboratories.

Histopathological Studies:

Birds were slaughtered and necropsied. Specimens from the liver and kidney were obtained from all sacrificed chicken. Samples were preserved in 10% formalin and embedded in paraffin wax. Sectioned at 5-micron thickness and stained with haematoxylin and eosin (H and E) (Bancroft *et al.*, 1990).

Statistic Alanalys:

The obtained data from treated groups were statistically analyzed in comparison to the control group for the mean and standard error using SPSS 10 (Coakes *et al.*, 2009). Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

A significant increase in live body weight, and non-significant a decrease in FCR with non-significant effect on feed intake in Yeast treated group compared to control(Table 1), this came in agreement with studies of Kalavathy *et al.* (2003) and Kabir *et al.* (2004).

Meanwhile, a significant increase in live body weight, body weight gain, and a significant decrease in FCR with a non-significant effect on feed intake in Sangrovit treated group compared to control this results agreed with those of Tschirner *et al.* (2003) and Vieira *et al.* (2008). *E.coli* infected chicken showed a significant decrease in live body weight, body weight gain and feed intake with a significant increase in FCR, this result agreed with Russell (2003) and Ask *et al.* (2006) who reported that colibacillosis had adverse effects on growth and health, growth retardation being the main problem, reduced eating behavior leading to reduce feed intake. While infected and treated groups with Yeast and Sangrovit showed a significant increase in body weight, body weight gain with a significant decrease in FCR when compared to infected non- treated group. Hence, Yeast and Sangrovit improved the intestinal health and growth performance of the chicken.

Table 1: The effect of Yeast and Sangrovit on MBW, BWG, FI, FCR in healthy and *E.coli* experimentally infected groups. (means ± standard error (SE); n=20).

Parameter	Days							
	1-21 day				21- day			
	MBW	BWG	FI	FCR	MBW	BWG	FI	FCR
G1	794.6 ±19.40a	327.4 ±16.2a	460.8 ±14.10a	1.4 ±0.07a	2164 ±18.50c	445.4 ±4.62c	675 ±8.66a	1.52 ±0.00b
G2	765.6 ±14.60a	307.4 ±10.2a	463.4 ±14.50a	1.5 ±0.05a	2261.8 ±21.10b	449.64 ±3.46c	676.9 ±4.41a	1.51 ±0.00b
G3	757.2 ±15.20a	302 ±24.7a	464.2 ±13.30a	1.5 ±0.11a	2323.1 ±23.80a	488.5 ±6.93a	675 ±3.46a	1.38 ±0.00c
G4	794.6 ±19.40a	327.4 ±16.2a	460.8 ±14.10a	1.55 ±0.07a	1928.2 ±17.00d	405.3 ±8.08d	660 ±2.89b	1.62 ±0.00a
G5	765.6 ±14.60a	307.4 ±10.2a	463.4 ±14.50a	1.5 ±0.05a	1975.2 ±24.60c	528.2 ±7.51b	661.84 ±4.18b	1.3 ±0.00c
G6	757.2 ±15.20a	302 ±24.7a	464.2 ±13.30a	1.5 ±0.11a	2087.8 ±18.40c	478 ±2.89c	657.6 ±2.89b	1.3 ±0.00c

Means within the same column with different superscripts are significantly different (P<0.05).

Regarding erythrogram results (Table 2); normocytic normochromic anemia has been shown in *E.coli* infected group. Meanwhile, there was a significant increase in RBCs, Hb and PCV values in the infected groups treated with Yeast and Sangrovit in comparison with the infected non- treated group. On the other hand, Yeast and Sangrovit treated groups showed non-significant changes compared to the control group, these results agreed with Marcel (1994), Fatma (2005), and Shimaa (2015).

There was a significant increase in TLC and heterophils(Tables 3 &4) in the infected non-treated group at the 7th day post-infection while on the 21st-day post-infection elicited increase in TLC and lymphocytes.

Concerning, Yeast and Sangrovit treated groups, there was a significant increase in total leukocytic and lymphocytic counts in comparison with control. Our results are in accordance with Sawsan *et al., (*2019); Ajit *et al., (*2014); Hanan (2002), and Fatma (2005).

Table 2: The effect of feeding Yeast and Sangrovit on erythrogram in healthy and *E.coli* experimentally infected groups at 4 and 6 weeks of age. (means ± standard error (SE); n=20).

Age	At 4 weeks of age			At 6 weeks of age		
Parameter / Group	RBCs ($\times10^6$/µL)	Hb (g/dl)	PCV (%)	RBCs ($\times10^6$/µL)	Hb (g/dl)	PCV (%)
G1 Control	4.36 ±0.51a	12.1 ±0.11a	36.3 ±0.32a	5.4 ±0.04a	11.1 ±0.97a	34.3 ±0.93a
G2 Yeast	4.5 ±0.43a	12.3 ±0.62a	36.5 ±1.88a	5.23 ±0.75a	11.3 ±0.08a	33.4 ±0.26a
G3 Sangrovit	4.4 ±0.93a	12.2 ±0.04a	36.4 ±0.14a	5.12 ±0.22a	11 ±0.23a	32.2 ±0.70a
G4 *E. coli*	2.9 ±0.17c	7.2 ±0.05c	23.78 ±0.16c	3.7 ±0.37b	7.5 ±0.66b	22.3 ±0.99b
G5 Yeast +*E.coli*	3.36 ±0.02b	9.9 ±0.04b	30.5 ±0.14b	5.13 ±0.72a	10.52 ±0.19a	31.2 ±.0.78a
G6 Sangrovit +*E. coli*	3.36 ±0.02b	9.4 ±0.12b	28.9 ±0.37b	5.1 ±0.76a	10.55 ±0.10a	31.3 ±0.30a

Table 3: The effect of feeding Yeast and Sangrovit on leukogram in healthy and *E.coli* experimentally infected groups at 4 weeks of age.(means ± standard error (SE); n=20).

Parameter / Group	TLC ($\times10^3/\mu L$)	Heterophils ($\times10^3/\mu L$)	Lymphocytes ($\times10^3/\mu L$)	Monocytes ($\times10^3/\mu L$)	Eosinophils ($\times10^3/\mu L$)	Basophils ($\times10^3/\mu L$)
G1 Control	6.7 ±0.96d	3.13 ±0.27c	3.28 ±0.42b	0.18 ±0.16b	0.11 ±0.02b	0.0 ±0.00a
G2 Yeast	7.49 ±0.42c	3.18 ±0.24c	4.02 ±0.29a	0.17 ±0.03b	0.12 ±0.02b	0.0 ±0.00a
G3 Sangrovit	7.39 ±0.58c	3.13 ±0.24c	3.96 ±0.26a	0. 18 ±0.03b	0.11 ±0.02b	0.0 ±0.00a
G4 *E. coli*	10.19 ±0.87a	6.98 ±0.46a	2.34 ±0.33c	0.64 ±0.05a	0.23 ±0.05a	0.0 ±0.00a
G5 Yeast + *E. coli*	8.9 ±0.93b	4.6 ±0.46b	3.4 ±0.39b	0.62 ±0.04a	0.28 ±0.07a	0.0 ±0.00a
G6 Sangrovit+*E.coli*	8.83 ±0.41b	4.4 ±0.16b	3.5 ±0.22b	0.66 ±0.03a	0.27 ±0.13a	0.0 ±0.00a

Means within the same column with different superscripts are significantly different($P<0.05$).

Table 4: The effect of feeding Yeast and Sangrovit on leukogram in health and *E.coli* experimentally infected groups at 6 weeks of age. (means ± standard error (SE); n=20).

Parameter / Group	TLC ($\times10^3/\mu L$)	Heterophils ($\times10^3/\mu L$)	Lymphocytes ($\times10^3/\mu L$)	Monocytes ($\times10^3/\mu L$)	Eosinophils ($\times10^3/\mu L$)	Basophils ($\times10^3/\mu L$)
G1 Control	6.87 ±0.19d	3.02 ±0.21a	3.2 ±0.05d	0.47 ±0.03c	0.18 ±0.07c	0.0 ±0.00a
G2 Yeast	8.71 ±0.73c	3.15 ±0.33a	4.95 ±0.48c	0.47 ±0.11c	0.14 ±0.04c	0.0 ±0.00a
G3 Sangrovit	8.74 ±0.69c	3.12 ±0.24a	4.9 ±0.39c	0.44 ±0.07c	0.18 ±0.02c	0.0 ±0.00a
G4 *E.coli*	10.85 ±0.63a	3.00 ±0.17a	6.6 ±0.44a	0.8 ±0.18a	0.45 ±0.13a	0.0 ±0.00a
G5 Yeast + *E.coli*	9.3 ±0.59b	3.03 ±0.12a	5.6 ±0.48b	0.65 ±0.01b	0.32 ±0.02b	0.0 ±0.00a
G6 Sangrovit+*E.coli*	9.88 ±0.68b	3.05 ±0.34a	5.9 ±0.39b	0.63 ±0.04b	0.3 ±0.04b	0.0 ±0.00a

Means within the same column with different superscripts are significantly different ($P<0.05$).

Regarding biochemical parameters (Tables 5 and 6) results showed a significant increase in the level of ALT and AST in the infected non-treated group. While the infected and treated groups with Yeast and Sangrovit showed a significant decrease compared to the infected group. These results were in agreement with Madian *et al*., (2008); Sharma *et al*., (2015) and Fatma (2005) who found that the levels of ALT and AST were significantly increased in *E.coli* infected broilers as compared to control. These elevations due to hepatocellular damage caused by *E.coli*. Supplementation of Yeast and Sangrovit did not significantly change the ALT and AST values compared to the control group, these results agreed with Sawsan *et al*. (2019).

A significant increase in the level of uric acid and creatinine in the infected group compared to the control group. While there was a significant decrease in infected treated groups compared to the infected group, these results agreed with Marcel (1994) and Hanan (2002) who reported that the experimental infection of chickens with *E.coli* caused

an elevation of serum uric acid and this may be attributed to the increase in the breakdown of plasma proteins. The increase in blood urea and creatinine could be due to the effect of the microorganisms and its toxins on the kidney (Obrig *et al*., 1987).

The Yeast and Sangrovit treated groups showed non-significant changes in the level of uric acid and creatinine compared to the control group, this agreed with Sawsan *et al*., (2019).

Table 5: The effect of dietary supplementation of Yeast and Sangrovit on some serum biochemical parameters in healthy and *E.coli* experimentally infected groups at 4 weeks of age.(means ± standard error (SE); n=20).

Parameter / Group	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	Uric acid (mg/dl)
G1 Control	3.80 ±0.18c	122.10 ±2.79c	0.22 ±0.02c	3.15 ±0.17c
G2 Yeast	4.20 ±0.16c	120.00 ±2.90c	0.22 ±0.01c	3.18 ±0.18c
G3 Sangrovit	3.70 ±0.19c	126.30 ±2.79c	0.21 ±0.01c	3.1 0.08c
G4 *E.coli*	6.90 ±0.06a	158.00 ±3.84a	0.39 ±0.04a	4.87 ±0.25a
G5 Yeast + *E.coli*	5.40 ±0.18b	139.10 ±3.31b	0.29 ±0.01b	3.8 ±0.16b
G6 Sangrovit+ *E. coli*	5.56 ±0.16b	136.90 ±1.98b	0.28 ±0.04b	3.78 ±0.12b

Means within the same column with different superscripts are significantly different (P<0.05).

Table 6: The effect of dietary supplementation of Yeast and Sangrovit on some serum biochemical parameters in healthy and *E.coli* experimentally infected groups at 6 weeks of age. (means ± standard error (SE); n=20).

Parameter / Group	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	Uric acid (mg/dl)
G1 Control	10.8 ±1.86c	203.8 ±10.20c	0.24 ±0.02c	3.12 ±0.12c
G2 Yeast	10.00 ±0.58c	200.2 ±8.66c	0.25 ±0.01c	3.15 ±0.13c
G3 Sangrovit	10.24 ±0.67c	204.00 ±12.60c	0.24 ±0.03c	3.17 ±0.11c
G4 *E.coli*	18.00 ±1.20a	276.00 ±5.77a	0.37 ±0.02a	5.12 ±0.07a
G5 Yeast + *E.coli*	13.1 ±0.26b	223.0 ±11.50b	0.33 ±0.02b	3.4 ±0.16b
G6 Sangrovit+*E.coli*	13.2 ±0.60b	225.1 ±12.30b	0.32 ±0.02b	3.38 ±0.12b

Means within the same column with different superscripts are significantly different (P<0.05).

Concerning the intestinal coliform count(Table 7) *E.coli* infected non- treated group showed a significant increase in the total coliform count of intestinal content compared to control group, meanwhile infected treated groups with Yeast and Sangrovit groups showed a significant decrease in the total coliform count of intestinal content compared with *E.coli* infected non treated group(G4), these results agreed with Ykhkeshi of coliform counts in the intestine.

The Yeast and Sangrovit treated groups showed a significant decrease in the total coliform count of intestinal content in comparison to the control group This result agreed with Natsir *et al*. (2013); Vidanarachchi *et al.* (2013) and Boka *et al.* (2014).

Table 7: The effect of dietary supplementation of Yeast and Sangrovit on the coliform bacterial count in healthy and *E.coli* experimentally infected groups at 4 and 6 weeks of age(means ± standard error (SE); n=20).

Parameter / Group	**Total coliform count Log CFU/g**	
	At 4 weeks	At 6 weeks
G1 Control	5.6±0.06c	6.7±0.05b
G2 Yeast	5.30±0.03d	6.35±0.03c
G3 Sangrovit	5.13±0.04d	6.3±0.05c
G4 Control+ *E.coli*	7.0±0.14a	8.6±0.12a
G5 Yeast + *E.coli*	6.8±0.05b	7.72±0.05b
G6 Sangrovit+*E.coli*	6.7±0.03b	7.7±0.06b

Means within the same column with different superscripts are significantly different (P<0.05).

Regarding the histopathological examination(Figs. 1 and 2) There were Improvements in tissues (liver and kidney) of Yeast and Sangrovit treated groups.

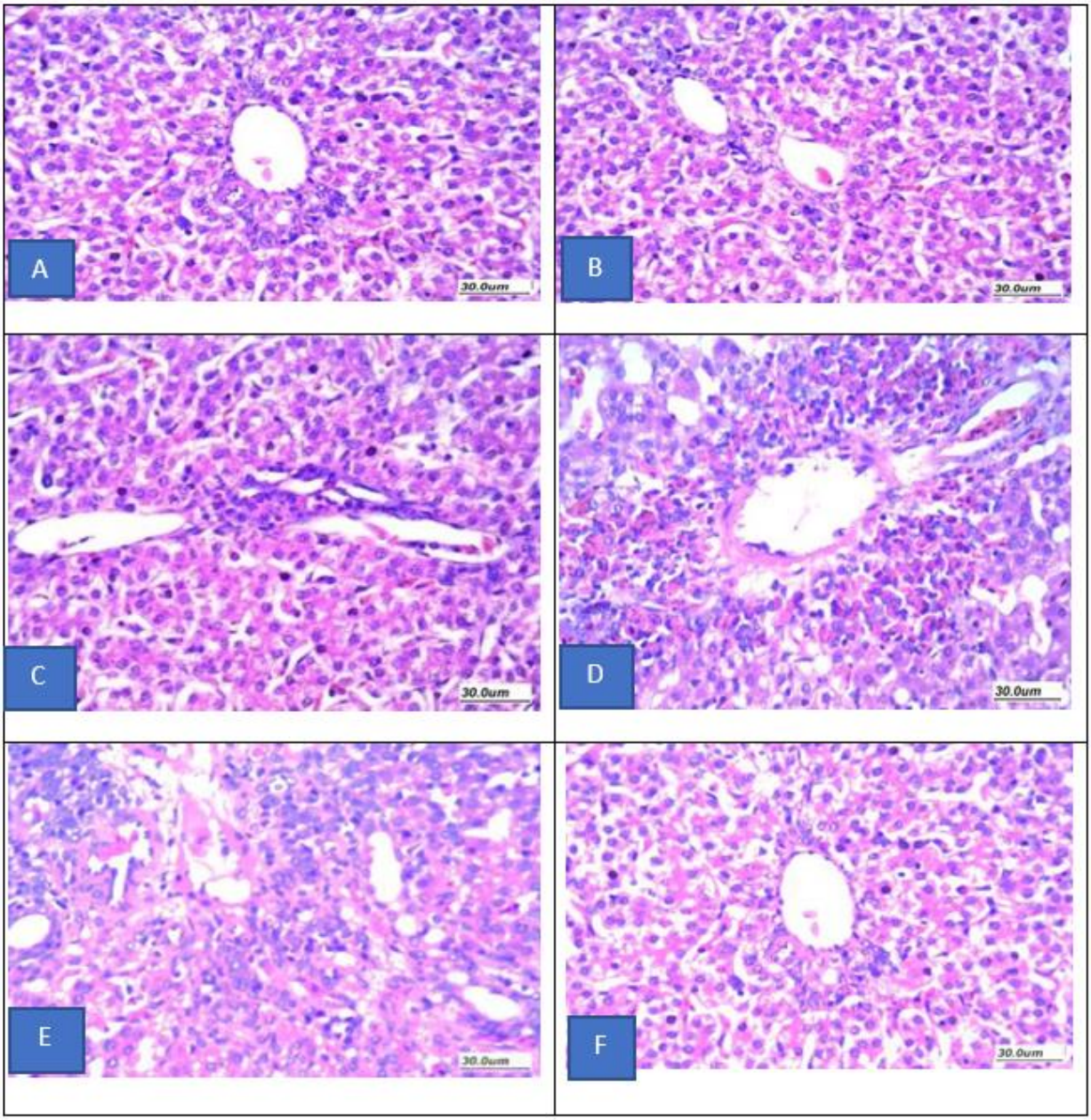

Fig. 1: Liver: (A) control group, (B) Yeast treated group, (C) Sangrovit treated group showing normally arranged hepatocytes radiating from a central vein. H &E x400. (D) *E.coli* group showing vacuolar degeneration of hepatocytes, lymphocytic infiltration around the central vein, (E) Yeast infected and treated group showing mild vacuolar degeneration and mild lymphocytic infiltration, (F) Sangrovit infected and treated group showing mild vacuolar degeneration of hepatocytes. H&E x400.

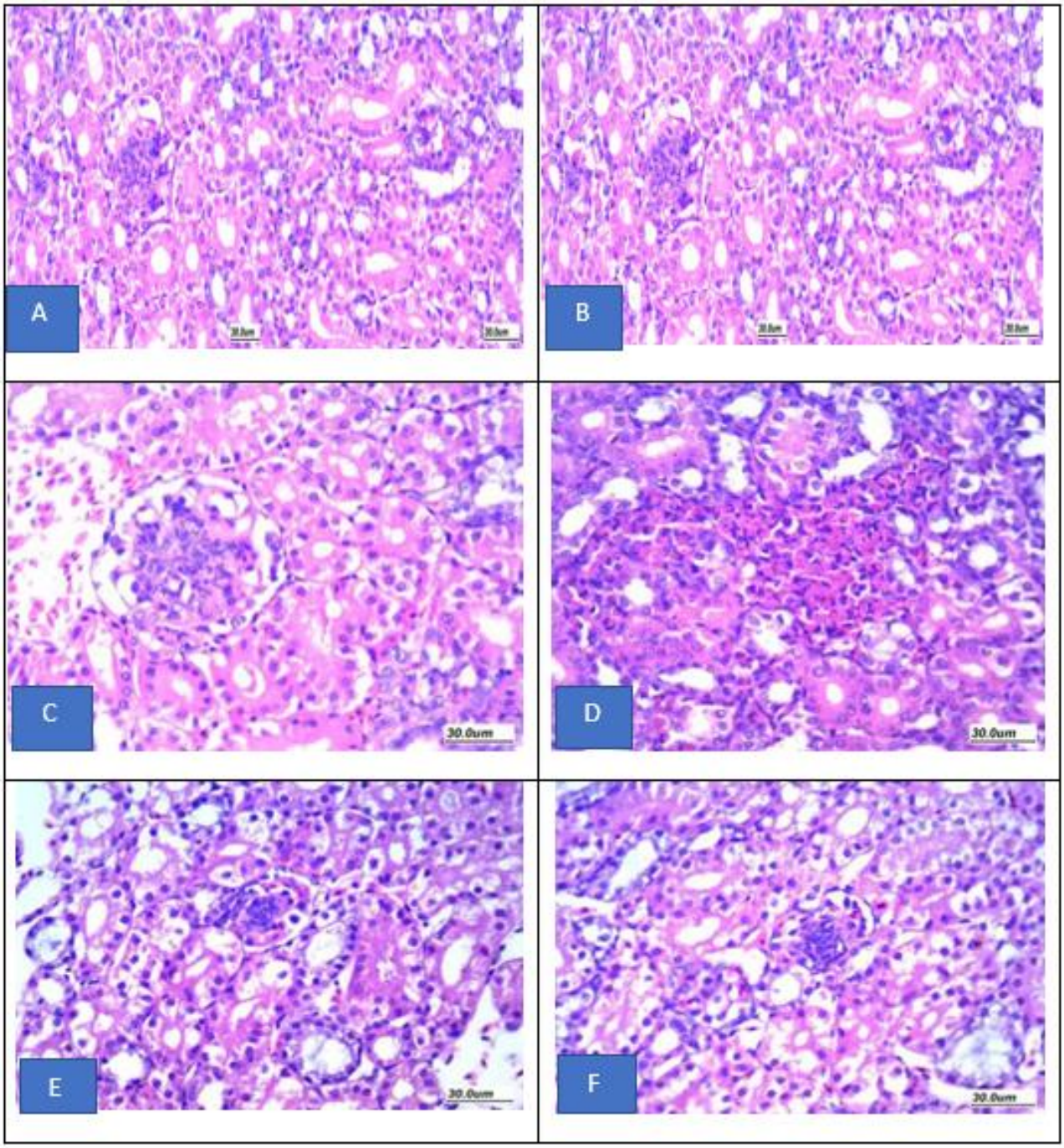

Fig. 2: Kidney: (A) control group, (B) Yeast treated group and (C) Sangrovit treated group showing normal renal histological structure of glomeruli, proximal and distal renal tubules. H&E x400. (D) infected *E.coli* group showing marked degenerative changes of tubular cells, (E) Yeast treated and infected group and (F) Sangrovit treated and infected group showing mild vacuolar degeneration of tubular epithelium. H&E x400.

CONCLUSION

It could be concluded that Yeast and Sangrovit as feed additives have marked impacts on growth-promoting, immunomodulating and antimicrobial effects with no adverse effects on haematological and serum biochemical parameters and can contribute in controlling and prevention of *E.coli* induced experimental infection in broiler chicken.

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