Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 63 (1): 110-125 Oct. 2019
DOI: 10.5455/ajvs.66926

Effect of Some Antibiotic Alternatives on Experimentally *Escherichia Coli* Infected Broiler Chicks

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Key words:
antibiotic alternatives, prebiotic, organic acid, *E. coli*, broiler chicks, growth performance, serum biochemistry

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Article History
Received: Aug 11 2019
Revised: Aug 30 2019
Accepted: Sept 10 2019

ABSTRACT

The present study was conducted to investigate the effect of antibiotic alternatives as prebiotic, organic acid and antibiotic supplementation to the broiler chicks on their protective effect against artificial infection with ciprofloxacin marked *E.coli* O25 that previously isolated from diseased chicken in Kafr elsheikh governorate, growth performance, survival rate, immunity, serum biochemistry and response to NDV. Three-hundred and fifty-day-old broiler chicks were divided into 7 equal groups, 50 chicks each; group 1 was kept as control. Chicks of group 2 was infected orally with 0.2 ml of *E.coli* O25 containing $1x10^8$ viable organism/ml in phosphate buffered saline (PBS) and kept as infected control group. Chicks of group 3 received organic acid (Butyric) from 1st day of age in water (2ml/liter). Chicks of group 4 received prebiotic (Hydrostar®) from 1st day of age in water (0.5ml/liter). Chicks of group 5 received antibiotic (Neomycin) in water (1gm/liter), Chicks of group 6 received prebiotic (Hydrostar®) and antibiotic (Neomycin). Chicks of group 7 received organic acid (Butyric) and antibiotic (Neomycin). Neomycin was applied for 5 successive days beginning from 48 hrs post infection. At 15 days of age chicks of treated groups (3-7) were orally inoculated with 0.2 ml of PBS containing 1×10^8 viable organism/ml of *E. coli* O25. Our results showed that the birds received organic acid (Butyric acid) (G3) and prebiotic (Hydrostar®) (G4) showed more favorable clinical signs, mortality rate, post mortem lesions, recovery rate, bacterial re-isolation results, growth performance and improved immune response to NDV. A decrease in the mean values of serum ALT and AST and creatinine levels and increase in mean values of albumin, globulin and antioxidant enzymes (SOD and CAT) were recorded that may provide evidence for the hepato and renoprotective effects of organic acid (Butyric acid) and prebiotic (Hydrostar®). It could be concluded that prebiotic (Hydrostar®) and organic acid (Butyric acid) can be used as antibiotic alternatives due to their high effective growth promoting, antibacterial and positive impact on liver, kidney functions and antioxidant enzymes. This study highly recommends the use of Hydrostar® and butyric acid as therapeutic agents in dealing with *E. coli* infection in chickens however, its concurrent administration with Neomycin in treatment of such case revealed the most favorable outcomes.

1. INTRODUCTION

Enteric disorders are one of the most important diseases which affect poultry causing high economic losses worldwide due to high mortality rates, decrease weight gain, increase medication costs and feed conversion rate (FCR) (Hafez, 2011).

E. coli is a normal inhabitant chicken's microflora in which some avian *E. coli* strains are pathogenic and cause diseases in domestic poultry, specially colibacillosis which considered one of the most important respiratory and systemic diseases causing high morbidity, mortality, loss of body weight, bad FCR, decrease in egg and meat production (Satyajit et al., 2013). Furthermore, *E.coli* is one of most

important food-borne pathogens of public health interest in poultry meat worldwide (Adeyanju and Ishola, 2014).

Antibiotics are routinely used to treat and prevent infections in humans and animals. However, scientific evidence suggests that the massive use of these compounds has led to increased problem of antibiotic resistance (Furtula et al., 2010 and Forgetta et al., 2012) and presence of antibiotics residues in feed and environment compromises human and animal health (Carvalho and Santos, 2016 and Gonzalez et al., 2017). For that reason, in addition to its residues in meat, development of resistant bacteria, and imbalance of normal microflora, there is a worldwide attempt to decrease antibiotic usage (Sorum and Sunde, 2001). So, there is a need for alternatives to antibiotic that ensure animal health and performance without compromising human health. Such alternatives have been based mostly such as probiotics, prebiotics, organic acids, phytogenic products, enzymes, betaine or mixtures (Hertrampf, 2001; O'Keefe, 2005; Plail, 2006 and Van , 2006).

Prebiotics are ordinarily fermentable feed additives that can directly or indirectly support a healthy intestinal microbiota and have gained increasing attention in the poultry industry as wariness toward antibiotic use has grown in the face of foodborne pathogen drug resistance. Their potential as feed additives to improve growth, promote beneficial gastrointestinal microbiota, and reduce human-associated pathogens, has been well documented. (Micciche et al., 2018).

The prebiotics are feed ingredients that are non-absorbable, and fermented by intestinal organisms, stimulating beneficial bacteria associated with animal health (Roberfroid, 2007). Moreover, prebiotics bind to pathogens in the intestinal lumen and block the adhesion of those bacteria to the epithelial cells (Spring et al., 2000) (Pourabedin and Zhao, 2015). also they increase digestion of nutrients in the feed by increasing the length and width of intestinal villi (Sinovec and Markovic, 2005). In addition, they activate innate immunity by the interaction of the sugars with certain receptors present on the surface of dendritic cells and macrophages which can then stimulate the production of cytokines, the proliferation of lymphocytes and activity of natural killer cells (Hashim, 2012 and Saad et al., 2013).

Several studies have been conducted to explore the effect of prebiotic on poultry performance and found that adding 1 mg/kg mannan oligosaccharide (MOS) in broiler chicks diets results in significantly higher feed intake and body weight over 14-28 d and overall period compared to control chicks.(Toghyani et al., 2011).

Since bacterial growth intolerant to pH changes, so organic acids have shown good results in poultry production by reducing the intestinal pH and reducing proliferation of pathogens in the gastrointestinal tract of poultry (Byrd et al., 2001) and (Chaveerach et al., 2004) providing better bird's intestinal health to obtain maximum nutrient absorption (Pirgozliev et al., 2008) and (Ao et al., 2009). The antibacterial activity of organic acids on *Campylobacter spp*, *E. coli*, *Salmonella*, *Clostridium perfringens*, and *Listeria monocytogenes* has been reported (Skrivanova et al., 2006) and (Over et al., 2009).

Moreover, the organic acids were considered as alternative to antibiotic growth promoters (Van et al., 2005). Butyric acid is one of such SCFA, which has higher bactericidal activity when the acid is undissociated (Lesson, 2007). Bacterial cell takes up undissociated fatty acids and once these acids dissociate, there is a change in the intracellular pH leading to death of bacterial cells. Butyrate also appears to play a role in the development of the intestinal epithelium. It was reported that butyrate derived from the fermentation of non-starch polysaccharides is important for normal development of epithelial cells and improved gastrointestinal health and reduced incidence of colon cancer in humans (Brouns et al., 2002).

This work aimed to investigate the comparative antibacterial, growth promoting and immune stimulating effects of prebiotic, organic acid and antibiotic on experimentally ciprofloxacin marked *E.coli* O25 infected broiler chicks.

2. MATERIAL AND METHODS

2.1. Samples collection:

Samples of cloacal swabs and internal organs were collected from poultry farms (broiler chickens) in kafr elsheikh governorate. All samples were cultured in nutrient broth at 37^{o}C for 18-24 hrs, and subcultured on MacConkey and Eosin Methylene Blue (EMB) agar. Colonies showing characteristic morphology of *E. coli* were biochemically identified based on standard microbiology techniques after subculturing on nutrient agar (Vandepitte et al., 2003).

In Vitro pathogenicity testing: This was performed by

2.1. a. Congo Red dye binding activity test:

Trypticase soya agar supplemented with 0.003% congo red dye and 0.15% bile salts was used for this test (Himedia, 2003). Each isolate was cultured on a separate plate and incubated at 37°C. After 24 hrs incubation, the cultures were left at room temperature for 48 hrs to facilitate observation of results. Appearance of red colonies was recorded as congo red (CR+) positive and colonies that did not bind the dye and remained white or grey were considered as congo red (CR -) negative (Sharda et al., 2010).

2.1.b. Hemolysis test:

E. coli isolates were cultivated on blood base agar supplemented with 5% washed sheep blood erythrocytes. Blood agar plates were, then incubated at 37℃ for 24 hrs and colonies producing clear zones of hemolysis, which were recorded as hemolysin positive (Fakruddin et al., 2013)

2.1. c. Serotyping of *E.coli* isolate:

One of congo red (CR+) positive and hemolytic *E.coli* isolates was serogrouped at the serology unit in Animal Health Research Institute, Dokki, Giza by slide agglutination test using polyvalent and monovalent diagnostic *E.coli* antisera using Mast diagnostics Kit (Mast Group Ltd., Merseyside, UK) according to Quinn et al. (1994).

2.1.d. Antibiotic sensitivity test of *E.coli* isolate

Antimicrobial susceptibility test using the disk diffusion method was performed. The isolate was tested for 10 different antimicrobial agents (Oxoid, Basingstoke, UK): amoxicillin (30µg), ampicillin (10µg), ceftriaxone (30µg), amikacin (30µg), colistin sulphate, neomycin, nitrofurantoin (NIT), ciprofloxacin, doxycycline (30µg) and streptomycin (10µg). The plate was incubated for 24 h at 37°C and inhibition zones were measured and classify the isolate as susceptible, intermediate or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016).

2.2. Preparation of bacterial cultures for experimental infection:

E. coli O25 was reconstituted in 5ml nutrient broth and incubated at 37 ^{0}C for 24 hrs. then sub-cultured on MacConkey's agar and incubated at 37 ˚C for 24 hrs.

2.3. Preparation of ciprofloxacin marked *E.coli* was carried out according to (Johnson et al., 2007).

E. coli O25 was grown on increased concentration of ciprofloxacin starting by the concentration of 80 µg /ml broth for 3 days then sub-cultured then increase the concentration till reached to 320 µg /ml broth. The concentration higher than 320 µg /ml broth was lethal to the bacteria.

2.4. Bacterial titration:

Tenfold dilution were prepared from 24 hrs cultures on peptone water to obtain 10^8 CFU/ml to be used for inoculation of chicks according to Sambrook et al., 1989. The chicks were challenged orally with 0.2 ml of saline containing 1x10^8 CFU /ml *E.coli* O25 on the 15th day of age (Cao et al., 2013).

2.5.Viral vaccines

1. Hitchner B1: on day 7, through intraocular route, Batch no.1084283A, 1000 dose.
2. Gumboro: on day 14, in drinking water, Batch no. 11623LJ01, 1000 dose.
3. Lasota vaccine: on day 21, in drinking water, Batch no. 94020030, 1000 dose.

2.6. Prebiotic (Hydrostar®):

Hydro star contain β-glucans, MOS (mannan oligosaccharide) and Hydrolyzed Saccharomyces cerevisiae yeast, Batch No.15963, Produced by Egy Euro animal health and expiry date 3/2020.

2.7. Organic acid (Butyric acid):

Batch no. 1201217, Produced by medical professions for veterinary products and feed additives (MUVCO) and expiry date 6/2020.

2.8. Antibiotic (Neomycin):

Contain neomycin sulphate 20 gm, Produced by medical professions for veterinary products and feed additives (MUVCO), and Batch no.1904122 and expiry date 4/2021.

2.9. Experimental chicks:

The present experiment was conducted on 350 one day old commercial broiler chicks in cages. Broiler chicks were divided into seven equal groups (n=50/group). All birds were subjected to the ordinary vaccination program for broilers against New Castle and Gumboro diseases. All birds were fed the balanced formulated starter and grower finisher rations that meet the nutritional requirements according to the (National Research Council nutrient requirements of poultry (NRC), 1994) as shown in table (1). Collected feed samples were analyzed for Dry Matter (DM), moisture and Ash contents according to (Association of Official Analytical Chemists (AOAC, 1985), and crude protein using Kjeldahl method according to (Randhir and Pradhan, 1981). Ether extract was determined according to (Bligh and Dyer, 1959) technique as modified by (Hanson and Olly, 1963). Fresh and clean drinking water and feed were supplied adlibtium.

2.10.Experimental design:

Table (1): Ingredients Proximate analysis of the experimental different dietary treatments

Ingredients	**Starter 0-3 weeks**	**Grower finisher 3-5 weeks**
Yellow corn (7%)	51.00	58.33
Soybean meal (44.63%)	31.4	25.51
Corn gluten (59.94)	9.0	8.00
Soybean oil	4.47	4.50
Dicalcium phosphate	1.80	1.50
Limestone	1.30	1.25
DL-methionine	0.140	0.050
Lysine	0.030	0.060
Common salt	0.300	0.300
Choline chloride	0.260	0.200
vitamins and mineral mixture*	0.300	0.300
Total (Kg)	100	100
Proximate analysis		
Dry matter	89.63	89.56
Moisture%	10.37	10.44
Crude protein%	23.096	20.36
Ether Extract%	5.06	6.94
Ash%	6.63	5.96
Carbohydrate%	54.84	56.30
Crude Fiber% (CF)	3.44	3.17
Nitrogen free extract (NFE)	51.40	53.13
Calcium %	0.99	0.89
Total phosphorus %	0.47	0.41
DL-methionine	0.50	0.42
Lysine	1.11	1.01
Metabolizable Energy**(ME kcal /kg)	3111.89	3227.75

* The used vitamins & mineral mixture* (Multivita Co.) composed of vitamin A 12000000 IU, vitamin D3 2200000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, Niacin 30000 mg, Biotin 50 mg, Folic acid 1000 mg, Pantothenic acid 10000 mg, Iron 30000 mg, Manganese 60000 mg, Copper 4000 mg, Zinc 50000 mg, Iodine 1000 mg, Cobalt 100 mg, Selenium 100 mg, calcium carbonate (CaCO3) carrier to 3000g. **Metabolisable energy (ME) estimation was done according to the equation of Lodhi et al., (1976).

***NFE = Nitrogen free extract and calculated by difference {100 – (moisture% + CP% + EE% + CF% + Ash%)}

Table (2): Outline of the experimental design

Group	Treatment
1	Control negative (non-challenged).
2	Control positive (Challenged*).
3	Challenged and treated with organic acid (Butyric acid) (2ml/liter) from the 1st day of age.
4	Challenged and treated with prebiotic (Hydrostar®) (0.5ml/1liter) from the 1st day of age.
5	Challenged and treated after infection by 48hrs with antibiotic (Neomycin)(1gm/liter) for 5 successive days .
6	Treated with prebiotic (Hydrostar®) (0.5ml/1liter) from the 1st day of age, challenged and treated after infection by 48hrs with antibiotic (Neomycin) (1gm/liter) for 5 successive days.
7	Treated with organic acid (Butyric) (2ml/liter) from the 1st day of age, challenged and treated after infection by 48hrs with antibiotic (Neomycin) (1gm/liter) for 5 successive days.

*The challenge was performed orally with 0.2 ml of PBS containing 1x108 CFU/ml E. coli O25 at 15th day of age according to (Cao et al., 2013).

2.11.Clinical signs and P/M lesions:

All groups kept under observation for symptoms, post mortem lesions, and mortality along the experimental period.

2.12.Bacteriological investigation:

For bacterial re-isolation, lungs, livers, gallbladder, spleen, and intestine were collected from 10 sacrificed birds in each group at the1st, 2nd and 3rd weeks after the challenge. The samples of all organs put in one tube and used as pooled

sample. Re-isolation was done as recommended by **(Hamm et al., 2016).**

2.13. Measurements:

2.13. a. Growth performance parameters:

Body weights (BW), weight gain, feed intake (FI) were estimated according to the equation by **Vohra and Roudybush (1971),** Feed conversion ratio (FCR) Ensminger (1980), feed efficiency ratio, Relative growth rate (RGR) was calculated according to the equation described by Brody (1968), protein efficiency ratio and mortality rate were recorded weekly.

2.13.b. Blood collection and biochemical analysis:

Blood was collected weekly post infection without anticoagulant from five randomly selected birds from each group through the wing vein. The blood samples were kept for 30 min at room temperature and the serum was collected through centrifugation at 3000 rpm for 15 min and were used for biochemical analysis and were assayed spectrophotometrically by using commercial diagnostic kits (LABOMED Co., Lab. American Inc., USA) for activities of Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) according to Reitman and Frankel (1957). Creatinine was determined according to Michael and Malcolm (2006). Total protein (TP) Lowry et al., (1951), Albumin (Alb) Henry et al., (1974), were determined in serum and Globulins concentration (Glob) was calculated by subtracting Albumin concentration from total proteins. Catalase (CAT) and super oxide dismutase (SOD) activities were determined according to Beers and Sizer (1952) and Martin (1987), respectively.

2.13.c.Immune response:

The antibodies titer against Newcastle disease were tested using the standard HI method according to the standard procedure described by Majiyagbe and Hitchner (1977) and the end point was estimated according to the scheme described by Kaleta and Siegmann (1971).

2.13.d. Chemical analysis of breast meat:

Breast meat was chemically analyzed for moisture, crude protein (CP) and total lipids according to (AOAC, 1990) and the values were expressed on a dry matter basis.

2.14. Statistical analysis:

Statistical analysis was performed using SPSS software (**SPSS, 2007**): SPSS for Windows release 16.0, SPSS Inc., U.S.A.). One way ANOVA followed by Duncan's multiple comparisons test was used to examine the statistically significant differences of different treatments (Groups) on the assessed parameters. Means with the different letters are significantly different according to Duncan's test ($P<0.05$).

3. RESULTS AND DISCUSSION

Colibacillosis is considered a huge economic problem in poultry farms (Alonso et al., 2011). It can be prevented by the use of antibiotics, however, antibiotics caused some harms such as bacteria acquiring resistance to antibiotics (Sorum and Sunde, 2001) and residues in the bird's meat (Burgat, 1991).

3.1. Antibiotic sensitivity test of *E.coli* isolate:

E.coli O25 was sensitive to neomycin, colistin sulfate, and streptomycin but resistant to amoxicillin, ciprofloxacin, amikacin and ceftriaxone while intermediate sensitivity to doxycycline, ampicillin, and nitrofuran was recorded.

3.2. Effect of *E.coli* challenge and different treatments on broilers (clinical signs, post mortum lesions and Mortality rate):

Experimentally infected groups with *E.coli* O25 showed different degrees of clinical symptoms vary from mild to severe (Table 3), depending on the immune system of the bird, these signs included depression, loss of appetite, ruffled feather, emaciation, conjunctivitis, rhinitis, and severe watery diarrhea. These signs agreed with that observed by Shen et al. (2002). On the other hand, the groups treated with prebiotic showed less clinical signs and chickens were more apparently healthy and this proved the great effect of prebiotics in control *E.coli* infection in broilers chicken and this agreed with that of (Cumminge and Macfarlane, 2002) who found that prebiotic alter intestinal microflora, alter the immune system, reduce the pathogen invasion including pathogens such as *salmonella enteritidis* and *E.coli.*

Also, the signs and the mortality rate were less in groups treated with organic acid and this agreed with (Amerah et al., 2012 and Cerisuelo et al., 2014) who reported that organic acids could

decrease mortality in experimentally infected chickens through reducing the concentrations of *Escherichia coli* in the small intestine.

Butyric acid is thus known to have an antimicrobial, anticatabolic and antioxidant effect together improving the lipid metabolism, mineral absorption and immune status of birds. It is also known to improve the carcass characteristics and overall performance of broiler birds (Deepa et al., 2018). Inoculated groups with *E.coli* O25 showed PM lesion (Table 4) as hyperemia of intestinal mucosa, congestion of liver, pericarditis and air saculitis. These results may be explained by the ability of *E. coli* to cross the mucosal barrier of the respiratory tract by their virulence factors and enter the bloodstream to disseminate throughout the body causing lesions in different organs Norhan et al. (2012), similar PM lesions recorded by Ameh et al. (2011). Treated groups with neomycin showed general improvement, but there were still mild lesions in some organs, which may be due to the long period needed by the inflamed tissues to heal This result was in agreement with that recorded by Fernandez et al. (1998).

Table (3): Clinical signs and their severity on different groups post challenge

Group	Treatment	General signs of illness[1]			Respiratory distress[2]			Diarrhea[3]		
		Weeks PC*			Weeks PC			Weeks PC		
		1	2	3	1	2	3	1	2	3
1	Non-challenged and non-treated	-	-	-	-	-	-	-	-	-
2	challenged and non-treated	++	+++	+++	++	+++	+++	+	++	+++
3	challenged and treated with butyric acid	++	++	+	++	++	++	+	++	+
4	challenged and treated with Hydrostar®	++	++	+	++	+++	+++	+	++	+++
5	challenged and treated with neomycin	++	+++	++	++	++	++	+	++	+
6	challenged and treated with neomycin +Hydrostar®	+	+	++	+	++	+	+	+	-
7	challenged and treated with neomycin+ butyric	+	+	++	+	++	+	+	+	-

*Post challenge, - no signs, +mild clinical signs,++moderate clinical signs and +++sever clinical signs. 1 listlessness, tendency to huddle together, loss of appetite, depression, ruffled feather and dropping of wings. 2gasping (mouth breathing), sneezing, rales. 3 fuel-smelling diarrhea.

Nonchallenged chickens showed no mortalities along the course of the experiment. In *E. coli* challenged groups, mortalities started at the 3rd day post-challenge and was the highest percentage (40%) that reduced by treatment with prebiotic (30%) , organic acid (24%) and Neomycin (20%), in combination between neomycin and prebiotic or organic acid (16%) (Table5).

Effect of different treatments on *E.coli* re-isolation rate from *E.coli* challenged broiler:

Infected and treated group with antibiotic (G5) revealed no re-isolation rate from the internal organs at the 3rd-week post infection but the re-isolation rate at the 1st and 2nd-week post infection was 40% and 20%, respectively as shown in table (6). The presence of *E. coli* post-treatment explained by Toutain et al. (2002) who mentioned that when infection reached the affected organ caused inflammation and production of exudate and other debris, which inhibit the antibiotic penetration or complete destruction of the organism. This result was similar to that recorded by Fernandez et al. (1998) and Zainab (2006). So the re-isolation rate was significantly higher in *E. coli* infected group than antibiotic-treated one.This result agreed with that recorded by Chansiripornchai and Sasipreeyajan (2002).

Table (4): Postmortem lesions in weekly scarified birds

Group	Treatment	Air sacculitis			Congested liver & spleen			Pericarditis, perihepatitis		
		Weeks PC*			Weeks PC			Weeks PC		
		1	2	3	1	2	3	1	2	3
1	Non-challenged and non-treated	-	-	-	-	-	-	-	-	-
2	challenged and non-treated	++	+++	+++	++	+++	+++	+	++	+++
3	challenged and treated with butyric acid	+	++	++	+	+	+	+	+	+
4	challenged and treated with Hydrostar®	+	++	++	+	+	-	+	++	+++
5	challenged and treated with neomycin	++	+++	++	+	++	++	+	++	+++
6	challenged and treated with neomycin +Hydrostar®	+	+	+	+	+	-	+	+	-
7	challenged and treated with neomycin+ butyric	+	+	+	+	+	-	+	+	+

*Post challenge, - no P/M lesions, +mild P/M lesions, ++moderate P/M lesion s and +++sever P/M lesions. P/M lesions (moderate to severe lesions of enteritis, air saculitis, pericarditis, congestion and hemorrhage in liver and congestion in spleen and other paranchymatus organs).

Table (5): The Mortality rate of infected groups by *E.coli* O25

Group	Treatment	Number of chicks	Mortality rate No.			Mortality rate%
			Weeks PC*			
			1	2	3	
1	Non-challenged and non-treated	50	0	0	0	0%
2	challenged and non-treated	50	12	5	3	40%
3	challenged and treated with butyric acid	50	6	4	2	24%
4	challenged and treated with Hydrostar®	50	8	4	3	30%
5	challenged and treated with neomycin	50	5	3	2	20%
6	challenged and treated with neomycin +Hydrostar®	50	5	3	0	16%
7	challenged and treated with neomycin+ butyric	50	5	2	1	16%

*Post challenge.

Table (6): *E.coli* re-isolation rate from *E.coli* challenged broilers on different treated groups

Group	Treatments	Weeks PC *(positive / total examined birds)		
		1st week.	2nd week.	3rd week.
1	Non-challenged + non-treated	0/10 (0%)	0/10 (0%)	0/10 (0%)
2	Challenged+ non-treated	9/10 (90%)	8/10 (80%)	6/10 (60%)
3	Challenged+ butyric	5/10 (50%)	3/10 (30%)	0/10 (20%)
4	Challenged +Hydrostar®	4/10 (40%)	3/10 (30%)	0/10 (20%)
5	Challenged + neomycin	4/10 (40%)	2/10 (20%)	0/10 (0%)
6	Challenged+ Hydrostar®+ neomycin	1/10 (10%)	0/10 (0%)	0/10 (0%)
7	Challenged + butyric + neomycin	2/10 (20%)	0/10 (0%)	0/10 (0%)

*Post challenge

The re-isolation rate in the group treated with organic acid started with 40% at the 1st-week post infection then decreased at the 2nd week to 30% until became 0% at the 3rd week and this agreed with Ricke (2003) who reported that organic acids have strong bacteriostatic effects. Also, in the group treated with prebiotic, the re-isolation rate decreased at the 2nd week post contamination until come to 0% in at the 3rd week and this concurred with (Spring et al, 2000 and

Huang et al., 2004) who cited recorded that prebiotic inspected beneath test circumstances to change the ways of these substances within the elimination of pathogens show within the bird's life forms. Van et al., (2004) have indicated significantly reduced levels of Salmonella in the ceca of birds fed organic acids, whereas Cox et al., (1994) showed butyric acid, in particular, was effective in reducing Salmonella colonization of the intestine. The groups treated with the combination between prebiotic and antibiotic together, and organic acid and antibiotic together recorded 0% re-isolation rate at the 2nd and 3rd-week post infection so the combination of antibiotic with prebiotic or organic acid succeeded in the elimination of the challenged *E.coli*.

Effect of *E.coli* infection and different supplementation on growth performance (Table 7&8):

By the end of the 3rd week post challenge, the best body weight gain was obtained in group (7) which was treated with butyric acid and neomycin and in group (5) which treated with neomycin alone. On the other hand, best results of feed conversion ratio, feed efficiency and protein efficiency were obtained in group (6) which was feed on Hydrostar® and neomycin followed by group (3) which was feed on butyric acid only. Relative growth rate was higher in group (5) which was treated with neomycin then in group (7) followed by group (3). By the end of 4th week post challenge, body weight gain was higher in group (7) and group (5) but feed conversion ratio, feed efficiency and protein efficiency were higher in group (3) followed by group (5). The relative growth rate was higher in group (2) and in both group (3) and (5). By the last week of the experiment, group (2) and (7) had the highest body weight gain while feed conversion ratio, feed efficiency, and protein efficiency were in maximum values in group (5) followed by group (7). The relative growth rate values at the 4th and 5th week of the experiment indicate a compensatory growth in group (2), table (7) and (8). The obtained results confirmed the previous findings of several researchers (Zhang et al., 2005; Angel et al., 2005; Nilson et al., 2004 and Santin et al., 2003). Also, in agreement with Onifade et al. (1999) who reported that prebiotic improved feed/gain ratio and BW gain. Pinchasov and Jensen (1989) reported that butyric acid, unlike other acids such as propionate, did not depress feed intake and increase feed conversion ratio and Bolton and Dewar (1965) indicate that free butyric acid is absorbed very quickly in the upper digestive tract.

Effect of *E.coli* infection and different supplementation on antioxidant enzymes (Table 9):

SOD (superoxide dismutase) catalyzes dismutation of superoxide radicals to hydrogen peroxide and oxygen, enhanced the capacity of scavenging free radicals and decreased damage of tissues or cells and CAT (catalase) catalyzes the breakdown of hydrogen-peroxide to water and molecular oxygen and is one of the key defense systems against oxidative stress (Ighodaro and Akinloye, 2018).

In the present study antioxidant enzymes (SOD and CAT) decreased in group (2) which inoculated by *E.coli* and increased in group (7) which treated with butyric and neomycin and (6) which treated with Hydrostar® and neomycin. These results agreed with (Zhang et al., 2011) who reported that at day 21, broiler birds supplemented with sodium butyrate up to 0.1 % elevated serum SOD and catalase, while reduced serum MDA levels but disagreed with Sukoyan et al., (2005) who reported that catalase activity decreased significantly and (Ciftci et al., 2010) stated that catalase activity remained unchanged.

Effect of *E.coli* infection and different supplementation on liver enzymes (Table 10):

In the present study liver enzymes (ALT and AST) decreased in group (2) which inoculated by *E.coli* and increased in group (7) treated with butyric acid and neomycin, group (6) treated with hydrostar® and neomycin, group (4) treated with Hydrostar and group (3) treated with antibiotics to reach to normal values these results indicate that prebiotic and butyric acid have the protective effect over hepatocytes.

Table (7): Effect of different treatments on growth performance parameters (weekly)

Parameter	Groups	Initial weight (g)	1st Week	2nd Week	3rd Week	4th Week	5th Week
Weight (g)	G1	46.85	115.9 ± 2.9 ^{r}	285.6 ± 2.8 ^{q}	690.4 ± 7.5 ^{k}	1160.3 ±5.4 ^{f}	1608.7 ± 8.7 ^{b}
	G2	46.65	121.5 ± 1.6 ^{r}	307.6 ± 1.2 ^{op}	556.7 ± 4.3 ^{m}	984.7 ± 5.4 ^{j}	1481.1 ± 11.4 ^{e}
	G3	46.8	120.1 ± 1.3 ^{r}	311.8 ± 1.4 ^{no}	657.7 ± 4.3 ^{l}	1136.3 ±2.7 ^{g}	1548.5 ± 8.6 ^{c}
	G4	46.4	129.2 ± 1.1 ^{r}	324.5 ± 1.5 ^{n}	668.4 ± 4.4 ^{l}	1072.0 ± 4.3 ^{i}	1503.2 ± 8.8 ^{d}
	G5	47.45	118.7 ± 0.85 ^{r}	296.6 ± 1.7 ^{pq}	660.5 ± 5.3 ^{l}	1141.6 ± 4.4 ^{g}	1535.5 ± 7.8 ^{c}
	G6	45.95	124.9 ± 0.63 ^{r}	326.2 ± 2.1 ^{n}	666.0 ± 3.7 ^{l}	1096.0 ± 4.5 ^{h}	1538.5 ± 7.2 ^{c}
	G7	45.3	120.0 ± 1.03 ^{r}	319.0 ± 1.7 ^{no}	684.4 ± 2.9 ^{k}	1170.0 ±2.89 ^{f}	1647.5 ± 6.6 ^{a}
Weight Gain (g)	G1	-----	69.1 ± 2.8 ^{n}	169.8 ± 3.61 ^{m}	404.7 ± 5.63 ^{fg}	469.9 ± 10.43 ^{b}	448.4 ± 6.58 ^{c}
	G2	-----	74.8 ± 2.11 ^{n}	186.1 ± 2.1 ^{klm}	249.1 ± 3.14 ^{j}	427.9 ± 9.73 ^{de}	496.4 ± 6.05 ^{a}
	G3	-----	73.3 ± 1.76 ^{n}	191.6 ± 1.65 ^{kl}	345.9 ± 2.89 ^{I}	478.5 ± 1.82 ^{ab}	412.2 ± 11.12 ^{ef}
	G4	-----	82.8 ± 1.85 ^{n}	195.2 ± 2.44 ^{kl}	343.9 ± 2.88 ^{I}	403.5 ± 8.23 ^{fg}	431.2 ± 11.89 ^{cd}
	G5	-----	71.2 ± 0.94 ^{n}	177.9l ± 2.48 ^{m}	363.8 ± 3.6 ^{h}	481.1 ± 1.17 ^{ab}	393.9 ± 11.94 ^{g}
	G6	-----	78.9 ± 1.24 ^{n}	201.2 ± 2.56 ^{k}	339.8 ± 2.91 ^{I}	430.0 ± 7.64 ^{d}	442.5 ± 11.42 ^{cd}
	G7	-----	74.7 ± 1.12 ^{n}	198.9 ± 1.33 ^{k}	365.4 ± 1.27 ^{h}	485.5 ± 5.28 ^{ab}	477.5 ± 9.46 ^{b}
FI (feed intake) (g)	G1	-----	119.21	293.42	594.98	753.72	947.81
	G2	-----	131.5	311.25	383.61	753.26	1201.31
	G3	-----	120.75	297.5	501.55	767.21	947
	G4	-----	115.75	303.5	508.97	690.1	977.65
	G5	-----	134.66	313.21	549.4	789.74	757.74
	G6	-----	132.7	310.75	462.13	730.2	960.29
	G7	-----	112.16	307.11	534.74	807.77	989.06
FCR (feed conversion ratio)	G1	-----	1.73 ± 0.07 ^{fg}	1.73 ± 0.04 ^{fg}	1.47 ± 0.02 ^{kl}	1.61 ± 0.04 ^{hij}	2.11 ± 0.03 ^{cd}
	G2	-----	1.76 ± 0.05 ^{f}	1.67 ± 0.02 ^{fgh}	1.54 ± 0.02 ^{ijk}	1.76 ± 0.04 ^{f}	2.42 ± 0.03 ^{a}
	G3	-----	1.65 ± 0.04 ^{ghi}	1.55 ± 0.01 ^{ijk}	1.45 ± 0.01 ^{klm}	1.6 ± 0.01 ^{hij}	2.3 ± 0.06 ^{b}
	G4	-----	1.4l ± 0.03 ^{m}	1.55 ± 0.02 ^{ijk}	1.48 ± 0.01 ^{kl}	1.71 ± 0.03 ^{fgh}	2.27 ± 0.06 ^{b}
	G5	-----	1.89 ± 0.02 ^{e}	1.76 ± 0.02 ^{f}	1.51 ± 0.01 ^{jk}	1.64 ± 0.01 ^{ghi}	1.93 ± 0.06 ^{e}
	G6	-----	1.68 ± 0.03 ^{fgh}	1.54 ± 0.02 ^{ijk}	1.36 ± 0.01 ^{m}	1.7 ± 0.03 ^{fgh}	2.17 ± 0.06 ^{c}
	G7	-----	1.5 ± 0.02 ^{jkl}	1.54 ± 0.01 ^{ijk}	1.46 ± 0.01 ^{klm}	1.66 ± 0.02 ^{fgh}	2.07 ± 0.04 ^{d}
FER (feed efficiency ratio)	G1	-----	0.58 ± 0.03 ^{hi}	0.58 ± 0.01 ^{hi}	0.68 ± 0.01 ^{cd}	0.62 ± 0.01 ^{fg}	0.47 ± 0.01 ^{k}
	G2	-----	0.57 ± 0.02 ^{I}	0.6 ± 0.01 ^{ghi}	0.65 ± 0.01 ^{def}	0.57 ± 0.01 ^{I}	0.41 ± 0.01 ^{m}
	G3	-----	0.61 ± 0.01 ^{gh}	0.64 ± 0.01 ^{ef}	0.69 ± 0.01 ^{bc}	0.62 ± 0.01 ^{fg}	0.44 ± 0.01 ^{lm}
	G4	-----	0.72 ± 0.02 ^{ab}	0.64 ± 0.01 ^{ef}	0.68 ± 0.01 ^{cde}	0.58 ± 0.01 ^{hi}	0.44 ± 0.01 ^{lm}
	G5	-----	0.53 ± 0.01 ^{j}	0.57 ± 0.01 ^{I}	0.66 ± 0.01 ^{cde}	0.61 ± 0.01 ^{gh}	0.52 ± 0.02 ^{j}
	G6	-----	0.6 ± 0.01 ^{ghi}	0.65 ± 0.01 ^{def}	0.74 ± 0.01 ^{a}	0.59 ± 0.01 ^{hi}	0.46 ± 0.01 ^{kl}
	G7	-----	0.67 ± 0.01 ^{cde}	0.65 ± 0.01 ^{def}	0.68 ± 0.01 ^{c}	0.6 ± 0.01 ^{ghi}	0.48 ± 0.01 ^{k}
PER (protein efficiency ratio)	G1	-----	2.51 ± 0.1 ^{ijk}	2.5 ± 0.05 ^{ijk}	3.34 ± 0.05 ^{b}	3.06 ± 0.07 ^{de}	2.32 ± 0.03 ^{lm}
	G2	-----	2.46 ± 0.07 ^{jkl}	2.59 ± 0.03 ^{ij}	3.19 ± 0.04 ^{cd}	2.79 ± 0.06 ^{h}	2.03 ± 0.02 ^{p}
	G3	-----	2.63 ± 0.06 ^{i}	2.79 ± 0.02 ^{h}	3.39 ± 0.03 ^{b}	3.06 ± 0.01 ^{de}	2.14 ± 0.06 ^{op}
	G4	-----	3.1 ± 0.07 ^{de}	2.79 ± 0.03 ^{h}	3.32 ± 0.03 ^{bc}	2.87 ± 0.06 ^{fgh}	2.17 ± 0.06 ^{nop}
	G5	-----	2.29 ± 0.03 ^{mn}	2.46 ± 0.03 ^{jkl}	3.25 ± 0.03 ^{bc}	2.99 ± 0.01 ^{ef}	2.55 ± 0.08 ^{ij}
	G6	-----	2.58 ± 0.04 ^{ij}	2.8 ± 0.04 ^{gh}	3.61 ± 0.03 ^{a}	2.89 ± 0.05 ^{fgh}	2.26 ± 0.06 ^{mno}
	G7	-----	2.89 ± 0.04 ^{fgh}	2.8 ± 0.02 ^{gh}	3.36 ± 0.01 ^{b}	2.95 ± 0.03 ^{efg}	2.37 ± 0.05 ^{klm}
RGR (relative growth rate)	G1	-----	84.8 ± 2.06 ^{gh}	84.56 ± 2.07 ^{gh}	82.93 ± 0.59 ^{h}	50.8 ±1.21 ^{no}	32.39 ± 0.42 ^{rs}
	G2	-----	89.01 ± 2.05 ^{cde}	86.76 ±1.15 ^{defg}	57.63 ±0.35 ^{l}	55.53 ±1.22 ^{lm}	40.26 ± 0.22 ^{q}
	G3	-----	87.81 ±1.8 ^{cdefg}	88.74 ±0.87 ^{cdef}	71.35 ± 0.18 ^{jk}	53.35 ±0.4 ^{mn}	30.7 ± 0.76 ^{rs}
	G4	-----	94.33 ± 1.83 ^{a}	86.06 ±1.02 ^{defgh}	69.26 ± 0.17 ^{k}	46.37 ±0.95 ^{p}	33.49 ± 0.86 ^{r}
	G5	-----	85.77 ±0.83 ^{efgh}	85.68 ± 1 ^{fgh}	76.02 ±0.2 ^{i}	53.4 ±0.4 ^{mn}	29.43 ± 0.85 ^{s}
	G6	-----	92.43 ± 1.45 ^{ab}	89.22 ± 0.82 ^{cd}	68.49 ±0.46 ^{k}	48.81 ±0.85 ^{op}	33.59 ± 0.83 ^{r}
	G7	-----	90.42 ± 0.96 ^{bc}	90.62 ± 0.52 ^{bc}	72.84 ±0.13 ^{j}	52.36 ±0.58 ^{n}	33.89 ±0.63 ^{r}

Values are expressed as a mean value ± standard error. Means within the same column of different superscript letters are significantly different at ($P \leq 0.05$).

Table (8): Effect of different treatments on growth performance parameters in all the experimental period (0- 35 days)

Parameters	Initial weight (g)	Final Weight (g)	Gain (g)	Feed intake (g)	FCR	FE	PE	RGR
G1	46.85	1608.8±8.7^{b}	1561.9±8^{b}	2709.1	1.7±0.009^{cd}	0.6±0.003^{ab}	2.8±0.014^{ab}	188.7±0.11^{b}
G2	46.65	1481.2±11.5^{d}	1434.5±12.1^{d}	2781.5	1.9±0.017^{a}	0.5±0.0044^{d}	2.5±0.021^{d}	187.8±0.28^{d}
G3	46.8	1548.5±8.7^{c}	1501.7±9.4^{c}	2634	1.8±0.011^{bc}	0.6±0.0036^{bc}	2.7±0.017^{bc}	188.3±0.24^{bcd}
G4	46.4	1503.2±8.9^{d}	1456.8±9.2^{d}	2596	1.8±0.012^{b}	0.6±0.0036^{c}	2.7±0.017^{c}	188±0.21^{cd}
G5	47.45	1535.6±7.9^{c}	1488.1±7.6^{c}	2544.8	1.7±0.009^{d}	0.6±0.003^{a}	2.8±0.014^{a}	188±0.04^{cd}
G6	45.95	1538.5±7.3^{c}	1492.6±6.7^{c}	2596.1	1.7±0.008^{cd}	0.6±0.0026^{ab}	2.8±0.012^{ab}	188.4±0.1^{bc}
G7	45.3	1647.5±6.6^{a}	1602.2±6.9^{a}	2750.8	1.7±0.008^{d}	0.6±0.0025^{a}	2.8±0.012^{a}	189.3±0.12^{a}

Values are expressed as a mean value ± standard error. Means within the same column of different superscript letters are significantly different at (P≤0.05).

Table (9): Effect of *E.coli* infection and different supplementation on antioxidant enzymes (n=5)

Group	SOD (U/L)			CAT (U/L)		
	1st week pc*	2nd week pc	3rd week pc	1st week pc	2nd week pc	3rd week pc
1	22.85±2.22^{b}	23.95±1.71^{ab}	25.45±1.37^{abc}	2.49±0.35^{b}	2.44±0.02^{b}	5.26±0.93^{abc}
2	26.75±3.07^{b}	16.7±1.96^{b}	17.35±1.60^{c}	2.3±0.15^{b}	2.31±0.03^{b}	4.1±0.78^{c}
3	31.85±3.52^{ab}	22.70±3.67^{ab}	26.90±3.72^{abc}	5.92±0.53^{a}	5.01±0.53^{a}	6.16±0.58^{ab}
4	31.50±5.38^{b}	21.25±2.93^{ab}	23.60±3.02^{bc}	3.34±0.44^{b}	2.33±0.03^{b}	4.86±0.72^{bc}
5	26.49±2.75^{b}	16.50±3.86^{b}	20.90±4.24^{bc}	2.80±0.29^{b}	2.36±0.06^{b}	4.43±0.82^{bc}
6	34.5±3.82^{ab}	31.5±4.49^{a}	34.65±2.79^{ab}	3.61±0.57^{b}	2.43±0.02^{b}	5.28±1.11^{abc}
7	43.5±6.97^{a}	32.95±1.90^{a}	36.10±1.99^{a}	6.8±0.97^{a}	6.69±0.54^{a}	7.08±0.72^{a}

*Post challenge, values are expressed as a mean value ± standard error. Means within the same column of different superscript letters are significantly different at (P≤0.05).

Alanine aminotransferase (ALT) present mostly inside hepatocyte so it is specific for the liver of human and other animal but not in birds (Lohr, 1975). Although serum AST present in the liver cell, intestine, and muscles, in acute infection it proceed ALT so it is not liver specific in birds, increased activity has been associated with hepatocellular damage in chicken and turkeys (Rivtez et al., 1977 and Pearson et al., 1979).

Elevated levels of serum ALT and AST indicates the deleterious effects of liver functions. Obtained results agreed with (Ali et al., 2014) and Aluwong et al. (2013) who recorded that supplementation of broiler feeds with 2.0% yeast decrease in activities of serum ALT and disagreed with (Abd-El-Rahman *et al.*, 2012) who stated a significant increase in AST and ALT enzyme activity of broilers that received prebiotic and with (Kamal and Ragaa (2014) and Adil et al., 2010 reported the butyric acid supplementation at 3.0 % did not influence the serum Alanine Transaminase (ALT) and Aspartate Transaminase (AST) levels in broilers but agreed with him in *Eimeria maxima* challenged-birds, inclusion of BAG at 0.4 % prevented the elevation of serum ALT and AST levels.

Table (10): Effect of *E.coli* infection and different supplementation on liver enzymes (n=5)

Group	ALT (U/L)			AST (U/L)		
	1st week pc*	2nd week pc	3rd week pc	1st week pc	2nd week pc	3rd week pc
1	8.2±0.37^{c}	8.4±0.51^{ab}	8±0.44^{b}	55.2±1.62^{c}	50.8±1.98^{d}	41.6±0.81^{d}
2	16.6±1.02^{a}	11±0.44^{a}	22.8±0.86^{a}	78.6±3.74^{a}	66.6±1.88^{a}	60.0±1.51^{a}
3	14.6±0.40^{b}	9.4±0.67^{a}	7.8±0.37^{b}	74.8±0.86^{a}	63.2±1.93^{ab}	53.8±1.35^{b}
4	9.4±0.50^{c}	8.4±0.67^{ab}	7.6±0.60^{b}	65.0±1.00^{b}	61.0±1.92^{abc}	47.8±0.72^{c}
5	15.2±0.58^{ab}	9.4±0.40^{a}	9±0.44^{b}	75.0±0.70^{a}	64.4±1.02^{ab}	57.8±0.58^{ab}
6	9.0±0.70^{c}	7.4±0.24^{b}	6.4±0.51^{b}	57.2±0.96^{c}	56.8±2.05^{c}	43.4±0.67^{cd}
7	9.2±0.66^{c}	7.6±0.60^{ab}	7.6±0.60^{b}	63.2±1.98^{b}	60.4±2.15^{bc}	44.4±0.67^{cd}

*Post challenge, values are expressed as a mean value ± standard error. Means within the same column of different superscript letters are significantly different at (P≤0.05).

Table (11): Effect of *E.coli* infection and different supplementation on serum Creatinine levels (n=5)

Groups	Creatinine (mg/dL)		
	1st week pc*	2nd week pc	3rd week pc
1	1.966±0.018[a]	1.988±0.004[a]	1.916±0.223[b]
2	2.010±0.008[a]	1.930±0.040[a]	2.256±0.016[a]
3	2.004±0.013[a]	1.922±0.049[a]	2.078±0.090[ab]
4	2.002±0.013[a]	1.948±0.026[a]	2.016±0.081[ab]
5	2.014±0.010[a]	1.984±0.006[a]	2.052±0.107[ab]
6	2.03±0.01[a]	1.944±0.023[a]	1.988±0.100[b]
7	2.020±0.013[a]	1.968±0.009[a]	1.996±0.089[b]

*Post challenge, values are expressed as a mean value ± standard error. Means within the same column of different superscript letters are significantly different at (P≤0.05).

Effect of *E.coli* infection and different supplementation on Creatinine (Table 11):

Creatinine is a product of protein metabolism, so its serum level increase indicates a defective excretion from the kidney. Creatinine is not a major nonprotein nitrogenous component of avian blood, the normal serum creatinine of the most birds' ranges from 0.5- 1.5 mg/dl (Rivtez et al., 1977). Increased level of creatinine in serum can be indicative of kidney damage (Yalcin et al., 2012).

In this study, there were no differences in creatinine level in first and second-week post infection between different groups and group 1 and group 2 but at the third week there was an increase in creatinine level in group 2 and values in group 6 and 7 returned as group 1. These results similar to Khakzadihe et al., 2014 who reported that the inclusion of 1% prebiotic inulin in dietary did not effect on TP, ALB, creatinine, glucose, and blood serum amylase on male Coturnix quails and disagreed with Hasan et al., 2014 and Huff et al., 1992 whom reported that supplementation of prebiotics increased uric acid and creatinine level.

Effect of *E.coli* infection and different supplementation on Albumin and Globulin (Table 12):

Albumin is synthesized by the liver and has a half-life about 2 weeks, so a decrease in albumin level may be due to decreased production by the liver or albumin loss either from the kidney (nephropathy) or loss from the intestine (enteropathy) (Levitt and Michael, 2016).

In the present study albumin and globulin decreased in group (2) which inoculated by *E.coli* and there were no differences in 1st and 2nd week post infection but in 3 rd week there were increase in albumin and globulin in group (4) treated with Hydrostar®, group (6) treated with Hydrostar and neomycin, group (7) treated with butyric and neomycin, group (3) treated with butyric and group (5) treated with neomycin respectively. The recent results were similar to that obtained by Griminger (1986) who studied that butyrate increases serum globulin concentrations and lowers albumin to globulin ratio also (Paryad and Mahmoudi, 2008) recorded that prebiotic increase of plasma total protein, albumin, globulin but (Khakzadihe et al., 2014) reported that 1% prebiotic inulin in dietary did not effects on TP, ALB, creatinine, glucose, and blood serum amylase on male Coturnix quails. Butyric acid at 0.4 % in broiler ration significantly increased serum total protein, albumin and globulin (Ali et al., 2014) in *E.coli* challenged birds. Butyric acid prevented the decrease of the serum albumin and globulin as reported with (Zhang et al., 2011).

The changes in liver metabolism caused by endotoxins treatment or live bacterial challenge have been observed in both mammals and birds (Curtis et al., 1980).

The liver is affected greatly due to infection or sepsis which in turn affects its function, (Kokosharov et al., 1997) who reported degenerative changes in the liver to which the decrease was attributed. Also, (Kokosharov, 2007) reported a significant decrease in either serum total protein or albumin due to *S. gallinarium* infection, also he added that the acute *S. gallinarium* infection caused a reduction in albumin whereas globulin fractions increased.

Effect of *E.coli* infection and different supplementation on moisture, dry matter, crude protein % and ether extract% content of breast muscle (Table 13 & 14):

In the recent study results showed that differences in moisture and dry matter between group (1) and (2) but in the other group's different treatment change moisture and dry matter percent to reach group (1). Crude protein % began to

decrease in group (2) in 2nd-week post infection and increased in other groups but in ether extract % increased only in group (7). These results agreed with (Paryad and Mahmoudi, 2008) who showed that breast meat of broiler chicks fed rations contains 1.5 and 2% S. cerevisiae had the higher (P<0.05) dry matter, crude protein and ether extract percentage compared with control. Crespo and Esteve (2001) who stated that changes in body fat deposition between broilers fed different dietary fatty acid profiles may be related to different rates of lipid synthesis or lipid oxidation.

Table (12): Effect of *E.coli* infection and different supplementation on serum Albumin and Globulin (n=5)

Group	Albumin (g/dL)			globulin (g/dL)		
	1st week pc*	2nd week pc	3rd week pc	1st week pc	2nd week pc	3rd week pc
1	5.12±0.06^{a}	5.01±0.01^{a}	4.84±0.15^{bc}	1.22±0.05^{a}	1.07±0.03^{a}	0.92±0.09^{bc}
2	4.92±0.07^{a}	4.89±0.03^{a}	4.77±0.17^{c}	1.01±0.05^{a}	0.82±0.10^{a}	0.88±0.02^{c}
3	5.00±0.03^{a}	5.00±0.01^{a}	5.07±0.08^{ab}	1.04±0.07^{a}	0.98±0.08^{a}	1.22±0.18^{ab}
4	5.06±0.07a	5.05±0.02^{a}	5.15±0.06^{a}	1.09±0.08^{a}	0.97±0.02^{a}	0.97±0.19^{abc}
5	4.99±0.07^{a}	4.93±0.07^{a}	4.95±0.17^{abc}	1.04±0.07^{a}	0.99±0.02^{a}	0.95±0.02^{abc}
6	5.10±0.06^{a}	5.01±0.01^{a}	5.17±0.03^{a}	1.16±0.08^{a}	0.94±0.03a	1.15±0.14^{abc}
7	5.09±0.05^{a}	5.01±0.01^{a}	5.11±0.04^{ab}	1.16±0.03^{a}	0.97±0.02^{a}	1.26±0.16^{a}

*Post challenge, values are expressed as a mean value ± standard error. Means within the same column of different superscript letters are significantly different at (P≤0.05).

Table (13): Effect of *E.coli* infection and different supplementation on moisture and dry matter content of breast muscle (n=5)

Group	Moisture%			Dry matter%		
	1st week pc*	2nd week pc	3rd week pc	1st week pc	2nd week pc	3rd week pc
1	75.54±0.21^{a}	75.53±0.22^{a}	73.35±0.68^{ab}	24.89±0.22^{a}	24.46±0.22^{a}	26.65±0.69^{b}
2	75.54±0.82^{a}	74.91±0.19^{a}	69.07±2.32^{b}	23.93±0.30^{a}	25.08±0.19^{a}	30.92±2.32^{a}
3	72.89±0.54^{a}	74.93±0.07^{a}	70.55±1.20^{b}	26.93±0.72^{ab}	25.06±0.07^{a}	29.45±1.20^{a}
4	74.92±1.04^{a}	74.60±0.72^{a}	69.07±0.03^{b}	24.90±0.87^{a}	25.39±0.72^{a}	30.92±0.03^{a}
5	75.05±0.37^{a}	75.05±0.37^{a}	74.58±0.32^{a}	25.01±0.44^{a}	24.94±0.37^{a}	25.41±0.32^{b}
6	75.46±0.73^{a}	73.89±0.83^{a}	75.18±0.69^{a}	23.97±0.17^{b}	26.10±0.83^{a}	24.82±0.70^{b}
7	74.48±0.17^{a}	74.76±0.45^{a}	73.96±0.22^{a}	24.99±0.34^{a}	25.23±0.45^{a}	26.04±0.22^{b}

*Post challenge, values are expressed as a mean value ± standard error. Means within the same column of different superscript letters are significantly different at (P≤0.05).

Table (14): Effect of *E.coli* infection and different supplementation on crude protein% and ether extract% content of breast muscle (n=5)

Group	CP (Crude Protein) %			EE (Ether Extract) %		
	1st week pc*	2 nd week pc	3rd week pc	1st week pc	2nd week pc	3rd week pc
1	70.73±1.41^{a}	73.7±0.39^{a}	76.59±0.89^{a}	15.09±0.15^{a}	16.36±0.31^{a}	19.28±0.33^{b}
2	71.73±0.49^{a}	61.41±0.97^{c}	63.33±2.76^{c}	14.64±0.35^{a}	14.14±0.19^{a}	17.60±0.25^{b}
3	70.52±0.63^{a}	66.00±2.59^{bc}	70.80±1.76^{ab}	15.00±0.23^{a}	16.42±0.63^{a}	19.48±0.94^{b}
4	67.03±2.17^{a}	66.82±2.66^{abc}	70.22±1.15^{ab}	14.81±0.18^{a}	14.70±0.58^{a}	18.60±1.01^{b}
5	71.9±0.22^{a}	63.44±1.72^{bc}	68.89±0.29^{bc}	14.72±0.26^{a}	14.53±0.07^{a}	17.16±0.18^{b}
6	65.52±1.27^{a}	73.36±5.22^{a}	72.24±2.64^{ab}	14.83±0.41^{a}	14.76±0.47^{a}	19.35±2.87^{b}
7	71.83±0.56^{a}	69.56±2.20^{ab}	73.66±1.96^{ab}	15.00±0.54^{a}	16.83±0.55^{a}	24.25±2.16^{a}

*Post challenge, values are expressed as a mean value ± standard error. Means within the same column of different superscript letters are significantly different at (P≤0.05).

Table (15): Effect of E.coli infection and different supplementation HI titer for ND as a log of base 2 (n=5)

Group	Treatments	Weeks after challenge		
		1st week	2 nd week	3rd week
1	Non-challenged + non-treated	1.51±0.057c	1.81 ±0.115c	2.11±0.175c
2	Challenged + non-treated	1.08±0.23e	1.51±0.057e	1.51±0.057e
3	Challenged + butyric	1.81±0.115b	2.11±0.175b	2.41±0.96b
4	Challenged + Hydrostar®	1.81±0.115b	2.11±0.175b	2.41±0.96b
5	Challenged + Neomycin	1.2±0.17d	1.81±0.115d	1.81±0.115d
6	Challenged+ Hydrostar® + neomycin	2.11±0.175a	2.41±0.96a	2.71±0.36a
7	Challenged + butyric + neomycin	2.11±0.175a	2.41±0.96a	2.71±0.36a

Values are expressed as a mean value ± standard error. Means within the same column of different superscript letters are significantly different at (P≤0.05).

Effect of *E.coli* infection and different supplementation HI titer for ND as a log of base 2 (Table 15):

Immune response against ND vaccination as evaluated by HI titer revealed differences in the log of the base 2. HI titer was superior in groups 6 and 7 followed by groups 3 and 4. Similar results were obtained by Sultan et al., (2014) who showed that immune response against ND and IBD was higher in group fed diet supplemented with Bio-mix (commercial herbal product) ($P<0.05$) and the increase of local IgA levels resulting from ingestion of the probiotic may contribute to enhancement of the mucosal resistance against GIT infections (Fukushima et al., 1998 and Fooks et al., 1999)

CONCLUSION

It could be concluded that, prebiotic (hydrostar®) and organic acid (butyric acid) can be used as antibiotic alternatives due to their high efficacy on promoting growth performance, antibacterial effect and positive impact on both liver, kidney functions, and antioxidant enzymes. The study highly recommends the use of hydrostar® and butyric acid as a prophylactic agent in dealing with *E.coli* infection in chicken, however, their concurrent administration with neomycin in the treatment of such case revealed the most favorable outcomes.

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