Incorporation Efficacy Comparison of Probiotic and Antibiotic on Growth Performance, Some Immunological and Biochemical Parameters in *Salmonella entertidis* Challenged Chicks

Fatma M. Abdel Hamid[1♣], Fatma A. El-Gohary[2] and Engy F. Risha[1]

Depts of Clinical Pathology[1] and Hygiene and Zoonoses[2].
Faculty of Vet. Med., Mansoura University. Egypt.
♣fatmamostafa980@yahoo.com

Abstract: The focus of this study was to investigate and compare the efficacy of probiotic and antibiotic for controlling of *Salmonella enteritidis* infection in newly-hatched chicks by monitoring their effect on body weight gains, some immunological and biochemical parameters. 150 newly hatched male Cobb chicks were divided into six equal groups (each of 25). Group 1 served as control, group 2 challenged with *Salmonella enteritidis*, group 3 treated only with probiotic (protexin), group 4 treated with protexin and challenged with *S. enteritidis*, group 5 challenged with *S. enteritidis* and treated with antibiotic meanwhile group 6 treated with antibiotic alone. Chicks were challenged with 0.5 ml phosphate buffered saline containing $8x10^8$ CFU of *S. enteritidis* /ml by oral gavage. Protexin probiotic was administered for birds before and after challenge for 3 weeks and sarafloxacin antibiotic was given after challenge at the recommended dose. Mortality, feed intake, body weight gain and feed conversion ratio were estimated. Blood samples were collected from birds at the end of first and second week post challenge. Our study showed that sarafloxacin and protexin were effective in the treatment of *Salmonella enteritidis* infection in newly-hatched chicks, but protexin seems to be more safe and effective without any deleterious effect on animal health.

[Fatma M. Abdel Hamid, Fatma A. El-Gohary and Engy F. Risha. **Incorporation Efficacy Comparison of Probiotic and Antibiotic on Growth Performance, Some Immunological and Biochemical Parameters in *Salmonella entertidis* Challenged Chicks.** *Life Sci J* 2013;10(4):3550-3558]. (ISSN:1097-8135). http://www.lifesciencesite.com. 473

Keywords: *Salmonella enteritidis*; chicks, body weight gains; probiotic; antibiotic; immunological; biochemical; parameters.

1. Introduction

Poultry industry is one of the most important sectors providing high quality protein for human consumption allover the world. Also challenging the poultry industry to find alternative means of control diseases as excessive use of antibiotic either for therapeutic or protective purposes led to the appearance of bacterial resistant strains **(Azza *et al.*, 2012)**. Salmonella is a facultative intracellular pathogen infecting wide range of hosts (**Ogunleye *et al.*, 2009)**. *Salmonella enteritidis* is one of the most salmonella serotype in poultry products that associated with human salmonellosis **(Haiqi *et al.*, 2013)** and consider an important international public health and economic problem resulting in syndromes such as enteric fever, bacteremia, focal infection, and enterocolitis. Therefore human health protection by the elimination of foodborne pathogens from food animals and their products has become very important for all sectors of the food production chain **(Thirabunyanon and Thongwittaya, 2012)**. Sarafloxacin is a synthetic antibiotic belonging to the fluoroquinolone that are used to control pulmonary, urinary and digestive bacterial infections in poultry and animals. They act by inhibiting bacterial DNA gyrase, a bacterial topoisomerase II that is essential for DNA replication and transcription (**Charleston *et al.*, 1998)**. Due to restriction in using antibiotic in poultry industry, probiotics represent an alternative tool for antibiotics. Probiotics are known as live microorganisms including bacteria and yeast that have a beneficial effect on the host health by improving its intestinal microbial balance **(Capcarova *et al.*, 2008)**. Probiotics can be effective as antibiotics, they have high efficacy in reducing colonization of salmonella, modulating immunological response and suppress inflammatory reactions in the intestinal walls preventing tissue damage **(Alloui *et al.*, 2013)**. As the bacteria present in probiotic prevent attachment of pathogenic bacteria by forming physical barrier on intestinal mucosa, also produce antibacterial compound and enzymes and increase phagocytic population **(Ribeiro *et al.*, 2007)**. The most common types of probiotic bacterium is lactic acid bacteria including genera *Lactobacillus*, *Pediococcus* and other that are found normally in the gastrointestinal tract of vertebrates and invertebrates. Other type of probiotic cultures are microorganisms that are not normally found in gastrointestinal tract like *Saccharomyces boulardii* **(Tellez *et al.*, 2012)**. Protexin is one of the commercial probiotics preparations that improved body weight gain and feed conversion rate in broilers **(Aftahi *et al.*, 2002)**. Aims

of our work are to investigate and compare the efficacy of probiotics and antibiotics in controlling of *Salmonella enteritidis* infection in newly-hatched chicks through evaluation of their efficiency on growth performance, some immunological and biochemical parameters.

2. Material and Methods

2.1. Chicks and Experimental Design:

One hundred and fifty one day-old, Cobb male broiler chicks were purchased from a local hatchery (Mansoura city-Egypt) for all trials. Upon arrival, the chicks were weighed and randomly assigned to six groups (25 of each). The chicks were reared in metal cages with wood shavings bedding material at an isolation unit (Faculty of Veterinary Medicine, Mansoura University, Egypt). They were maintained under strict hygienic conditions, with free water and feed access during the experiment up to 3 weeks. The experimental groups were as follow: Gp1: control negative (non treated), Gp2: Control positive (salmonella challenged), Gp3: probiotic treated, Gp4: fed probiotic supplemented diet and salmonella challenged, GP5: salmonella challenged and antibiotic treated and Gp6: antibiotic treated alone. Chicks in groups 3, 4 were fed probiotic at a dose rate 1g/liter of drinking water for 3 weeks. Meanwhile, those in groups 5, 6 were received antibiotic at dose 40 ppm (40 µg/mL) in drinking water for 5 consecutive days post challenge. Chicks were observed daily for any clinical signs and mortality.

2.2. Bacterial Strain and Inoculum Preparation:

A *Salmonella enteritidis* nalidixic acid resistant strain obtained from (Animal Health research institute, Giza, Egypt) used as challenged organism in this study. Bacterium was inoculated in brain heart infusion broth (BHI) and incubated at 37°C for 12 h. Chicks in groups 2, 4 & 5 were challenged at 2 day of age by 0.5 ml of PBS containing $8x10^8$ CFU/ml of *Salmonella entertidis* by oral gavage. The number of CFU in the inoculum was determined according to **(Yamawaki *et al.*, 2013)**. Phosphate buffer saline (PBS) was used as a placebo in unchallenged groups.

2.3. Probiotic and Antibiotic Treatment:

Protexin® commercial available probiotic was added as a lyophylyzed mix containing 2 X 10^8 CFU/g of *Lactobacillus rhamnosus; L. plantarum, L. delbruekii spp. bulgaricus, L. acidophilus, Bifidobacterium bifidum, Streptococcus thermophilus, and Enterococcus faecium*. Protexin was reconstituted with phosphate-buffered saline to protect the freeze-dried bacteria from osmotic shock. (Protexin®, Probiotics International Ltd., South Petherton, Somerset, UK).

We used Sara Flox WSP (sarafloxacin water-soluble powder; Abbott Laboratories, North Chicago, IL).

2.4. Growth Performance

All birds were weighed at 0, 7, 15 and 21 days of age. The feed intake was recorded throughout the experimental period, after which the body weight gain (BWG) feed/gain (F/G) were calculated, feed consumption, and feed conversion ratio were also calculated.

2.5. Sample Collection

At the end of first and second week post challenge randomly 10 chicks from each group were picked up and blood samples were collected individually from wing vein. Blood samples were taken in plain centrifuge tube for separation of serum to be used in estimation of serum lysozyme activity, bactericidal activity and some biochemical parameters.

2.6. Lysozyme Activity

Serum lysozyme was determined by the turbidimetric assay according to **Parry *et al.* (1965)**. The lysozyme substrate was 0.75 mg of gram positive bacterium Micrococcus Lysodeikticus Lyophilized Cells (Sigma-Aldrich) which was suspended in 1 ml of PBS, pH 5.8. In round bottom microtitre plate 25 µl of serum was added to each well with 175 µl of substrate solution at 25°C. The reduction in absorbance at 450 nm was read after 0 and 20 min using microtitre plate ELISA reader. The unite of lysozyme in serum in µg /ml was obtained from lysozyme curve made by Lyophilized hen egg-white lysozyme (Sigma-Aldrich).

2.7. Serum Bactericidal Activity

Bactericidal activity was measured according to **Welker *et al.* (2007)** 200 UL of serum or Hank's Balanced Salt Solution as control was added to duplicate wells of 96 round bottom well microtiter plate and incubated for 2.5 hr at 37 °C with 50 µL of suspension live a 24 hrs culture of *E.coli* $3X10^8$. To each well, 25 µL diphenyltetrazolium bromide solution ((MTT; 2 mg/ml) (Sigma) was added and incubated for 30 min at room temperature to allow the formation of formazan. Then the supernatant was discarded and the precipitate was dissolved in 200 µL of dimethyl sulfoxide (DMSO). The absorbance of the dissolved formazan was read at 560 nM with microtitre plate ELISA reader and reported as absorbance units.

2.8. Serum Biochemical Analysis:

Prepared frozen serum samples were analyzed for, asprtate aminotransferase (AST), alkalin phosphatase (ALP), lactate dehydrogenase (LDH), glucose, total protein, albumin, cholesterol, triglyceride, nitric oxide (NO) and superoxide dismutase (SOD) with semi-automatic spectrophotometer (BM-Germany,5010) using commercial test kits (Randox Co. UK and Biodiagnostic, Egypt.) according to enclosed pamphlets.

3. Statistical Analysis:

Our results were analyzed by (ANOVA) using SPSS software statistical program (SPSS for windows (ver.20.00, USA). Two groups were significantly different if *P* value was statistically lower than 0.05.

4. Results and Discussion:

Salmonella is the most common agents of foodborne diseases and poultry products still the main source of *S. enteritidis* associated with food borne infections in humans **(Setta *et al.*, 2012)**. The effect of probiotic and antibiotic on body weights in birds challenged with *S.enteritidis* are presented in (Table 1). Body weights and body weight gains were significantly decreased in Gp2 (challenged with $8x10^8$ CFU of *S.enteritidis*) from 1 to 3 weeks of age comparing with all experimental groups. Birds that were treated with probiotic and antibiotic (Gp 3-6) showed an improvement in BW and BWG ($P < 0.05$) compared to the control group. However, probiotic treated groups (3, 4) showed significant improvements at ($P < 0.05$) in BW and BWG than antibiotic treated ones (Groups 5 &6). It is evident form these results that birds fed on probiotic at a level 1 g/kg exhibited higher body weights and body weight gains among all groups allover this trial. These weights indicated a protective effect of probiotic treatments on birds experimentally infected with *S.enteritidis*. Antibiotics as would be expected are more effective in improving performance when the animal is producing well below its genetic potential and may have only statistically significant improvements 80% of the time **(Rosen, 1995)**. However, many studies by **Fuller, (2001); Higgins *et al.* (2008)** and **Mountzouris *et al.* (2010)** proved that the inclusion of various probiotic products is useful in maintaining the intestinal ecosystem in birds by inhibiting the pathogens and fortifying the beneficial members of the intestinal microflora. The recent usage of probiotics instead of antibiotics as sub-therapeutic antibiotics not only influence intestinal microbial population and activities but also affect animal metabolism and specifically alter intestinal function **(Anderson *et al.*, 2000)**. **Ignatova *et al.* (2009)** found that probiotic addition improved final body weight by 14.4%, feed intake by 7.7% and increased feed utilization by 8.1%. Similarly, **Chen *et al.* (2013)** found that the inclusion of probiotics increased body weight gain and feed intake throughout the experimental period, but did not affect feed conversion and thus confirmed the positive effect of probiotics on growth performance in broilers. Our results are in accordance with that found by **Mohnl *et al.* (2006); Mountzouris *et al.* (2007); Samli *et al.* (2007)** and **Abaza *et al.* (2008)**. Moreover, several studies investigated that probiotic supplemented to the birds improve the body weight and daily weight gain **(Khaksefidi and Ghoorchi, 2006; Timmerman *et al.*, 2006; Liu *et al.*, 2007; Mountzouris *et al.*, 2007; Torres-Rodriguez *et al.*, 2007)**. Meanwhile, these results are contrary to the findings of other studies. **Mohan *et al.* (1996)** reported that probiotic improve body weights and body weight gains in chickens only after the 4^{th} week of growth. Also, **Yeo and Kim (1997)** revealed that chickens fed probiotics showed significant increase in average daily weight gain during the first 3 weeks but not during the $4–6^{th}$ weeks of growth. **Zhou *et al.* (2010)** found that inclusion of Bacillus coagulans at two levels in bird's diet improved body weight and feed conversion ratio in Guangxi Yellow chickens. **Zhang *et al.*, (2012)** reported that broilers fed diets supplemented with 10^8 CFU *B. subtilis*/kg had higher body weight gains. Broiler performance was beneficially enhanced by dietary inclusion of B.subtlis probiotic **(Mountzouris *et al.*, 2010; Zhou *et al.*, 2010)**, whereas, a few studies did not report positive effects **(Willis and Reid,2008 : Lee *et al.*, 2010)**.

Table 2 showed the effect of probiotic and antibiotic treatments on feed consumption, feed conversion ratio and mortality % in all treated birds. Higher feed intake ($p<0.05$) was found in Gp1 followed by GP4, Gp3&5 and the lesser feed intake was in Gp2. However, the highest feed conversion ratio was observed in Gp2 followed by Gp1, Gp5, Gp6 and Gp4. Meanwhile, birds in Gp3 showed the least feed conversion ratio ($p<0.05$). There was a significant mortality (60%) in the birds challenged with *S. enteritidis* in Gp2 followed by 32% in Gp5, 24% in Gp4, 12% in Gp6 &Gp1. While, significant reduction in mortality (8%) was observed in probiotic fed birds Gp3. The results in this experiment revealed that probiotic supplementation to birds increased feed consumption, lowered feed conversion ratio and reduced mortality to such comparable degree with other groups especially to control group. Growth performance of birds might be improved by the addition of antibiotic, but the real improvement with no antibiotic resistant bacteria is obtained by using of probiotic. Our results come in agreement with those found by **Wiedmer and Hadorn (1999)** who found that supplementation of Ross Hybrid chicks diet with either probiotic or antibiotic resulted in small but non-significant improvement in body weights, feed conversion rate, and litter quality compared to a control diet up to 41 days. Also, **Ayed *et al.* (2004)** reported that replacement of avilamycin antibiotic by activis probiotic in the broiler diets improved growth performance and lowered food conversion index and such improvement was essentially felt in the early in the growth period when chicks begin to develop their lean tissues. **Willis *et al.* (2007)** observed a significant difference in feed consumption and efficiency due to addition of probiotic to broiler diet. Various studies showed the superiority of probiotic to antibiotic in

improving growth performance as **Maiorka *et al.* (2001)** found that the use of a synbiotic composed of *Saccharomyces cerevisiae* and *Bacillus subtilis* increased feed conversion compared with antibiotic and control treatments at 45 days of age. Similarly, **Cavazzoni *et al.* (1998)** reported that feeding *B. coagulans* strain as a probiotic to broiler chickens improved body weight and feed conversion in comparing to virginiamycin. Also, **Khaksefidi and Ghoorchi (2006)** recorded improvements in the body weight, daily weight gain, feed consumption and feed conversion ratio in birds fed diet supplemented with 50 mg/kg of probiotic from 22 to 42 days than birds fed the control diets, this was attributed to the increased efficiency of digestion and nutrient absorption processes due to presence of the probiotic bacteria. The inclusion of probiotics in the diet allows the rapid development of beneficial bacteria in the digestive tract of the host, improving its performance. Meanwhile **Zhang *et al.* (2013)** who compared the efficiency of antibiotic and probiotic effects on growth performance, found that feed conversion ratio was enhanced by dietary supplementation of enramycin.In agreement with results reported by **Pedroso *et al.* (2006); El- Husseiny *et al.* (2008)** and **Hassan *et al.* (2010)**. As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes **(Edens, 2003)**. Regarding mortality results, cumulative mortality rates were lower in the probiotic fed birds at the level of 1 g/kg than the other groups over the period 3weeks of age. Broilers given Lactobacillus preparations in similar trials, the effects on mortality were inconsistent **(Jin *et al.*, 1998; Zulkifli *et al.*, 2000).** Our results agreed with those reported by **Yo¨ ru¨ k *et al.* (2004)** who found that supplementation of probiotic (containing *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *and Enterococcus* species) during the late laying period in layer hens reduced mortality. Also, **Amerah *et al.* (2011)** and **Amerah and Gracia (2011)** recorded similar response using *B. subtilis* on performance in maize and wheat based diets. Meanwhile, **O'Dea *et al.* (2006)** found no significant differences in broiler mortality between the probiotic treatments and the control group.

The biochemical analysis in our study revealed that salmonella infection in newly-hatched chicks resulted in liver damage manifested by increase enzymes activities of AST and LDH either at first or second week post challenge with *S. enteritidis* comparing with control group while ALP and blood glucose level insignificantly changed comparing with the control one (Table,3). This was resulted by increase lipid peroxidation of hepatocytes as *Salmonella enterica serovar enteritidis* bacterial LPS (endotoxin) induces extensive damage to a variety of organs, including liver due to the increased production of reactive oxygen intermediates **(Benzer *et al.*, 2009)**. Our result agree with **Freitas Neto *et al.*, 2007** who found an increase in AST level in commercial laying hens infected with *Salmonella gallinarum* correlated with hepatic lesions that ranged from vacuolar degeneration to multifocal necrosis. Also **Azza *et al.*, 2012** showed a significant increase in the previous enzyme activities in the infected group at the 5th and 6th weeks of the experiment which may reflect development of hepatic lesions at that time.

As shown in Table 3 the activities of liver enzymes AST and LDH in protexin treated groups (3 & 4) are the same as in the control group which indicate that protexin has no side effect as it not alter biochemical parameters. Our result agree with **Thirabunyanon** and **Thongwittaya** (**2012**) who found that the potential of *B. subtilis* NC11 as a spore-former is safe for animals as it not affect blood biochemical parameters. Also protexin improved liver function in Gp4, as the main benefits of probiotics may occurred by preventing production and or uptake of lipopolysaccharides in the gut reducing levels of low-grade inflammation (**El-Jakee *et al.*, 2010**). Sarafloxacin treatment also improve liver enzyme at first week post challenge but at second week improve only AST while LDH and ALP were significantly increased in group 5 comparing with control one (Table 3).

The current investigation showed an increase in total protein and globulin level in the infected group (Gp2) while albumin and A/G ratio insignificantly changed comparing with control one at first week post challenge (Table 3). The increase in total protein level may be attributed to increase globulin due to either antigenic stimulation of infectious agent or associated with development of liver disease (**Azza *et al.*, 2012**). Also **Xie *et al.* (2000)** stated that *S. typhimurium* LPS treated birds resulted in increased levels of blood protein concentration due to an altered production of proteins related to the acute phase response as known in other species. Total protein, albumin, globulin and A/G ratio are insignificantly changed in both protexin and sarafloxacin treated groups either at first and second week post challenge except in GP4 total protein and globulin levels are increased comparing with control group at 2nd week post challenge (Table3).

The cholesterol and triglycerides levels were insignificantly changed in salmonella treated group at 1st week post infection but significantly decreased comparing with control group at 2nd week post infection (Table 3). This is explained by **Garcia *et al.* (2010)** who mentioned that *Salmonella gallinarum* infection in commercial layers decrease triglycerides and cholesterol levels either due to less food ingestion by the birds or due to alterations in the lipid

metabolism by hepatic lesions as they reported an increase in enzyme activity of ALT and GGT which indicate hepatic lesions. This is also documented by **Xie *et al.* (2000)** who found that *S. typhimurium* LPS induced hypocholesterolemia due to changes in cholesterol and lipoprotein metabolism in the liver during acute phase response. Our result show that probiotic treatment decreased the concentrations of total cholesterol level at 2nd week post treatment but TG level insignificantly changed with control group. This is may be referred as probiotic bacteria ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut that decrease the systemic levels of blood lipids either by inhibiting hepatic cholesterol synthesis and or redistributing cholesterol from plasma to the liver. As well as some bacteria may interfere with cholesterol absorption from the gut by deconjugating bile salts that necessary for cholesterol metabolism or by directly assimilating cholesterol **(Capcarova *et al.*, 2008)**.

NO are well-known as antimicrobial chemicals produced by macrophages in response to infection **(Setta *et al.*, 2012)**. In our study *Salmonella enteritidis* infection resulted in increase both nitric oxide and lysozyme activity at 1st week post infection (PI) but insignificantly changed at 2nd one comparing with the control group. Also SOD activity decrease either at 1st or 2nd week PI meanwhile bactericidal activity insignificantly changed (Table, 4). As known the cell has protective agents against damage induced by oxygen-reactive species including GSH-Px, CAT and SOD that are constitute an antioxidant cellular enzymatic system. LPS-induced increase in oxygen-reactive species resulted in increase lipid peroxidation and nitric oxide levels and decrease in the antioxidant activity in tissues **(Benzer *et al.*, 2009)**. Plasma lysozyme activity was increased by both LPS and B-glucan as the lysozyme gene transcription was increase in all organs following intraperitoneal injection of both LPS and B-glucan in Atlantic salmon (**Lowry *et al.*, 2005)**. This is agree with our result as we found a significant increase in lysozyme activity in GP 4 (treated with protexin and challenged with *S. enteritidis*) comparing with control one either at 1st or 2nd week PI. This was also reported by **El-Jakee *et al.* (2010**) who found a significant increase in lysozyme activity in mice after 2nd and 10th day of oral challenge with *Salmonella typhimurium* and treatment with mixed culture of probiotic strains. So the increase in lysozyme activity may occur either due to probiotics treatment which indicated an immune stimulation or due to Infections or invasion by foreign material **(Magda *et al.*, 2011)**. Our result showed that protexin treatment alone increase SOD and bactericidal activity comparing with control group where some lactobacilli has antioxidant activity that reduce accumulation of ROS during the ingestion of food and degrade the superoxide and hydrogen peroxide anions **(El-Jakee *et al.*, 2010)**. But in sarafloxacin treated groups GP 5 & 6 SOD activity is decreased comparing with control group at 2nd week PI meanwhile lysozyme activity increased in GP 5 either at 1st or 2nd week PI (Table,4).

5. Conclusion

Probiotic supplementation improved performance, reduced mortality,increase serum lysozyme and has antioxidant activity sometimes, better than antibiotics, which favor its usage for birds in an attempt to find antibiotic alternative and reducing appearance of antibiotic-resistant strains. We concluded that probiotic seems to be more safe and effective without any deleterious effect on animal health.

Table 1: The effect of probiotic and antibiotic on body weight and weight gains(gm) in newly-hatched chick challenged with Salmonella enteritidis.

Treatments	1wk		2wk		3wk	
	BW	**BWG**	**BW**	**BWG**	**BW**	**BWG**
GP1	139.68±2.8^{ab}	100.5±2.0^{ab}	362.3 ± 3.5^{b}	222.6 ± 1.5^{bc}	534.5 ± 4.3^{c}	172.2 ±2.0^{c}
(Cont)						
GP2	95.14±3.1^{c}	55.3 ± 1.8^{d}	198.7 ± 2.8^{e}	103.6± 1.4^{e}	312.1± 3.3^{e}	113.41±2.5^{d}
(Inf.)						
Gp3	151.2±5.2^{a}	110.9 ± 2.2^{a}	394.6 ± 4.1^{a}	243.4± 2.6^{a}	637.4± 4.5^{a}	242.81±3.0^{a}
(Prob.)						
Gp4	138.4±4.5^{ab}	99.5± 3.4^{c}	311.2 ± 3.3^{d}	172.8 ± 2.0^{d}	531.6± 3.8^{c}	220.41±2.4^{b}
(Prob.Inf)						
Gp5	141.3±3.4^{b}	102.9± 2.1^{ab}	341.4± 2.5^{c}	200.1 ± 1.6^{c}	520.4±5.2^{cd}	179±3.1^{c}
(Ab.Inf)						
Gp6	154.8±3.8^{a}	115.8± 1.8^{a}	389.7 ± 4.5^{ab}	234.9± 3.2^{b}	618.3± 4.3^{b}	228.61±2.8^{b}
(Ab.)						

a-d Values represent the mean ± SEM. Values within a column with different superscripts differ significantly ($P \leq 0.05$).

Table 2: The effect of probiotic and antibiotic on feed consumption, feed conversion ratio and mortality % in newly-hatched chick challenged with Salmonella enteritidis.

Treatments	Feed Consumption (gm)	FCR	Mortality %
(N. died/ total;%)			
Gp1	440 ± 10.3^{a}	2.6 ± 0.12^{ab}	3/25^{c} (12)
(Cont.)			
Gp2	312± 11.5^{d}	2.8 ± 0.1^{a}	15/25^{a} (60)
(Inf.)			
Gp3	425± 12.5^{c}	1.8 ± 0.1^{d}	2/25^{c} (8)
(Prob.)			
Gp4	432± 10.3^{b}	1.96 ± 0.13^{c}	6/25^{b} (24)
Pro.Inf)			
Gp5	423± 11.5^{c}	2.4 ± 0.16^{b}	8/25^{b} (32)
(Ab.Inf)			
Gp6	435 ± 13.3^{ab}	1.9 ± 0.15^{c}	3/25^{c} (12)
(Ab.)			

a-d Values represent the mean ± SEM. Values within a column with different superscripts differ significantly ($P \leq 0.05$).

Table.3: Some selective serum biochemical parameters (Mean ± S.E) at the end of 1st and 2nd week post challenge with Salmonella enteritidis in newly-hatched chick.

	Groups	AST U/L	ALP U/L	LDH U/L	Glucose mg/dl	T. Protein g/dl	Albumin g/dl	Globulin g/dl	A/G ratio	CHO mg/dl	TG mg/dl
At the end of 1st week	GP1 (Cont.)	43.50 ±1.7^{bc}	43.00 ±2.7^{a}	1402 ±44.87^{bc}	251.5 ±3.96^{a}	3.37 ±0.37^{b}	2.02 ±0.43^{a}	1.35 ±0.09^{b}	1.57 ±0.40^{a}	208.75 ±2.05^{b}	85.75 ±1.93^{b}
	GP2 (Inf.)	60.00 ±5.32^{a}	38.25 ±4.76^{a}	1826 ±65.02^{a}	267.5 ±17.7^{a}	4.47 ± 0.49^{a}	1.84 ±0.16^{a}	2.62 ±0.39^{a}	0.75 ±0.13^{a}	190.0 ±39.66^{b}	118.50 ±6.38^{b}
	GP3 (Prob.)	42.57 ±5.99 ^{c}	47.75 ±6.20^{a}	1527.00±80.18^{b}	263.00 ±12^{a}	3.21 ±0.31^{b}	1.53 ±0.22^{a}	1.68 ±0.13^{b}	0.90 ±0.11^{a}	114.25 ±16.52^{b}	101.75 ±9.58^{b}
	Gp4 (Pro.Inf)	41.00 ±5.99^{c}	42.5 ±4.87^{a}	1322.00±59.88^{c}	256.25 ±29.1^{a}	3.33 ±0.28^{b}	1.62 ±0.14^{a}	1.71 ±0.38^{b}	1.23 ±0.46^{a}	187.00 ±66.78^{b}	124.75 ±8.8^{a}
	GP5 (Ab.Inf)	46.25 ±5.48^{bc}	53.00 ±7.53^{a}	1420.2 ±76.29^{bc}	256.0 ±22.07^{a}	3.48 ±0.14^{ab}	1.43 ±0.23^{a}	2.04 ±0.34^{ab}	0.89 ±0.39^{a}	357.50 ±28.67^{a}	257.0 ±31.42^{a}
	GP6 (Ab.)	56.00 ±3.65^{b}	53.50 ±3.22^{a}	1313.00±48.64^{c}	291.59 ±13.41^{a}	3.44 ±0.42^{ab}	1.75 ±0.23^{a}	1.69 ±0.26^{b}	1.07 ±0.13^{a}	181.75 ±23.77^{b}	236.75 ±5.51^{a}
At the end of 2nd week	GP1 (Cont.)	44.37 ±1.43^{b}	38.75 ±2.01^{b}	1387 ±46.13^{b}	275.0 ±10.34^{ab}	3.85 ±0.62^{b}	1.40 ±0.27^{a}	2.44 ±0.52^{b}	0.67 ±0.23^{a}	231.25 ±8.03^{b}	85.75 ±1.43^{bc}
	GP2 (Inf.)	54.5 ±2.75^{a}	43.00 ±7.22^{b}	2032 ±61.61^{a}	264.5 ±6.35^{ab}	3.98 ±0.13^{b}	1.69 ±0.08^{a}	2.28 ±0.21^{b}	0.77 ±0.12^{a}	168.75 ±20.25^{c}	50.75 ±7.85^{d}
	GP3 (Prob.)	45.75 ±1.65^{b}	38.75 ±3.44^{b}	1409 ±22.69^{b}	263.5 ±11.06^{ab}	3.67 ±0.34^{b}	1.47 ±0.18^{a}	2.29 ±0.22^{b}	0.65 ±0.08^{a}	171.25 ±5.48^{c}	73.00 ±4.61^{c}
	GP4 (Pro.Inf)	43.50 ±2.39^{b}	42.50 ±4.8^{b}	1416 ±42.30^{b}	259.25 ±13.80^{ab}	5.43 ±0.48^{a}	1.62 ±0.14^{a}	3.81 ±0.38^{a}	0.43 ±0.03^{a}	137.00 ±7.92^{c}	72.75 ±9.76^{c}
	GP5 (Ab.Inf)	44.50 ±2.10^{b}	72.75 ±2.83^{a}	2010.75±33.09^{a}	248.25 ±13.80^{b}	3.48 ±0.14^{b}	1.43 ±0.23^{a}	2.04 ±0.34^{b}	0.89 ±0.39^{a}	420.25 ±22.03^{a}	165.25 ±12.71^{a}
	GP6 (Ab.)	48.00 ±2.67^{ab}	45.25 ±7.33^{b}	1304.75±53.61^{b}	288.25 ±8.29^{a}	4.05 ±0.22^{b}	1.46 ±0.26^{a}	2.58 ±0.33^{b}	0.62 ±0.17^{a}	145.25 ±10.97^{c}	109.65 ±5.94^{b}

Table.4: Some selective immunological parameters (Mean ± S.E) at the end of 1st and 2nd week post challenge with Salmonella enteritidis in newly-hatched chick

	Groups	Nitric oxide µ mol/ L	SOD U/ml	Lysozyme µg /ml	Bactericidal Activity (absorbance unite)
At the end of 1st week	GP1 (Cont.)	1.97 ±0.47^{b}	250.15 ±25.81^{b}	25.54 ±3.51^{c}	3.12 ±0.54^{b}
	GP2 (Inf.)	3.88 ±0.18^{a}	180.87 ±8.39^{c}	44.96 ±3.85^{b}	3.27 ±0.24^{b}
	GP3 (Prob.)	1.53 ±0.25^{b}	313.58 ±20.56^{a}	23.52 ±4.52^{c}	5.88 ±0.27^{a}
	GP4 (Pro.Inf)	2.4 2±0.25^{b}	224.02 ±5.95^{bc}	59.22 ±6.20^{a}	3.83 ±0.42^{b}
	GP5 (Ab.Inf)	1.67 ±0.55^{b}	219.94 ±15.27^{bc}	41.46 ±3.85^{b}	3.15 ±0.18^{b}
	GP6 (Ab.)	1.26 ±0.30^{b}	187.62 ±8.02^{c}	27.65 ±4.53^{c}	3.29 ±0.25^{b}
At the end of 2nd week	GP1 (Cont.)	2.27 ±0.63^{a}	227.32 ±25.86^{a}	20.00 ±2.3^{bc}	4.37 ±1.04^{bc}
	GP2 (Inf.)	2.23 ±0.33^{a}	162.27 ±8.92^{b}	12.51 ±1.54^{c}	3.58 ±0.71^{c}
	GP3 (Prob.)	1.82 ±0.22	204.48 ±20.8^{a}	36.43 ±5.36^{a}	10.76 ±1.45^{a}
	GP4 (Pro.Inf)	1.46 ±0.27^{a}	241.06 ±5.12^{a}	34.36 ±4.51^{a}	6.82 ±1.33^{b}
	GP5 (Ab.Inf)	1.37 ±0.16^{a}	141.7 ±17.72^{b}	41.46 ±3.85^{a}	3.39 ±0.22^{c}
	GP6 (Ab.)	2.52 ±0.58^{a}	134.03 ±3.33^{b}	25.15 ±3.64^{ab}	2.93 ±0.45^{c}

References

1. Abaza IM, Shehata MA, Shoieb MS and Hassan II (2008): Evaluation of some natural feed additive in growing chick diets. International Journal of Poultry Science; 7 (9): 872-879.
2. Aftahi A, Munim T, Hoque MA and Ashraf MA (2002): Effect of yoghurt and protexin boost on broiler performance. International Journal of Poultry Science; 5(7):651-655.
3. Alloui MN,Szczurek W and Swiatkiewcz S (2013): The usefulness of prebiotics and probiotics in modern poultry nutrition: a review. Ann. Anim. Sci.; 13(1): 17–32.
4. Amerah AM and Gracia MI (2011): Influence of three *Bacillus subtilis* strains combination on the performance, intestinal morphology and blood parameters of broilers fed wheat-based diet. International Poultry Scientific Forum, Atlanta, Georgia.
5. Amerah AM, Jansen van Rensburg C, Plumstead PW (2011): Effect of feeding diets containing a probiotic or antibiotic on broiler performance and litter water-soluble phosphorus. Poult. Sci.; 90 (E-Suppl. 1), 15.
6. Anderson DB, McCracken VJ, Aminov RI, Simpson JM, Mackie RI, Verstegen, MWA and Gaskins HR (2000): Gut microbiology and growth-promoting antibiotics in swine. Pig News Inf; 20:1115N–1122N.
7. Ayed MH, Laamari Z and Rekik B (2004*):* Effects of incorporating an antibiotic " avilamycin" and a probiotic "activis" in broiler diets. Proceedings, Western Section, American Society of Animal Science; 55: 237-242.
8. Azza HA, Kamel HH, Walaa M A, Olfat SHM and Amira HM (2012): Effect of bactocell® and revitilyte-plustm as probiotic food supplements on the growth performance, hematological, biochemical parameters and humoral immune response of broiler chickens. World Applied Sciences Journal; 18 (3): 305-316.
9. Benzer F, Kılıç A, Yılmaz S, Erişir M, Timurkaan N and Ertaş HB (2009): Influence of enrofloxacin administration on oxidative stress and antioxidant enzyme activities of experimentally infected broilers with *Salmonella enterica serovar enteritidis*. E-Journal of New World Sciences Academy Veterinary Sciences; 4(2):24-33.
10. Capcarova M, Kolesarova A, Massanyi P and Kovacik J (2008): Selected blood biochemical and haematological parameters in turkeys after an experimental probiotic *enterococcus faecium* m-74 strain administration. International Journal of Poultry Science; 7 (12): 1194-1199.
11. Cavazzoni V, Adami A and Castrovilli C (1998): Performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic. Br. Poult. Sci.; 39: 526–529.
12. Charleston B, Gate JJ, Aitken IA, Stephan B and Froyman R (1998): Comparison of the efficacies of

three fluoroquinolone antimicrobial agents, given as continuous or pulsed-water medication, against *Escherichia coli* infection in chickens. Antimicrobial Agents and Chemotherapy; 42(1):83-87.
13. Chen W, Wangb JP, Yanc L and Huanga YQ (2013): Evaluation of probiotics in diets with different nutrient densities on growth performance, blood characteristics, relative organ weight and breast meat characteristics in broilers British Poultry Science; 54(5): 635-641.
14. Edens FW (2003): An alternative for antibiotic use in poultry probiotics. Brazilian Journal of Poultry Science; 5: 75–97.
15. El-Husseiny OM,Abdallah AG and Abdel-Latif KO (2008):The influence of biological feed additives on broiler performance.Int.J.Poult.Sci.7, 862–871.
16. El-Jakee J, Moussa M, Nada SA, Mohamed KF, Ashgan MH and Mohamed ML (2010) : Influence of probiotics mixture on *Salmonella typhimurium* in mice. International Journal of Microbiological Research; 1 (2): 50-61.
17. Freitas Neto OC, Arroyave W, Alessi AC, Fagliari JJ and Berchieri A (2007): Infection of commercial laying hens with *salmonella gallinarum*: clinical, anatomopathological and haematological studies. Brazilian Journal of Poultry Science; 9(2):133-141.
18. Fuller R (2001): The chicken gut microflora and probiotic supplements. Poultry Science; 38: 189–196.
19. Garcia KO, Santana AM, Freitas Neto OC, Simplício KMMG, Júnior AB and Fagliari JJ (2010): Experimental infection of commercial layers using a *Salmonella enterica* sorovar Gallinarum strain: blood serum components and histopathological changes. Brazilian Journal of Veterinary Pathology; 3(2):111-117.
20. Haiqi H, Kenneth JG, Christina LS, David JN and Michael HK (2013): Nitric oxide as a biomarker of intracellular salmonella viability and identification of the bacteriostatic activity of protein kinase a inhibitor H-89. PLoS One ;8(3): e58873.
21. Hassan HMA, Mohamed MA, Youssef AW and Hassan ER (2010): Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. Asian–Aust.J. Anim. Sci;23: 1348–1353.
22. Higgins SE, Higgins JP, Wolfenden AD, Henderson SN, Torres-Rodriguez A, Tellez G and Hargis B (2008): Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella* Enteritidis in neonatal broiler chicks. Poult. Sci. 87:27–31.
23. Ignatova, M, Sredkova V and Marasheva V (2009): Effect of dietary inclusion of probiotic on chickens performance and some blood indices. Biotechnology in Animal Husbandry; 25(5-6):1079-1085.
24. Jin LZ, Ho YW, Abdullah N and Jalaludin S (1998): Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing *Lactobacillus* cultures. Poult. Sci.; 77:1259-1265.
25. Khaksefidi A and Ghoorchi T (2006): Effect of probiotic on performance and immunocompetence in broiler chicks. Journal of Poultry Science; 43(3): 296-300.
26. Lee KW, Lee SH, Lillehoj HS, Li GX, Jang SI, Badu US, Park MS, Kim DK,Lillehoj EP, Neurnann AP, Rehberger TG and Siragusa GR (2010): Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. Poult. Sci.; 89:203–216.
27. Liu JR, Lai SF and Yu B (2007): Evaluation of an intestinal Lactobacillus reuteri strain expressing rumen fungal xylanase as a probiotic for broiler chickens fed on a wheat-based diet. British Poultry Science ; 48: 507–514.
28. Lowry VK, Farnell MB, Ferro PJ, Swaggerty CL, Bahl A and Kogut MH (2005): Purified beta-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica serovar Enteritidis.* International Journal of Food Microbiology; 98: 309– 318.
29. Magda ME, Sabry SE, Mohamed AE, Santoch L, Said MD and Neven AE (2011): The viability of probiotics as a factor influencing the immuneresponse in the Nile tilapia, *Oreochromis niloticus*. Egypt J. Aquat. Biol. & Fish; 15(1): 105 – 124.
30. Maiorka A, Santin E, Sugeta SM, AlmeidaJG and Macari M (2001): Utilization of prebiotics, probiotics, or symbiotics in broiler chicken diets. Brazilian Journal of Poultry Science; 3: 75-82.
31. Mohan B, Kadirvel R, Natarajan A and Bhaskaran M (1996): Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broilers. British Poultry Science; 37: 395–401.
32. Mohnl M, Nitsch S and Schatzmayr G (2006): Effect of a combination of probiotics, prebiotics and immune-modulating substances on the performance of broiler chickens. The XII European Poultry Conference. Verona, Italy.
33. Mountzouris KC, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G and Fegeros K (2007): Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poultry Science; 86: 309-317.
34. Mountzouris KC, Tsitrsikos P, Palamidi I, Arvaniti A, Mohnl M, Schatzmayr G and Fegeros K (2010):Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins,and cecal microflora composition. Poult. Sci.; 89:58–67.
35. O'Dea EE, Fasenko GM, Allison GE, Korver DR, Tannock GW and Guan LL (2006): Investigating the effects of commercial probiotics on broiler chick quality and production efficiency. Poultry Science; 85: 1855–1863.
36. Ogunleye AO, Ajuwape ATP and Adetosoye AI (2009): Hematological changes in *salmonella* paratyphi A infected pullets. Revue Élev. Méd. vét. Pays trop; 62 (1): 23-26.
37. Parry RM, Chandau RC, Shahani RM (1965): A rapid and sensitive assay of muramidase. Proceedings of

the Society for Experimental Biology and Medicine; 119:384-386.
38. Pedroso AA, Menten JF, Lambais MR, Racanicci AM, Longo FA and Sorbara, JO (2006):Intestinal bacterial community and growth performance of chickens fed diets containing antibiotics. Poult.Sci.; 85: 747–752.
39. Ribeiro AML, Vogt LK, Canal CW, Cardoso MR, Labres RV, Streck AF and Bessa MC (2007): Effects of prebiotics and probiotics on the colonization and immune response of broiler chickens challenged with *Salmonella enteritidis*. Brazilian Journal of Poultry Science; 9(3):193-200.
40. Rosen GD (1995): Antibacterials in poultry and pig nutrition. Pages 143–172 in Biotechnology in Animal Feeds and Animal Feeding. R. J. Wallace and A. Chesson, ed. VCH, New York.
41. Samli H, Senkoylu N, Koc F, Kanter M and Agma A.(2007): Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and microbiota. Arch Anim Nut; 61:42–49.
42. Setta A, Barrow PA, Kaiser P and Jones MA (2012): Immune dynamics following infection of avian macrophages and epithelial cells with typhoidal and non-typhoidal *Salmonella enterica serovars*; bacterial invasion and persistence, nitric oxide and oxygen production, differential host gene expression, NF-KB signalling and cell cytotoxicity. Vet Immunol Immunopathol.; 146 (3-4):212-24.
43. Tellez G, Pixley C, Wolfenden RW, Layton SL and Hargis BM (2012)**:** Probiotics/direct fed microbials for Salmonella control in poultry. Food Research International; 45: 628–633.
44. Thirabunyanon M and Thongwittaya N (2012): Protection activity of a novel probiotic strain of *Bacillus subtilis* against *Salmonella enteritidis* infection. Research in Veterinary Science; 93: 74–81.
45. Timmerman HM, Veldman A, Elsen van den E, Rombouts FM and Beynen AC (2006): Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. Poultry Science; 85: 1383–1388.
46. Torres-Rodriguez A, Donoghue AM., Donoghue DJ, Barton JT, Tellez G and Hargis BM (2007): Performance and condemnation rates analysis of commercial turkey flocks treated with a Lactobacillus spp-based probiotic. Poultry Science; 86: 444-446.
47. Welker TL, Chhorn L, Mediha YA, Klesius HP (2007): Growth, immune function, and disease and stress resistance of juvenile Nile tilapia (Oreochromis niloticus) fed graded levels of bovine lactoferrin. Aquaculture; 262(1):156-162.
48. Wiedmer H and Hadorn R (1999): Les effets de différents additifs alternatifs dans les aliments d'engraissement comparés avec l'effet d'un antibiotique et un témoin zero. 3e Journées de la recherche avicole; pp. 109-111, Saint-Malo, France.
49. Willis WL and Reid L (2008): Investigating the effects of dietary probiotic feeding regimens on broiler chicken production and Campylobacter jejuni presence. Poult. Sci.; 87(4): 606–611.
50. Willis WL, Isikhuemhen OS and Ibrahim SA (2007) Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotic. Poultry Science; 86: 1856–1860.
51. Xie H, Rath NC, Huff GR, Huff WF and Balog JM (2000): Effects of *Salmonella typhimurium* lipopolysaccharide on broiler chickens. Poultry Science; 79:33–40.
52. Yamawaki RA, Milbradt EL, Coppola MP, Rodrigues JCZ, Andreatti Filho RL, Padovani CR and Okamoto AS (2013): Effect of immersion and inoculation in ovo of *Lactobacillus* spp. in embryonated chicken eggs in the prevention of *Salmonella* Enteritidis after hatch1. Poultry Science; 92 :1560–1563.
53. Yeo J and Kim K (1997): Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broilers chicks. Poult. Sci.; 76:381–385.
54. Yo¨ ru¨ k MA, Gu¨ lM, Hayirli A and Macit M (2004): The effects of supplementation of humate and probiotic on egg production and quality parameters during the late laying period in hens. Poultry Science; 83: 84–88.
55. Zhang ZF, Cho JH and Kim IH (2013): Effects of Bacillus subtilis UBT-MO2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. Live stock Science; 155: 343–347.
56. Zhang ZF, Zhou TX, Ao X and Kim IH (2012): Effects of β-glucan and Bacillus subtilis on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize-soybean meal based diets. Live stock Science 150(2):419–424.
57. Zhou X, Wang Y, Gu Q and Li W (2010):Effect of dietary probiotic, *Bacillus coagulans*, on growth performance,chemical composition, and meat quality of Guangxi yellow chicken.Poult.Sci.;89:588–593.
58. Zulkifli I, Abdullah N, Azrin NM and Ho YW (2000): Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. Br. Poult. Sci.; 41:593–597.

12/23/2013