Egypt. J. Chem. Environ. Health, 2 (2): 298 -314 (2016)
On line ISSN: 2536-9164.

Effect of Probiotics on *Salmonella Enteritidis* Infection in Broiler Chickens

Dina, M.W. Shibat El-hamd[1] and Hams, M. A. Mohamed[2]

[1]Department of Poultry Diseases, Animal Health Research Institute, Qena Laboratory & [2]Department of Microbiology, Faculty of Veterinary Medicine, South Valley University

Abstract

Probiotics are live cultures of microorganisms administered orally and acted beneficially on host health. The addition of probiotics to the diet of poultry has been found to improve growth performance, feed conversion efficiency, immune responses and help in combating enteric pathogens. Therefore, this study was carried out to determine the role of probiotics for preventing *Salmonella Enteritidis* infection and its effect on the performance as well as the immune response of broiler chickens. The studied *Salmonella Enteritidis* isolate was isolated from chickens from Qena Provence, Upper Egypt. One hundred and thirty, one day old Ross broiler chicks were divided into four equal groups; the first group (G1) was fed on a balanced ration and considered a negative control group.The second group (G2) was fed on a balanced ration and provided with the probiotic (**Micro- Procell, cheil- Bio.com. LTD)** containing Lactobacillus Plantarum 1×10^8cfu, Lactobacillus Acidophilus 1×10^8cfu and Saccharomyces Cerevisiae1×10^7 cfu, in drinking water for 5 successive days. The third group (G3) was challenged with *Salmonella Enteritidis* 10^9Cfu / ml after Probiotics treatment and the fourth group (G4) was challenged with *Salmonella Enteritidis* 10^9Cfu / ml at 6 days old and considered the positive control group. All groups were kept under complete observation for 4 weeks. Throughout the time of the experiment, both clinical signs and post mortum lesions were recorded for all groups, body weight (BW), food conversion ratio (FCR), total bacterial count, differential leucocytic count, phagocytic activity, serum biochemical parameters and humoral immunity (IgG and IgM) using ELISA technique were investigated. Results revealed high performance parameters, as an increase in body weight and FCR. Neither clinical signs nor PM appeared in both non infected group and the probiotics treated groups. The infected, probiotic treated group showed mild decrease in the performance parameters and mild degree of clinical sings and PM lesions for *Salmonella Enteritidis* infections. While the infected non probiotics treated group showed significant decrease in body weight, low of the performance parameters and characteristics sings and pm lesions for *Salmonella Enteritidis* infections along the experiment. Total bacterial count were decreased in infected treated group than infected one, differential leucocytic count showed increase monocyte and lymphocyte in propiotic treated group also the immune status assessment clarified that both phagocytic percentage and index significantly increased ($P\leq0.05$) in the

Egypt. J. Chem. Environ. Health, 2 (2): 298 -314 (2016)
On line ISSN: 2536-9164.

probiotic treated group as compared with their negative control group. Serum biochemical parameter showed elevated total proteins and albumin in probiotic treated group when compared with other groups. Results of ELISA assay revealed significant elevation in humoral immune response in the Probiotics and infected treated groups respectively when compared with control groups. The study concluded that the use of probiotics improve the performance parameters which including weekly feed consumption, weekly body weight gain, main weekly body weights and FCR and improve the immune response of birds against *Salmonella Enteritidis* infection.

Key words: Broilers Chickens, Probiotics, Performance parameters, Serum Biochemistry, Immune response.

Introduction

Salmonella infections are recognized worldwide as an important food borne human diseases. Approximately 13 million cases of paratyphoid infections occur worldwide annually **(Murugkar *et al.*, 2005).** *S. Enteritidis* in poultry causes serious economic losses due to high rate of mortality (4-50%), loss of weight and decreased in egg production in addition to the public health impact due to infection with *S. Enteritidis* **(Haider *et al.*, 2004)**. Several methods have been currently employed to reduce *S. Enteritidis* infections in poultry farms such as using of preventative feed medication or antibiotic growth promoters **(Dekich 1998),** an increase in the use of antibiotics for therapeutic, prophylactic and growth promotion purposes led to presence of antibiotic residues in poultry meat and eggs which have deleterious effects on human consumers. It can cause resistance of human flora and pathogenic microbes to those antibiotics so that there is increasing interest in finding alternatives to antibiotics for poultry production. Probiotics can be listed among these products**. Azza *et. al.*, (2012)**. The use of probiotics is becoming more and more popular and proves to be a useful tool in the fight against *Salmonella* infections. **(Soncini 2011 and Herich *et al.*, 2010).**Additionally, improved performance has been reported with probiotic cultures **(Huang *et al.*, 2004; Higgins *et al.*, 2005 and Timmerman *et al.*, 2006).**

Probiotics are "live microorganisms when administered in adequate amounts conferring a health benefit to the host". The most important advantage of a probiotic is that it neither has any residues in animal products, nor exerts any antibiotic resistance by consumption and it has been reported that probiotics have a good impact on the poultry performance **(Koenen, *et al*. 2004 and Mountzouris *et al.*, 2007).**

Egypt. J. Chem. Environ. Health, 2 (2): 298 -314 (2016)
On line ISSN: 2536-9164.

There are many studies have observed an immunomodulatory effects from probiotic treatments. **Yurong *et al.* (2005)** reported increases in the number of Ig-producing cells (IgM and IgG) detected in Peyer's patches and the cecal tonsils of chicks by day 7 and 10, respectively, following administration of a probiotic culture in the drinking water containing *Bacillus Subtilis*, *Candida Utilis*, and *Lactobacillus Acidophilus*. Probiotics have previously been associated with activation of innate immunity through phagocytic cells. Recently, **Farnellet *et al.*, (2006**) reported that specific isolates of probiotic bacteria increased the oxidative burst capacity and degranulation of heterophils isolated from chicks treated 24 h following probiotic treatment, indicating that the innate immune system may also be activated through probiotic treatment. **Olivares *et al.* (2006)** reported an increase in both the number of circulating phagocytic cells and their activity in humans following consumption of either 2 lactic acid bacteria or a commercial yogurt.

Macrophages are present in most organs and possess effectors functions such as Phagocytosis, antigen processing and presentation, and cytokine secretion **(Qureshi *et al.*, 2000).** Because *Salmonella* spp. has a dynamic relationship with macrophages, we hypothesized that macrophages may play a role in the reduction of *Salmonella* following probiotic treatment. So the following study was aimed to evaluate the effect of probiotic on growth performance and their beneficial effects on immunity of broilers against *Salmonella* infection.

Materials and Methods

2.1. The probiotics:

Commercial preparation (Micro- Procell (cheil- Bio.com. LTD) containing Lactobacillus Plantarum 1×10^8cfu- Lactobacillus Acidophilus 1×10^8cfu – Saccharomyces Cerevisiae1×10^7cfu. It was given in the drinking water at one day of age for 5 consecutive days in a dose of 0.5gm/ 25 liter of the drinking water as recommended by manufacturer.

2.2 Preparation of the *S. Enteritidis* challenge strain:

S. Enteritidis field strain which was previously isolated from Qena province and identified serologicaly in faculty of Veterinary Medicine, Banha University and molecular identification in molecular unit in Assuit University **(Dina 2013)** was centrifuged at 3000 r.p.m for 10 min. Sediment was diluted with sterile buffer saline and adjusted using MacFerland 0.5 tube to contain 10^9 CFU/ml. The challenge inoculum was prepared according to the method of **Timms *et al.*, (1990)**.

2.3 Experimental Chicks

A total of one hundred and thirty, day-old Ross broiler chicks of mixed sex were used for evaluation of the protective value of a probiotic against *S. Enteritidis* challenge. The chicks were taken from a breeder flock free from Salmonellosis.

Chicks were randomly divided into four equal groups, each group contain 30 birds. All birds were subjected to the ordinary vaccination program for broilers against New castle using live Hitchner B1 and La Sota vaccine strains at 6 and 17 days of age, respectively, and Gumboro diseases was applied using live intermediate strain (228 E) at 14 days of age. All the vaccines were given via eye drop instillation. All birds were fed balanced commercial starter and growing rations (21% and 18% protein respectively) and water ad-libitum. The birds were housed in floor-pen and clean well ventilated separate experimental rooms.

2.4 Experimental Design

One hundred and thirty, day-old Ross broiler chicks of mixed sex were used. At first day, ten chicks were taken randomly, sacrificed and then examined bacteriologically to prove their freedom from *S. Enteritidis* infection. Chicks were randomly divided into four equal groups, each group contain 30 chicks as the followings:

Group (1): negative control (non infected-non treated chicks).

Group (2): Probiotic treated chicks.

Group (3): Probiotic treated then infected with *S. Enteritidis* chicks.

Group (4): positive control infected non treated (*S. Enteritidis* infected) chicks.

At 6 days of age, each chick in the experimentally infected groups was inoculated orally with 1 ml / containing 10^9 CFU *S. Enteritidis* **(Okamoto *et al.*, 2007).** The period of the experiment extended for 3 weeks after infection.

2.5 Evaluation of the used Probiotics.

2.5.1 Clinical Signs, Mortalities and Gross Lesions

All chicks were kept under daily observation for mortality, clinical signs and post mortem lesion.

2.5.2 The Performance

At arrival, the chicks were weighed and then the chicks in each group were subjected to weekly determination of the production parameters that include; the body weight (BW), feed intake (FI) and Feed conversion ratio (FCR) which was calculated as the ratio between feed intake and body weight gain at the end of each week. These measures were taken till the end of the study (4 weeks of age).

2.5.3 Bacterial count of *S. Enteritidis:*

Three chicks from the infected treated and infected non treated groups were taken randomly at 14, 21 and 28 days of age, scarified and one gram of cecal contents was aseptically removed and grinding in a sterile mortar then placed into sterile tubes containing 9 ml of buffer peptone water and incubated overnight at 37°C for 24 hours then ten fold serial dilution up to 10^{-6} was prepared.1ml of each dilution was plated on XLD agar, incubated for 24 hours at 37°C and the CFU *of S. Enteritidis* per gram of cecal content was determined **(ISO, 2011).**

2.5.4 Immune status assessment:

2.5.4. A. Collection of Blood Samples:

Blood samples were collected via the wing vein from all groups on the 2nd, 3rd and 4th week of age. Blood was collected into two tubes, the first containing the anticoagulant (Heparin), for phagocytic assay and differential leukocytic count and the second without anticoagulant for serum separation. The obtained serum was used for biochemical and immunological examination.

2.5.4. B. Hematological examination:

Differential leukocyte counts were determined according to the methods described by **Bernard *et al.*, (2000).**

2.5.4. C. Phagocytosis assay: (phagocytic activity and phagocytic index).

For measurement of phagocytic assay, *Candida albicans* culture which was molecularly identified by mycology unite in faculty of science, Assuit University (AUMC 8758) was added to heparinized blood clloected from 5 randomly selected chicks at 14, 21, 28 weeks from all groups at a rate of 50µg/ml and shaken in water bath at 23-25˚C for 3-5 hours, smears of the whole blood were made then stained with Wright-Giemsa stain as described by **Kawahara *et. al.*, (1991).** Phagocytosis was calculated by determining the proportion of macrophages, which contained intracellular yeast cells in a random count of 200 macrophages and expressed as percentage of phagocytic activity (PA) while the numbers of phagcytozed organisms were counted in the phagocytic cells and called phagocytic index (PI).

Phagocytic activity (PA) = Percentage of phagocytic cells containing yeast cells.

$$\textbf{Phagocytic index (PI)} = \frac{\text{Number of yeast cells phagocytized}}{\text{Number of phagocytic cells}}$$

2.5.4. D. Detection of the Humoral Immune Response

At the age of 14 and 28 days, 5 broilers per treatment were randomly selected and blood samples were collected from the wing vein of the birds in tubes. Blood samples were allowed to clot overnight at 4°C then centrifuged at 3000xg for 10 min. The separated sera were stored at -20°C till used in the serological tests. IgG and IgM were determined using Indirect Enzyme Linked Immune-Sorbent Assay (ELISA) test using ELISA kits (Biomerieux, France) as described by **Gaca *et al.*, (1999).**

2.5.5. Biochemical parameters:

Serum total protein and albumin were determined using commercial diagnostic kits (Stanbio, USA) according to the method of **Doumas *et al.*, (1971).**

Egypt. J. Chem. Environ. Health, 2 (2): 298 -314 (2016)
On line ISSN: 2536-9164.

2.6 Statistical Analysis

The statistical analysis was examined using One-Way analysis of variance (ANOVA) according to **Shott (1990).**

Results and Discussion

Commercial poultry is one of the fastest growing sectors of the animal agricultural industry, especially broiler production **Herren (2000)**, an increase in consumption of meat and poultry increases the potential risk for exposure to *Salmonella* through contamination. There is an increasing interest in evaluating non-medical alternatives for antimicrobials in terms of their ability to improve disease resistance, and enhance overall animal health and production in poultry. In the present study, attempts were made to evaluate the use of probiotic and investigate the influence of such feed supplements on *Salmonella enteritidis* infection due to their antibacterial properties.

Clinical Signs and Mortality Rate:

Non infected and non treated chicks (G1) as well as Probiotic treated groups (G2) appeared normal, displaying no abnormal clinical signs during the time of the experiment. Groups of infected non treated chicks (G4) showed decreased appetite, depression, ruffled feather, tendency to huddle together and white diarrhea, while the most post mortem lesions were distended gall bladder congested and enlarged, swollen liver with focal necrosis and distention of ureters with ureates. Clinical signs of the infected chicks treated with Probiotic group (G3) were less severe than those of infected non treated chicks (G4). Mortality rate was 10% in G3 during the experimental period (Table1). The protective efficacy of the probiotics which contained *Lacobacillus* spp. against *S. Enteritidis* infection was evaluated by **Samanta and Biswas (1995); Soomro *et al.*, (2002); Timmerman *et al.*, (2006) and Wafaa *et al.*, (2006)** who detected significant decrease in mortality in *S. Enteritidis* infected chickens and treated with probiotic than infected ones. **Higgins *et al.*, (2007 a, b)** and **Vicente *et al.*, (2007 a, b)** concluded that effective probiotics may accelerate the development of normal microflora in chicks and increased the resistance to infection by some enteric bacterial pathogens.

Growth performance: Concerning the results of the performance parameters (final weight (BW), average weight gain, feed intake (FI) and feed conversion ratio (CFC)), table (2 and 3). The birds in G2 (probiotic treated birds) had the best performance in average weight gain because probiotics are made up of lactobacillus predominantly, which are favourably disposed to good gut health, thereby facilitating the growth of beneficial group of gut microbes and depressing the potentially pathogenic and harmful group **Jeurissen *et al.*, 2002**, this will in turn favour digestion of food and assimilation of the end products of that, which will be used for

muscle or flesh formation needed for weight gain , which is seen as lowest value for the feed conversion ratio (FCR). In case of *S. Enteritidis* infection, **Tellez *et al.*, (2001); Wafaa *et al.*, (2006); Wilkie (2006) and Rahimi *et al.*, (2007)**demonstrated that probiotics containing *lactobacilli* could overcome the growth depressing effect caused by this infection. The improvement in the performance parameters caused by probiotic administration may be due to stimulating the host's appetite **(Nahashon *et al.*, 1992)**,improving feed conversion ratio **(Cavit 2003 and Haj *et al.*, 2004),** producing digestive enzymes **(Saarela *et al.*, 2000)** andthe beneficial effect on the health of the host **(Soomro *et al.*, 2002).**

Bacterial count of *S. Enteritidis:*

There were reduction in the total bacterial count of *S. Enteritidis* isolated from G3 (infected treated with probiotic group) as recorded in table (4). The reducing effect of probiotics on the total count of *Salmonella* spp. was studied comprehensively by several researchers and there are many hypotheses that explain the mechanism of action of Probiotics containing lactic acid bacteria against *Salmonellae* colonization in birds; one of them is that production of lactic acid which is unfavorable pH for growth of *Salmonellae* **(Alkoms *et al.*, 2000; Rolfe 2000 and Johanssen *et al.*, *2004*)**, the competition between *Lactobacilli* and the enteric bacteria which is called competitive exclusion **(Heres *et al.*, 2003)**, also the production of bacteriocin which is antibacterial substances that kill *Enterobacteriacae* **(Pascual *et al.*, 1999).**

Differential leukocyte counts

There was a significant increase in total leukocyte and lymphocytes count (leukocytosis and lymphocytosis) in G2 (Probiotic group) without change in heterophils count compared to the control G1 (non infected non treated group) as shown in table (5). This may be as a result of the stimulation of the immune system by the immunogenic property of the probiotic used in this treatment **(Stanley *et al.*, 2011)**. While there was a significant increase in total leukocyte count and heterophils count (heterophilia) and decrease in lymphocytes count (lymphopenia) in G4 (Infected non treated group) compared to the G1, control group, On the other hand, there was significant increase in lymphocyte count heterophils count in G3, infected treated group, compared to G1, non infected non treated group,. With regard to Neutrophils which are the first type of defense cells that will appear during acute infection causing due to the *Salmonella enteritidis* challenge and they are part of the immune system for protection of the birds **Nathan (2006),** explained why the value is high in G4 followed by G3 because the birds in this treatment groups have high level of immunity to microbial invasion **Stanley *et al.*, 2011**, while G1 and G2 having the least value.

Egypt. J. Chem. Environ. Health, 2 (2): 298 -314 (2016)
On line ISSN: 2536-9164.

Phagocytosis assay: (phagocytic activity and phagocytic index).

There was significant increase in phagocytic activity and Phagocytic index of G2 (probiotic treated birds) compared to the control group (G1) and these results agree with **Shareef and Al-Dabbagh (2009)** who recorded a significant increase in the phagocytic activity of leukocytes and the phagocytic index in experimental birds after the application of *Lactobacillus* probiotic. While in G3 (infected treated birds) there was significant increase in Phagocytic activity and Phagocytic index compared to G4 (infected non treated birds), and there was significant decrease in Phagocytic activity and Phagocytic index of G4 (infected non treated birds) compared to the control group (G1) as seen in table (6). These results were in agreement with **Borchers *et al.* (2009**) who reported that probiotics stimulate natural resistance of the organism through increasing the number of antibodies and increasing the effectiveness of macrophages and the boost produced by the colonization of probiotics are essential for the development of functional immune system including the presence of T and B lymphocytes in the lamina propria and the expansion and maturation of IgA and also induction of tolerance by the present antigens.

Detection of the Humoral Immune Response

For evaluation of humoral immune response of chickens to probiotic, indirect ELISA test was done. The results reveal that during the experimental period, the control non infected group (G1) shows no significant ($P \leq 0.05$) differences in the mean IgG and IgM values. In the probiotic treated birds, gradual and significant ($P \leq 0.05$) increase in IgG and IgM values. These values are increased significantly ($P \leq 0.05$) at 28 days of age as shown in table (7). **Barrow and Lovell (1991) and Olabisi and Peter (2008)** reported the production of high level of serum IgG after oral inoculation of *S. Enteritidis* in layer chickens and it is believed that Probiotics can enhance the immune response in broilers. **(Chaturverdi *et al.*, 1997)** so that probiotics resulted in an enhancement of broiler humoral immune response **(Huang *et al.*, 2004; Kabir *et al.*, 2004 and Koenen *et al.*, 2004)** and could therefore be regarded as an improved capacity of the humoral immune system of birds.

Biochemical parameters

There was significant increase in Serum total protein without change in albumin in G2 and there was decrease in both total protein and albumin in (G4) compared to G1. Significance increase in total protein in G3 compared to G4 was observed as shown in table (8). The high level of globulin which is a precursor for immunoglobulin (antibodies) is responsible for the protective functions of probiotic **Berndt *et al.*, 2007.** The low levels of total protein and albumin in G4 causing hypoproteinemia generally leading to a fall in level of immunoglobulin (antibodies) and declining of immune response of birds (**Obidi *et al.*, 2008 and Fasanmi, 2011).**

From these results it can concluded that probiotic supplementation improve performance, increase the immunity of the birds to *Salmonella* challenge as probiotics can be considered as an immune potentiates due to stimulation of immune system, does not have adverse effects on kidney functions and it has the ability to reduce the adverse effect of *Salmonella Enteritidis* infection in broiler chicks. It is recommended to use probiotics in poultry as they do not require withdrawal period, they can make a valuable contribution to flock health and safety of poultry products as food. This may also provide a significant tool for the poultry industry in controlling the major enteric infections and in reduction of food borne pathogens such as Salmonellosis.

Table (1): Mortality rate in different experimental groups:

Groups	No. of chicks	No. of dead bird / week				Total mortality	Percent
		1st week	2nd week	3rd week	4th week		
G1	30	0	0	0	0	0	0%
G2	30	0	0	0	0	0	0%
G3	30	0	2	1	1	4	13%
G4	30	0	6	3	1	10	33%

Group (1): negative control (non infected-non treated chicks). Group (2): Probiotic treated chicks.
Group (3): Probiotic treated then infected with *S. Enteritidis* chicks.
Group (4): positive control infected non treated (*S. Enteritidis* infected) chicks.

Table (2): The effect of probiotic on body weights (g) in different experimental groups

Groups	Initial B. W.	Age/ week			
		1st week	2nd week	3rd week	4th week
G1	46 ±1.2	180±2.3	450±3.2	860±4.3	1470±7.3
G2	46 ±1.2	185.6±1.5	475±2.2	900±7.2	1490± 4.3
G3	46 ±1.2	179±1.5	425±3.2	820±4.3	1435± 4.6
G4	46 ±1.2	175±1.3	400±4.1	630±4.2	840±7.3

Group (1): negative control (non infected-non treated chicks). Group (2): Probiotic treated chicks.
Group (3): Probiotic treated then infected with *S. Enteritidis* chicks.
Group (4): positive control infected non treated (*S. Enteritidis* infected) chicks.

Egypt. J. Chem. Environ. Health, 2 (2): 298 -314 (2016)
On line ISSN: 2536-9164.

Table (3): The performance parameters of different experimental groups

Groups	Age/ week	FI (g)	Body weight gain	FCR
G1	1st	166. 56	134	1.2
	2nd	373	270	1.4
	3rd	689	410	1.67
	4th	938	610	1.53
G2	1st	178	193.6	0.91
	2nd	401	289.4	1.38
	3rd	756	425	1.78
	4th	788	590	1.34
G3	1st	165	133	1.24
	2nd	340	246	1.38
	3rd	675	395	1.71
	4th	898	615	1.46
G4	1st	165	129	1.28
	2nd	320	225	1.42
	3rd	465	230	2.02
	4th	597	210	2.82

Group (1): negative control (non infected-non treated chicks).
Group (2): Probiotic treated chicks.
Group (3): Probiotic treated then infected with *S. Enteritidis* chicks.
Group (4): positive control infected non treated (*S. Enteritidis* infected) chicks.

Table (4): Bacterial count of *S. Enteritidis* isolated from 1g of intestinal content of G3 and G4

Groups	Bacterial count in 1g of intestinal content post infection		
	2nd week	3rd week	4th week
G3	7.4×10^4	6.5×10^4	4.4×10^4
G4	8.5×10^6	8.3×10^9	8.0×10^{12}

Group (3): Probiotic treated then infected with *S. Enteritidis* chicks.
Group (4): positive control infected non treated (*S. Enteritidis* infected) chicks.

Egypt. J. Chem. Environ. Health, 2 (2): 298 -314 (2016)
On line ISSN: 2536-9164.

Table (5) Differential leukocyte count of different experimental groups::

Group (1): negative control (non infected-non treated chicks).

Groups	Age/ week	WBCS (10^3/µl)	Lymphocytes (10^3/µl)	Neutrophils (10^3/µl)	Heterophil (10^3/µl)	Monocytes (10^3/µl)
G1	2nd	22.2 ± 0.56	11.7 ± 0.31	7.63 ± 0.20	4.45 ± 1.34	1.90 ± 0.16
	3rd	22.2 ± 0.56	11.8 ± 0.3	7.34 ± 0.76	4.06 ± 1.38	1.80 ± 0.10
	4th	22.2 ± 0.56	11.6 ± 0.3	7.58 ± 0.52	4.53 ± 1.52	2.02 ± 0.10
G2	2nd	24.6 ± 0.55	13.44 ± 0.34	8.04 ± 1.05	4.71 ± 1.40	1.97 ± 0.17
	3rd	25 ± 0.55	14.37 ± 0.29	6.96 ± 0.70	4.21 ± 1.97	2.30 ± 013
	4th	24.4 ± 0.51	13.82 ± 0.26	8.61 ± 0.90	4.75 ± 1.89	2.01 ± 0.18
G3	2nd	24.6 ± 0.75	9.87 ± 0.36	11.81 ± 0.97	6.94 ± 1.18	1.72 ± 0.06
	3rd	25.5 ± 0.98	11.48 ± 0.21	10.75 ± 0.91	5.87 ± 1.92	1.84 ± 0.07
	4th	25.4 ± 0.8	10.30 ± 0.09	11.83 ± 1.08	4.84 ± 1.66	1.98 ± 0.13
G4	2nd	24.4 ± 0.5	8.78 ± 0.20	13.45 ± 0.36	6.77 ± 1.04	1.37 ± 0.10
	3rd	24.6 ± 0.83	10.17 ± 0.16	11.40 ± 0.88	4.53 ± 1.52	1.84 ± 0.05
	4th	25.6 ± 0.81	9.52 ± 0.19	13.28 ± 0.7	4.56 ± 1.98	1.88 ± 0.08

Group (2): Probiotic treated chicks.
Group (3): Probiotic treated then infected with *S. Enteritidis* chicks.
Group (4): positive control infected non treated (*S. Enteritidis* infected) chicks.

Table (6): Phagocytic percent (P %) and phagocytic index (PI) in different experimental groups

Groups	Age / week					
	2nd		3rd		4th	
	P %	PI	P %	PI	P %	PI
G1	75± 2.3	1.2±0.3	75±2.1	1.1±0.2	75±2.2	1.0±0.01
G2	80±3.8*	1.4±0.1	82±3.9 *	1.6±0.1	84±2.6*	1.5 ±0.2
G3	70±1.3≠	1.2±0.2	75±1.2≠	1.3±0.2	77±3.4≠	1.2± 0.2
G4	56±1.7*	1.0±0.02	51±1.7*	0.8±0.04 *	50±1.3*	0.8 ± 0.03 *

Group (1): negative control (non infected-non treated chicks).
Group (2): Probiotic treated chicks.
Group (3): Probiotic treated then infected with *S. Enteritidis* chicks.
Group (4): positive control infected non treated (*S. Enteritidis* infected) chicks.
≠ Significant when compared with +ve control
*Significant when compared with –ve control

Egypt. J. Chem. Environ. Health, 2 (2): 298 -314 (2016)
On line ISSN: 2536-9164.

Table (7) Immunoglobulins (IgG and IgM) values in different experimental groups

Groups	Age/ day	IgG mg/ ml	IgM mg/ml
G1	14	2.75	2.00
	28	2.75	2.00
G2	14	3.78	2.28
	28	4.83	3.20
G3	14	3.77	3.25
	28	4.81	3.85
G4	14	2.00	1.95
	28	1.80	1.75

Group (1): negative control (non infected-non treated chicks).
Group (2): Probiotic treated chicks.
Group (3): Probiotic treated then infected with *S. Enteritidis* chicks.
Group (4): positive control infected non treated (*S. Enteritidis* infected) chicks.

Table (8): Serum T. Protein (g/dl) and Albumin (g/dl) in different experimental groups:

Groups	Age/ week	T. Protein (g/dl)	Albumin (g/dl)
G1	2nd	3.71 ± 0.12	1.62 ± 0.09
	3rd	3.72 ± 0.11	1.54 ± 0.11
	4th	3.76 ± 0.12	1.58 ± 0.08
G2	2nd	3.95 ± 0.07	1.65 ± 0.08
	3rd	3.96 ± 0.20	1.56 ± 0.12
	4th	4.05± 0.12	1.53 ± 0.09
G3	2nd	3.51± 0.04	1.49 ± 0.03
	3rd	3.56 ± 0.04	1.38 ± 0.02
	4th	3.6 ± 0.09	1.32 ± 0.08
G4	2nd	3.16 ± 0.05	1.29 ± 0.03
	3rd	3.19 ± 0.06	1.22 ± 0.03
	4th	3.22 ± 0.09	1.13 ± 0.37

Group (1): negative control (non infected-non treated chicks).
Group (2): Probiotic treated chicks.
Group (3): Probiotic treated then infected with *S. Enteritidis* chicks.
Group (4): positive control infected non treated (*S. Enteritidis* infected) chicks.

References

Alkoms, H. L.; Skytta, E.; Saaela, M.; Maltila, T.; Latra, K. and Helender, I. M. (2000): Lactic acid permeabilizes Gram-negative bacteria by disturbing the outer membrane. Appl. Environ. Microbiol., **66**, 2001–2005.

Azza, H. Abd-El-Rahman; Walaa, M. Ahmed; Olfat, S.H.; and Amira, H. Mohamed (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,

Barrow, P. A. and Lovell, M. A. (1991): Experimental infection of egg laying hens with *Salmonella Enteritidis* phage type 4. Avian Pathol., **20**, 335–348.

Bernard, F.F.; Joseph, G.Z. and Nemi, C.J. (2000): Schalm's Veterinary Hematology. 5th Ed., USA.

Berndt, A.; Wilhelm; A., Jugert, C.; Pieper, J. and Sachse, K. (2007): Chicken Cecum Immune Response to *Salmonella enteric* Serovars of Different Levels of Invasiveness. Infect Immun. **75** (12): 5993-6007.

Borchers, A. T.; Selmi, C.; Meyers, F. J.; Keen, C. L. and Gershwin, M. E. 1H.H.(2009): Probiotics and immunity. J Gastroenterol.**44**: 26-46

Cavit, A. (2003): Effect of dietary probiotic supplementation on growth performance in the chickens. Turkish J. Vet. Anim. Sci., **28**, 887–891.

Chaturverdi, M. M.; Kumar, A.; Darnay, B.G.; Chainy, G. B.; Agarwal, N. S. and Aggarwal, B. B. (1997): Sanguinarine (Pseudochelerythrine) Is a Potent Inhibitor of NP-kappa B Activation, I- kappa B Alpha Phosphorylation, and Degradation. J. Biol.Chem., **48**: 30129-30134.

Dekich, M. A. (1998): Broiler industry strategies for control of respiratory and enteric diseases. Poult. Sci., **77**, 1176–1180.

Dina, M. W. Shibat El-hamd; Ahmed, I. A. and Ibrahim A. A. (2013): Epidemiological and molecular studies on *Salmonella* infections in chickens in Qena Province. PhD thesis in Veterinary medical Science, poultry diseases, Faculty of Vet.Med.South Valley University, Qena

Doumas, B. T.; Watson, W. A, and Bigg, H.G. (1971): Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta **31**: 87- 96.

Farnell, M. B.; Donoghue, A. M. F.; Solis de los Santos, P. J. Blore, B. M.; Hargis, G. T. and Donoghue, D. J. (2006): Upregulation of oxidative burst and degranulation in chicken heterophils stimulated with probiotic bacteria. Poult. Sci**.85:**1900–1906.

Fasanmi, O. G. (2011): Health Management Practices in Tropical Poultry Production. (1st edn), TAF Printronic, Ibadan, Nigeria, p. 58-62.

Gaca, M..D.A.; Pickering, J.A.; Arthur, M.J.P. and Benyon R.C. **(1999):** Human and rat hepatic stellate cells produce stem cell factor: A possible mechanism for mast cell recruitment in liver fibrosis. J. Hepatol. **30**:850–858.

Haider, M. G.; Hossain, M. G.; Hossain, M. S.; Chowdhury, E. H.; Das, P.M. and Hossain, M. M. (2004): Isolation and characterization of Enterobacteria Associated with Health and Disease in Sonali Chickens. Banganladesh Journal of Veterinary Medicine **2** (1):15-21.

Haj, A. M.; Laamari, Z. and Rekik, B. (2004): Effects of incorporating an antibiotic "Avilamycin" and a probiotic"Activis" in broiler diets. American Society of Animal Science **55**:237-240.

Heres, L.; Wagenaar, J. A.; van Knapen, F. and Urlings, B. A. P. (2003): Passage of *Salmonella* through the crop and gizzard of broiler chickens fed with fermented liquid feed. Avian Pathol.,**32**, 173–181.

Herich, R.; Kokinčakova, T.; Laukova, A. and Levkutova, M. (2010): Effect of preventive application of *Enterococcus faecium*EF55 on intestinal mucosa during Salmonellosis in chickens. Czech Journal of Animal Science, **55**, 42–47.

Herren, R. (2000): The science of animal agriculture. (2nd ed.), Delmar, Albany, NY, USA

Higgins, J. P.; Higgins, S. E.; Salvador, V.; Wolfenden, A. D.; Tellez, G. and Hargis, B. M (2007a): Temporal effects of lactic acid bacteria probiotic culture on *Salmonella* in neonatal broilers. *Poult. Sci.*, ***86***, 1662–1666.

Higgins, S. E.; Erf, G. F.; Higgins, J. P.; Henderson, S. N.; Wolfenden, D.; Gaona-Ramirez, G. and Hargis, B. M. (2007b): Effect of probiotic treatment in broiler chicks on intestinal macrophage numbers and phagocytosis of *Salmonella Enteritidis* by abdominal exudate cells. Poult. Sci.,**86** (11), 2315–2321.

Higgins, S. E.; Torres, A.; Rodriguez, J. L.; Vicente, C. D.; Sartor, C. M.; Pixley, G. M.; Nava, G.; Tellez, J.; Barton, T. and Hargis, B. M. (2005): Evaluation of intervention strategies for idiopathic diarrhea in commercial turkey brooding houses. J. Appl. Poult. Res. **14**:345–348.

Huang, M. K.; Choi, Y. J.; Houde, R.; Lee, J. W.; Lee, B.; and Zhao, X. (2004): Effects of Lactobacilli and an acidophilic fungus on the production performance and immune responses in broiler chickens. Poult. Sci. **83**:788–795.

ISO (2011): Standards catalogue 07.100.30. Food Microbiology.

Jeurissen, S. H.; Lewis, F; vanderKlis, J. D.; Mroz, Z. and Rebel, J.M. (2002): Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality. Curr Issues Intest. Microbio l**3** (1): 1-14.

Johanssen, S. A.; Griffith, R. W.; Wesley, I. V. and Scanes, C. G. (2004):*Salmonella enterica*serovartyphimurium colonization of the crop in the

domestic turkeys: Influence of probiotic and prebiotic treatment (*Lactobacillus acidophilus* and lactose). Avian Dis., **48,** 279–286.

Kabir, S. M. L.; Rahman, M. M.; Rahman, M. B.; and Ahmed. S. U. (2004): The dynamics of probiotics on growth performance and immuneresponse in broilers. Int. J. Poult. Sci. 3:361–364.

Kawahara, E.; Ueda, T. and Nomura, S. (1991): In vitro phagocytic activity of white spotted shark cells after injection with *Aeromonas salmonicida* extracelluar products. GyobyoKenkyu, Japan, **26** (4): 213-214.

Koenen, M.E.; Karmer, J.; Vander Hulst, R.; Heres, L.; Jeurissen, S. H.; and Boersma, W. J. (2004): Immunomodulation by probiotic Lactobacilli in layer and meat-type chickens. British Poultry Science **45**: 355-366.

Mountzouris, K. C.; Tsirtsikos, P.; Kalamara, E.; Nitsch, S.; Schatzmayr, G.; and Fegeros, K. (2007): fermentation by lactic acid bacteria could contribute to the Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus and Pediococcus strains in promoting broiler performance and modulating cecalmicroflora composition and metabolic activities. Poultry Science, **86**: 309-317.

Murugkar, H.V., Rahman, H. and Dutta, P. K. (2005). Distribution of virulence genes in *Salmonella* serovars isolated from man and animals. Indian Med. Res., **177**, 66–70.

Nahashon, S. N.; Nakaue, H. S. and Mirosh, L.W. (1992): Effect of direct-fed microbials on nutrient retention and production parameters of laying pullets. Poult. Sci., **71**(Suppl.1), 111.

Nathan, C. (2006): Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol **6** (3): 173-182.

Obidi, J. A.; Onyeanusi, B.I.; Rekwot, P. I.; Ayo, J. O. and Dzenda, T. (2008): Seasonal variation in seminal characteristics of Shikabrown breeder cocks. Int J Poult. Sci. **7** (12): 1219-1223.

Okamoto, A. S.; AndreattiFilho, R. L.; Lima, E. T.; Pereira, R. E. P.; Menconi, A.; Rocha, T. S. and Marietto-Gonçalves, G. A. (2007): Immunological evaluation of the intestinal mucosa of broiler chicks treated with *lactobacillus* spp. and challenged with *Salmonella Enteritidis*. Brazilian J. Poult. Sci., **9** (4), 259–262.

Olabisi, O. I. and Peter, S. (2008): *Salmonella* Enteritidis experimental infection in chickens: Effects of challenge dose on serum immunoglobulin G antibody response. African J. Biotechnol., **7** (20), 3783–3787.

Olivares, M.; Diaz-Ropero, M. P.; Gomez, M.; Lara-Villoslada, F.; Sierra, S.; Maldonado, J. A.; Martin, R.; Rodriguez, J. M. and Xaus, J. (2006): The consumption of two new probiotic strains *Lactobacillus gasseri* CECT 5714 and *Lactobacillus coryniformis* CECT 5711, boosts the immune system of healthy humans. Int. Microbiol. **9**:47–52.

Pascual, M., Hugas, M., Badiola, J. I., Monfort, J. M. &Garriga, M. (1999): *Lactobacillus salivarius*CC2197 prevents *Salmonella* Enteritidis colonization in chickens. Appl. Environ. Microbiol., 65, 4981–4986.

Qureshi, M. A.; Heggen, C. L.; and Hussain, I. (2000): Avian macrophage: Effector functions in health and disease. Dev. Comp. Immunol. **24**:103–119

Rahimi, S.; Shiraz, Z.; ZahraeiSalehi, T.; KarimiTorshizi, M. A. and Grimes, J. L. (2007): Prevention of *Salmonella* infection in poultry by specific egg-derived antibody. Inter. J. Poult. Sci., **6**(4), 230–235.

Rolfe, R. D. (2000): The role of probiotic cultures in the control of gastrointestinal health. J. Nutr., **130,** 396–402.

Saarela, M.; Mogensen, G.; Fondens, R.; Matto, J. and Mattila-Sandholm, T. (2000): Probiotic bacteria: safety, functional and technological properties. *J. Biotechnol.,* **84,** 197–215.

Samanta, M. and Biswas, P. (1995): Effect of feeding probiotic and lactic acid on the performance of broiler. Indian J. Poult. Sci., 30, 145–147.

Shareef, A.M. and Al-Dabbagh, A.S.A. (2009): Effect of probiotic (*Saccharomyce scerevisiae*) on performance of broiler chicks. Iraqi J. Vet. Sci. **23**: 23-29.

Shott, S. (1990): Statistical for health professionals. W.B. Saunders Co. Philadelphia, 313–336.

Soncini, R. A. (2011): Alternativaspararedu cirriesgos de infecciónpor Salmonelas en planteles de aves. Congresso Latinoamericano de Avicultura, Buenos Aires.

Soomro, A. H., Masud, T. and Rathore, H. A. (2002): Application of probiotic culture. *J. Amer. Vet. Advances, **1**,* 40–42.

Stanley, L; Schrier, M. D.; Stephen, A. and Landaw, M. D. (2011): Mean corpuscular volume.

Tellez, G.; Petrone, V. M.; Escorcia, M.; Morishita, T. Y.; Cobb, C. W. and Villasenor, L. (2001): Evaluation of avian-specific probiotic and *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Heidelberg-specific antibodies on cecal colonization and organ invasion of *Salmonella* Enteritidis in broilers. *J.Food Prot., **64***, 287–291.

Timmerman, H. M.; Veldman, A.; van den Elsen, E.; Rombouts, F. M. and Beynen, A. C. (2006): Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. Poult. Sci. **85**:1383–1388.

Timms, L. M.; Marshall, R. N. and Breslin, M. F. (1990): Laboratory assessment of protection given by an experimental *S. Enteritidis* PT4 inactivated adjuvant vaccine. *Vet. Rec*., **127**(25-26), 611–614.

Vicente, J. L.; Aviña, L.; Torres-Rodriguez, A.; Hargis, B. and Tellez, G. (2007a): Effect of a *Lactobacillus* spp. based probiotic culture product on broiler chicks performance under commercial conditions. *Inter. J. Poult. Sci.*, **6**, 154–156.

Vicente, J. L.; Wolfenden, A.; Torres-Rodriguez, A.; Higgins, S.; Tellez, G. and Hargis, B. M. (2007b): Effect of probiotic culture candidates on *Salmonella* prevalence in commercial turkey houses. *J. Appl. Poult. Res.*, ***16***, 55–58.

Wafaa, A. A.; Madian, K.; Ebtehal, A. and Gehan, M. K. (2006): The effect of combined competitive exclusion culture with mannan-oligosacharides and ciprofloxacin on *Salmonella Enteritidis* colonization in broiler chickens. *12th Cong. Fac. Vet. Med., Assiut Univ., Egypt.*

Wilkie, D. C. (2006): Non-antibiotic approaches to control pathogens in the gastrointestinal tract of the broiler chicken. Ph. D. Thesis, College of Graduate Studies and Research in Partial Fulfilment of the Requirements.

Yurong, Y.; Ruiping, S. ; Shimin, Z. and Yibao, J. (2005): Effect of probiotics on intestinal mucosal immunity and ultrastructure of cecal tonsils of chickens. Arch. Anim. Nutr. **59**:237–246.