

A Trial to Prevent *Salmonella* Enteritidis Infection in Broiler Chickens Using Autogenous Bacterin Compared with Probiotic Preparation

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Abstract

This study was carried out to investigate the efficacy of the locally prepared autogenous *Salmonella* Enteritidis (*S. Enteritidis*) bacterin as well as a probiotic preparation in the prevention of broiler chickens from *S. Enteritidis* infection. A total of three hundred and ten, one day-old Hubbard broiler chicks were used. At day old, ten chicks were sacrificed and examined bacteriologically to prove their freedom from *S. Enteritidis* infection. Three hundred birds were divided into four equal groups. Chickens in group (1) were kept as blank control negative non infected-non treated birds, while those of group (2) were challenged non treated birds. Group (3) was vaccinated intramuscularly by the autogenous bacterin at the first day of age in a dose of 0.2 ml/bird and boosted as a second dose at 10 days of age in a dose 0.5 ml/bird, however, group (4) was given a commercial probiotic preparation as 1gm/ 4 liter of the drinking water from the first day of age and continued for 5 successive days. All birds in groups 2, 3, and 4 were challenged orally by 0.5 ml containing 10^9 CFU/ml *S. Enteritidis* at 20 days of age. All the groups were kept under complete observation for three weeks for recording signs, mortalities, gross lesions, shedding rate of *S. Enteritidis*, re-isolation of the organism, the performance as well as detection of the titer of antibodies serologically using microagglutination test and enzyme linked immunosorbant assay (ELISA) test. The results showed that the both the bacterin and the probiotic are equally effective in reducing signs, mortalities, gross lesions, the shedding rate and the re-isolation of *S. Enteritidis* and also increasing in the performance of chickens. The effect of the bacterin and the probiotic was significant ($P \leq 0.05$) when compared with the infected non treated chickens. Moreover, the serological investigation revealed an improvement in the titer of antibodies after vaccination and probiotic treatment. In conclusion, double doses of locally prepared autogenous *S. Enteritidis* bacterin and the probiotic preparation were effective and safe methods for prevention of *S. Enteritidis* infection in broiler chickens.

Keywords: *Salmonella* Enteritidis, Prevention, Bacterin, Probiotic

1. Introduction

Salmonella typhoid and paratyphoid caused by several species of *Salmonellae* infections are recognized worldwide as important food borne human diseases. Approximately 13 million cases of paratyphoid infections occur worldwide annually (Murugkar et al., 2005). Unfortunately, poultry meat is the major source of food borne *Salmonella* paratyphoid infection (Mayrhofer et al., 2003). Riemann et al., (1998) demonstrated an increase in the number of *Salmonella* Enteritidis (*S. Enteritidis*) isolates from humans in United States from 1.12 to 7.16 isolates per 100,000 person per year as this serotype accounted for 28% of all *Salmonellae* isolates for human. As well, Herikstad et al., (2002) considered *S. Enteritidis* the most common species of *Salmonellae* that isolated worldwide.

In addition to the public health impact, infection with *S. Enteritidis* in poultry causes serious economic losses due to high rate of mortality (4-50%), loss of weight and decreased in egg production (Hoop 1997).

Besides good hygiene and bird's husbandry practice, 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), using of competitive exclusion products like probiotics and development of vaccines (Lillehoj et al., 2000).

Control of *S. Enteritidis* infection using antimicrobials emerges the problem of antimicrobial resistance (Zhang-Barber et al., 1999). Therefore, alternative safe strategies are emerged to overcome this important problem.

Priyantha (2009) reported that vaccination is only alternative to control salmonellosis in chickens and other precaution like bio-security and good management practices must be taken to consideration first. There are numbers of live and killed *S. Enteritidis* vaccines used commercially worldwide with some success. The efficacy of live attenuated *S. Enteritidis* vaccines was studied (Arnon et al., 1983; Barrow et al., 1990; Barrow 2000 and 2007; Atterbury et al., 2010; Penha et al., 2010; Methner et al., 2011) and the vaccine proved its efficacy but it could be hazardous because of residual virulence caused by insufficient attenuation, reversion to virulence and uncertain safety (Barbezange et al., 2000), so its use is prohibited in several countries. However, inactivated *S. Enteritidis* vaccine had been developed and can confer partial protection against intestinal colonization, fecal shedding, systemic spread and egg contamination (Timms et al., 1990; Gast et al., 1993; Nakamura et al., 1994; Timms et al., 1994; Davison et al., 1999; Miyamoto et al., 1999; Liu et al., 2001; Khan et al., 2003; Okamura et al., 2003; Nakamura et al., 2004; Haider et al., 2007; Cima 2010).

Reducing intestinal colonization with *S. Enteritidis* using competitive exclusion compounds (probiotics) during the grow-out period is crucial to improve the microbiological quality at processing as well as for consumer health (Vugia 1993). Probiotics consist of live or dead organisms and spores (Patterson and Burkholder 2003), non-traditional chemicals (Moore et al., 2006), bacteriophages (Higgins et al., 2005), organic acids (Jarquin et al., 2007; Wolfenden et al., 2007) and others have emerged in the last decades as some of the tools that could be potentially useful in the near future for pathogen control and poultry performance. Probiotics are used now safely to prevent enteric infections especially with *S. Enteritidis* in newly hatched chicks with successful results (Soerjadi et al., 1981; Fuller 1997; Tellez et al., 2001; Ayed et al., 2004; Wafaa et al., 2006; Higgins et al., 2007a, b; Revollo et al., 2009).

So, the present work was designed to evaluate the role of the locally prepared killed *S. Enteritidis* bacterin compared with a commercial probiotic preparation in the prevention of *S. Enteritidis* infection in broiler chickens.

2. Material and Methods

2.1 Experimental Chickens

A total of three hundred and ten, day-old Hubbard broiler chicks of mixed sex that obtained from Cairo Poultry Company in 10th Ramadan city were used for evaluation of the protective value of the prepared *S. Enteritidis* bacterin in comparison with a probiotic against *S. Enteritidis* challenge. The chicks were taken from a breeder flock free from salmonellosis. The birds were kept under complete observation for six weeks (experimental period) in separate thoroughly cleaned and disinfected houses and provided with feed and water *ad libitum*. All the birds were vaccinated against Newcastle disease (ND) using live Hitchner B1 and La Sota vaccine strains at 6 and 19 days of age, respectively, against infectious broncheitis (IB) disease using live H 120 strain at 6 day old and also against avian influenza (AI) disease using inactivated H5 N1 reassortant virus vaccine strain a 7 days old. Vaccination against infectious bursal disease (IBD) was applied using live intermediate strain (228 E) at 14 days

of age. All the vaccines were given via eye drop instillation except (AI) vaccine which given through subcutaneous route at the back of the neck.

2.2 The Ration

Commercial starter and grower broiler chicken ration were given till 21 and 35 days of age, respectively, while finisher ration was used till 42 days of age (end of the experiment). The used commercial balanced ration based on yellow corn or soyabean that met the National Research Council (NRC) (1984) broiler chicken requirements. The starter ration contained crude protein-not less than 21%, crude fat-not less than 2.94%, crude fibers-not less than 2.35%, metabolizing energy-not less than 3054 Kcal/kg ration and used for the first 3 weeks of age. The grower and finisher ration contained crude protein-not less than 17.15%, crude fat-not less than 2.5%, metabolizing energy-not less than 3020 Kcal/kg ration and used for the remaining of the experimental period. The ration contained coccidiostat (semduramicin) while no antibiotics were added to it.

2.3 The Used Probiotic

The probiotic that used in the experiment was a commercial preparation containing (*Lactobacillus acidophilus*, *Enterococcus faecium*, *Lactobacillus plantarum* and *Lactobacillus casei*) plus potassium, vitamins A, D3, E and K, riboflavin, pantothenic acid, thiamine and niacinamide. That product was manufactured by Bomac Vets Plus, USA. Batch No., A704. It was given in the drinking water at the age of one day for 5 consecutive days in a dose of 1gm/ 4 liter of the drinking water as recommended by manufacturer.

2.4 The Challenge Inoculum

Broth culture of *S. Enteritidis* field strain was centrifuged at 3000 r.p.m for 10 min. Sediment was diluted with sterile buffer saline and adjusted using MacFerland matching tube to contain 10^9 CFU/ml. The challenge inoculum was prepared according to the method of *Timms et al.*, (1990). At 20 days of age, each bird in the experimentally infected groups was inoculated orally with 0.5 ml/ containing 10^9 CFU/ml *S. Enteritidis* (*Okamoto et al.*, 2007).

2.5 Preparation of Local Salmonella Enteritidis Bacterin

The bacterin was prepared from *S. Enteritidis* field strain according to the technique of *Timms et al.*, (1990). The strain of *S. Enteritidis* was transferred from semi-solid media to nutrient broth and incubated at 37°C for 24 hr, then to nutrient agar and incubated at 37°C for 24 hr, after which it was grown on brain heart infusion agar and kept at 37°C for 48 hr in Roux flask. Growth was harvested in normal saline and inactivated with 1 % formol saline for 24 hr at room temperature. Using MacFerland matching tube, washed concentrates of inactivated bacteria were suspended in saline and adjusted to contain 10^{11} CFU/ ml. Sterile bacterin was obtained by adding equal volume of incomplete Ferund's adjuvant to adjusted washed concentrate of inactivated bacteria and kept at refrigerator until used. The prepared whole cell inactivated *S. Enteritidis* bacterin was given for the experimental chicks at the first day of age in a dose of 0.2 ml/bird and boosted as a second dose at 10 days of age in a dose 0.5 ml/bird. The bacterin in the two shots was given intramuscularly (I/M) in the thigh muscles.

2.6 Quality Control Tests of the Prepared Bacterin

The prepared *S. Enteritidis* bacterin was tested for purity, complete inactivation, sterility and safety according to the Standard International Protocols as described by the *British Veterinary Codes* (1970) as follows:

2.6.1 Purity Test

The test was done before formalin inactivation of *S. Enteritidis* strain. It was applied to confirm that the broth culture of *S. Enteritidis* strain did not contain any contamination by other organisms before inactivation. Such purity was detected by inoculation of the broth culture onto Salmonella Shegella (*S. S*) agar and incubated at 37°C for 24 hr. Appearance of pure colonies of *S. Enteritidis* and pure *S. Enteritidis* organism after Gram staining of the organism indicated culture purity.

2.6.2 Completion of *S. Enteritidis* Inactivation

In assurance that the used *S. Enteritidis* organisms were completely inactivated, *S. S* agar media was inoculated with formalin inactivated bacteria. After 24-48 hrs of incubation at 37°C, no visible growth of *S. Enteritidis* indicated complete inactivation of the organism.

2.6.3 Sterility Test

The prepared *S. Enteritidis* bacterin was confirmed to be free from any fungal contaminants by inoculation of it onto Sabouraud Dextrose Agar (SDA) plates and incubated at 25°C for 7 days. Also the bacterin was inoculated

on Pleuropneumonia Like Organism (PPLo) broth tubes and agar plates and incubated at 37°C for 72 hrs and 14 days, respectively in CO₂ incubator to ensure the freedom of the bacterin from mycoplasma organisms.

2.6.4 Safety Test

Ten, day old broiler chicks were inoculated intramuscularly (I/M) with a large dose of the prepared bacterin (ten fold the normal bacterin dose). The chicks were observed daily for 7 successive days for any signs of local reactions, clinical signs or deaths.

2.7 Experimental Design

Three hundred and ten, one day-old Hubbard broiler chicks of mixed sex were used. At arrival, randomly ten chicks were sacrificed and then examined bacteriologically to prove their freedom from *S. Enteritidis* infection. A completely randomized design was used as three replicates, each consists of 25 chicks and the groups were divided as the followings;

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

Group (2): Infected non treated (*S. Enteritidis* infected birds).

Group (3): (Vaccinated and *S. Enteritidis* infected birds).

Group (4): (Probiotic treated and *S. Enteritidis* infected birds).

2.8 The Measured Parameters for Evaluation of the Bacterin Efficacy in Comparison with the Probiotic

2.8.1 Clinical Signs, Mortalities and Gross Lesions

Birds in the challenged groups were observed daily for three weeks post challenge till the end of the study (6 weeks of age) for the clinical signs or deaths. Dead birds were subjected to necropsy for recording the lesions of *S. Enteritidis* (O'Brien 1988).

2.8.2 Detection of the Shedding of *S. Enteritidis*

Cloacal swabs were taken from birds in each group just before experimental infection (at 20 days of age) to ensure that the birds free from *S. Enteritidis* infection. Weekly after the challenge up to 6 weeks of age, cloacal swabs were collected from each of the infected as well as control group and examined bacteriologically for the presence of *S. Enteritidis* organism. Sterile cotton swab was inserted into the cloaca of each bird and rotated gently to collect the cloacal contents. Each swab was transferred to 10 ml tube of tetrathionate broth and incubated overnight at 37°C. A loopful from the broth was streaked on S.S agar for *Salmonella* isolation. Suspected colonies were identified morphologically and biochemically.

2.8.3 Re-isolation of *S. Enteritidis*

Ten birds from each group post challenge were weekly randomly selected, sacrificed and the liver, heart, spleen and caecum were collected for *S. Enteritidis* re-isolation. Samples were inoculated into tetrathionate broth, incubated at 37°C for 24 hr, streaked onto S.S agar and incubated at 37°C for 24 hr. Suspected colonies were identified morphologically and biochemically.

2.8.4 The Performance

At arrival, the chicks were weighed and then the birds in each group were subjected to weekly determination of the production parameters that include; the body weight (BW), the cumulative feed conversion (CFC) and the European Production Efficiency Factor (EPEF) according to Sainsbury (1984). These measures were taken till the end of the study (6 weeks of age).

2.8.5 Detection of the Humoral Immune Response

Just before the first *S. Enteritidis* vaccinal dose (at zero hour), a randomly ten identified birds were selected and the blood samples were collected via the brachial vein. Also just before the second booster dose of *S. Enteritidis* vaccination (10 days after the first dose), another blood; samples were collected as previously from the same marked birds. Blood samples were collected weekly after the second vaccination till 6 weeks of age (end of the study). The collected 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. The antibody titer against *S. Enteritidis* was determined using Microagglutination test (MAT) and indirect Enzyme Linked Immuno-Sorbent Assay (ELISA) test.

Micro agglutination test (MAT)

It was done as Williams and Whittemore (1971) and Brown et al., (1981).

Indirect Enzyme-Linked Immunosorbent Assays (ELISA)

This test was applied according to the method of *Kim et al., (1991)* and *Barrow (1992)*.

2.9 Statistical Analysis

The statistical analysis of data of protection, faecal shedding and re-isolation was carried out using Chi-Square (*Greenwood and Nikulin 1996*). The statistical analysis of average body weight and ELISA data were examined using One-Way analysis of variance (ANOVA) according to *Shott (1990)*.

3. Results and Discussion

Although *S. Enteritidis* infection in chicken broilers or layers sometimes sub-clinical, but poultry products are considered as important sources for human food borne outbreaks (*Bielke et al., 2003*). In terms of the loss to producers annually, it is difficult to estimate, however any strategies which reduce the incidence of salmonellosis in poultry are clearly important to all facts of the industry. Reducing *Salmonella* incidence has become monitored and regulated by Food Safety and Inspection Service (*Helmick et al., 1994*). *Wilkie (2006)* and *Rahimi et al., (2007)* suggested using of alternatives rather than antimicrobial therapy for controlling enterobacteria. It has been accepted worldwide that vaccination to prevent or reduce *Salmonella* infection in poultry is practical (*Barrow 2007*). In big poultry producing countries, commercial vaccines are commonly used in layers as well as broilers to control the outbreaks of salmonellosis (*Paiva et al., 2009*). Antimicrobial feed additives such as antibiotics and synthetic antimicrobial agents have been used extensively in poultry feed to suppress or eliminate harmful intestinal pathogens and to improve both growth and feed efficiency (*Jin et al., 1997*). However, the continued feeding of antibiotics at sub-therapeutic levels usually increase the possibilities of antibiotic residue, the development of drug-resistant bacteria and additionally a reduction in the ability to cure bacterial infections in humans (*Jensen 1998*), because of that, the routine use of antibiotics as feed additives and growth promoters has been banned in some European countries since 1986 (*Van Belle 2001*). Probiotics, such as lactic acid-producing bacteria and yeast have been reported to improve gastrointestinal balance through bacterial antagonisms, competitive exclusion and immune stimulation (*Havenaar and Huisint 1992*).

The quality control tests of the locally prepared whole cell inactivated *S. Enteritidis* bacterin revealed that the bacterin was pure (contained only *S. Enteritidis* organisms) as confirmed by Gram staining. Microscopically, there were Gram negative, straight and non spore forming *S. Enteritidis* organisms. The bacterin was completely inactivated as there was no growth on *S. S* agar media. Also, the prepared bacterin was sterile without fungal or mycoplasmal growth contamination as indicated by absence of growth on SDA and PPLO broth or agar, respectively. No local reactions, signs or deaths were observed after bacterin I/M inoculation in the chicks and that ensure the safety of the bacterin. *Young and Kang (2005)* reported on the safety and efficacy of *S. Gallinarum* bacterin when applied in young layer chicks.

In the present study, Three hundred, day old broiler chicks were used and divided into four groups and submitted for vaccination with locally prepared *S. Enteritidis* bacterin or treated with probiotic, and then experimentally infected with *S. Enteritidis* to evaluate the efficacy of both tools in protection from infection.

The results of experimental infection of broiler chickens at 20 days old after vaccination with the locally prepared *S. Enteritidis* bacterin and treatment with the probiotic indicated that infected groups (2, 3 and 4) manifested signs of depression, anorexia and watery diarrhea 3 days post challenge. The severity of signs was highly in the birds that infected only (group 2) than those vaccinated or probiotic treated (group 3 and 4). The morbidity rates were 80, 30 and 20% in the only infected, vaccinated infected and probiotic treated and infected groups, respectively. *S. Enteritidis* may produce clinical disease in chicks up to six weeks of age and occasionally in adult laying birds. Affected birds are depressed, reluctant to move and commonly have diarrhoea (*Wray et al., 1996*).

Table (1) shows that the mortality rate 30.67% in infected non treated birds, 5.33% in vaccinated infected birds and 12% in probiotic treated infected ones. Dead birds were subjected to post-mortem examination for specific *S. Enteritidis* lesions. Lower mortality rate in broilers was recorded by *Samanta and Biswas (1995)* after treatment with probiotics. The most predominant lesions were congestion of muscles and internal organs and inflammation of the intestinal mucosa with mucoid intestinal contents. The lesions were markedly severe in infected non treated broilers than treated ones. *Lister (1988)* recorded nearly similar lesions caused by *S. Enteritidis* in broiler chickens.

From table (1), the results clear that there is a significant ($P \leq 0.05$) difference in the protection rate between the infected non treated group and the vaccinated and probiotic treated groups. The protection rate of the locally prepared *S. Enteritidis* bacterin and the probiotic in birds are 82.61% and 60.87%, respectively which is

significantly ($P \leq 0.05$) higher than infected non treated birds (0%). These results agreed with *Gast et al., (1993); Timms et al., (1994); Feberwee et al., (2000 and 2001a, b)* who proved that *S. Enteritidis* bacterin was highly protective for broiler or layer chicken flocks when using signs, mortalities and post-mortem lesions as criteria for measuring the protective index. *Ghosh (1989)* reported that vaccination of broilers with *S. Virchow* formalin killed bacterin reduced mortalities from 85 to 0%. Also, *Timms et al., (1990)* found decline in mortalities from 100 to 50% in *S. Enteritidis* bacterin inoculated broiler chickens.

The protective efficacy of the probiotics which contained *Lactobacillus* spp. against *S. Enteritidis* infection was evaluated by *Samanta and Biswas (1995); Soomro et al., (2002); Timmerman et al., (2006); 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. *Lactobacilli* have been widely reported to produce antibacterial compounds called bacteriocins and the effect of bacteriocins have been hypothesized to be the mechanism by which *Lactobacilli* exert cytotoxic effects *in vivo* (*Bogovic-Matijasic et al., 1998; Ocan et al., 1999*). They also hypothesized that a soluble peptide could mediate the reduction of *S. Enteritidis*.

The results of the faecal shedding rate of *S. Enteritidis* in broiler chickens after vaccination with the locally prepared bacterin and treatment with probiotic is illustrated in table (2). The results declare that there are significant ($P \leq 0.05$) differences between the vaccinated and probiotic treated groups and the infected non treated one along three weeks observation period. Gradual and significant ($P \leq 0.05$) decrease in the shedding rate is observed within each group until reached the last week of observation period. The faecal shedding rate in the vaccinated birds collectively is 9.24% and in the probiotic treated one is 17.5% which is significantly ($P \leq 0.05$) lower than infected non treated birds (40.6%). There was a considerable difference between the carriage of *Salmonella* in the cecal contents, indicating that broilers were able to clear systemic infections but could remain intestinal carriers (*Bjerrum et al., 2003*). So, the intestinal carrier status was most important in control of *S. Enteritidis* contamination during transportation and processing of broilers, where cross contamination played a major role (*Gurtler et al., 2004; Dorea et al., 2010*).

The role of formalin inactivated whole cell *S. Enteritidis* oil emulsion bacterin in reducing the shedding rate of the organism after examination of the cloacal swabs was tested by *Barbour et al., (1993); Gast et al., (1993); Nakamura et al., (1994); Liu et al., (2001); Clifton-Hadley et al., (2002); Davis and Breslin (2003); Okamura et al., (2003); Davis and Breslin (2004); Radwan (2007)* and they concluded that this type of vaccine was very effective or significant in reducing the *S. Enteritidis* faecal shedding rate in vaccinated chickens when compared with non vaccinated control birds.

Concerning the reducing effect of the probiotics on *S. Enteritidis* shedding rate, the results of *Tellez et al., (2001) and Rahimi et al., (2007)* in broilers and *Fulton et al., (2002)* in duckling are parallel with our results.

The results of the re-isolation rate of *S. Enteritidis* from different organs of sacrificed broiler chickens after vaccination with the locally prepared bacterin and treatment with the probiotic are shown in table (3). These results indicate that along the whole three weeks observation period, the highest and significant ($P \leq 0.05$) re-isolation rate in the infected non treated broiler chickens is (46.67%), while this rate is significantly ($P \leq 0.05$) lower in the probiotic treated birds (21.67%) until it reaches its lowest and significant ($P \leq 0.05$) level in the vaccinated ones (11.67%). The results of *Bolder and Palmu (1995)* proved the possibility of *S. Enteritidis* to become extra-intestinal and invade the liver week post infection. It was documented that the frequency of *S. Enteritidis* re-isolation from the internal organs (liver and spleen), the intestine (caecum) and the eggs (shells or contents) was significantly reduced in vaccinated broilers and layers with multiple doses of *S. Enteritidis* oil emulsion bacterin than non vaccinated controls (*Gast et al., 1993; Nakamura et al., 1994; Timms et al., 1994; Davison et al., 1999; Miyamoto et al., 1999; Khan et al., 2003; Okamura et al., 2007; Radwan et al., 2007*). *Young et al., (2007)* detected that vaccination against *Salmonellae* in breeders could reduce the colonization of the organism in the hatched broiler chicks. As well, *Penha et al., (2009)* and *Priyantha (2009)* found that vaccination of layers and breeders with inactivated *S. Enteritidis* vaccine induced significant reduction of organ (spleen, liver and caeca) colonization after challenge. Nevertheless, *Clifton-Hadley et al., (2002)* mentioned that there was no effect of vaccination on internal organs colonization after oral challenge with *S. Typhimurium*.

The reducing effect of probiotics on the colonization by *Salmonella* spp. was studied comprehensively by several researchers. It was reported that probiotics maintained or increased the normal intestinal flora which are normally found in the intestinal tract of hatched birds and these flora can exclude *Salmonellae* colonization (*Goren et al., 1988; Starvic 1992; Barbour et al., 1993; Nisbet et al., 1996; Mead 2000; Seo et al., 2000*).

Contrary results were mentioned by *Seuna et al., (1980)* who found that supplementation of the birds with normal avian gut microflora didn't prevent or only partially prevent *Salmonella* colonization. This inconsistent may be due to the difference in the strains of bacteria that present in the culture. There are many hypotheses that explain the mechanism of action of 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; Johanssen et al., 2004*), the competition between *Lactobacilli* and the enteric bacteria which is called competitive exclusion (*Heres et al., 3003*), also the production of bacteriocin which is antibacterial substances that kill enterobacteriaceae (*Pascual et al., 1999*).

Concerning the results of the performance of each group after vaccination with the locally prepared *S. Enteritidis* bacterin and treatment with the probiotic, they are seen in table (4). The measured parameters including the average body weight (BW), the cumulative feed conversion (CFC) and European production efficiency factor (EPEF) show significant ($P \leq 0.05$) improvement in the vaccinated and probiotic treated groups than infected non treated one along the whole course of the study (6 weeks of age). The best CFC is observed in the vaccinated and probiotic treated broilers (1.89 and 1.78, respectively), while the worst one is in the infected non treated birds (2.43).

Our results are in agreement with that recorded by *Mohrah and Zaki (1995)* who demonstrated that vaccination of chickens with *Salmonella Gallinarum Pullorum* bacterin induced significant increase in the body weight of birds.

Most of the published studies regarding the effect of probiotics (especially those containing *Lactobacillus* spp.) on bird's performance in case of absence of enteric infections revealed that these compounds are effective as they improve the growth of the broiler chickens (*Mohan et al., 1996; Jin et al., 1998; Zulkifli et al., 2000; Kalvathy et al., 2003; Gracia et al., 2004; Huang et al., 2004; Opalinski et al., 2007; Midilli et al., 2009*). On the other hand, *Angle et al., (2005)* found no improvement in performance but there were increased protein, calcium and phosphorus retention. In case of *S. Enteritidis* infection, *Fairchild et al., (2001); Tellez et al., (2001); Wafaa et al., (2006); Wilkie et al., (2006); 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; Ayed et al., 2004*), producing digestive enzymes (*Saarela et al., 2000*), synthesizing of vitamins (*Coates and Fuller 1977*), stimulating lactic acid production (*Bailey 1987*) and the beneficial effect on the health of the host (*Soomro et al., 2002*).

Results listed in table (5) show the titer of antibodies against *S. Enteritidis* after vaccination with the locally prepared bacterin and treatment with the probiotic using microagglutination test. After the 1st dose of bacterin administration and probiotic treatment, the geometric mean titer (GMT) of antibodies increases to reach 65 in vaccinated group and 60.6 in the probiotic treated birds. After booster dose of vaccination (before challenge), GMT increases to reach 98 and 74.5 in vaccinated and probiotic treated birds, respectively. A week after *S. Enteritidis* challenge, the GMT increases to reach 60.6, 211.1 and 113.1 in the infected non treated, vaccinated and probiotic treated birds, respectively. Moreover, two weeks post challenge, GMT increases in the infected treated chickens and the vaccinated ones up to 139.3 and 242.5, respectively, but it declines in the probiotic treated chickens to reach 171.

For evaluation of humoral immune response of chickens to *S. Enteritidis* vaccination and probiotic, ELISA test was done. Table (6) reveals that during the whole experimental period, the blank control non infected group shows no significant ($P \leq 0.05$) differences in the mean optical densities values. However, there is no significant ($P \leq 0.05$) difference in optical densities values in *S. Enteritidis* infected, non treated birds till 20 days of age (before challenge) but these values increase significantly ($P \leq 0.05$) at 27 days of age to reach (1.781) then decrease at 34 and 41 days of age to 1.457 and 1.274, respectively. Vaccinated group shows gradual and significant ($P \leq 0.05$) increase in optical densities values from 0.234 prior vaccinations to 1.614 before booster dose (10 days old) and to 2.543 after booster dose (at 20 days old), then significant ($P \leq 0.05$) increase in these values at 27, 34 and 41 days old is observed. Furthermore, in the probiotic treated birds, gradual and significant ($P \leq 0.05$) increase in optical densities values to reach 0.234 then 0.561 at day and 10 days old, respectively is detected. These values are increased significantly ($P \leq 0.05$) at 27, 34 and 41 days of age.

Barrow and Lovell (1991) and *Olabisi and Peter (2008)* reported on production of high level of serum IgG after oral inoculation of *S. Enteritidis* in layer chickens. The enhancement of the immune response of *S. Enteritidis* infected birds to vaccination by the locally prepared bacterin was mentioned by *Barbour et al., (1993); Gast et al., (1993); Methner et al., (1994); Methner and Steinbach (1997); Okamura et al., (2003); Davies and Breslin*

(2004); Pakpinyo *et al.*, (2008) as they detected that *S. Enteritidis* bacterin induced high level of circulating specific IgG against un-specified protein antigen.

Most of the studies pertaining to using of bacterins have investigated post-vaccine *S. Enteritidis* shedding over time (Woodward *et al.*, 2003; Piao *et al.* 2007). Only a few have looked at the immune response following vaccination, and these studies were limited to young chickens (less than four weeks old) (Liu *et al.*, 2001; Babu *et al.*, 2004; Okamura *et al.*, 2004).

Penha *et al.*, (2009) detected that vaccination of layers and breeders with inactivated *S. Enteritidis* vaccine induced significant increased in the antibody titers as measured by ELISA. Furthermore, Tran *et al.*, (2010) demonstrated that immunization of chickens with inactivated *S. Enteritidis* vaccine in two shots induced increasing in ELISA seroconversion (serum IgG response) which persisted up to from 3 to 32 and 34 week post-vaccination. Contrary findings were obtained by Babu *et al.*, (2004) which had different opinions on the protective effect of vaccination against *S. Enteritidis*. The serum antibody titers were of uncertain values as indicators of the extent of protection against *Salmonella*, even if a killed vaccine could induce a high *S. Enteritidis*-specific antibody level, it did not necessarily protect the chickens against *S. Enteritidis* infection.

Our findings concerning the immunopotantiation caused by probiotic administration concur with Pollmann *et al.*, (1980); Zulkifli *et al.*, (2000); Toms and Powvie (2001); Huang *et al.*, (2004); Koenen *et al.*, (2004). Also, Seville *et al.*, (1996) and Panda *et al.*, (2000) demonstrated an increase in the serum immunoglobulins IgG and IgA after administration of competitive exclusion cultures. Abdel-Alim *et al.*, (2006) demonstrated an increase in the haemagglutinating antibody titers in ochratoxicated immunosuppressed chickens after treatment with a probiotic than non treated birds. Moreover, Lee *et al.*, (2007); Rowghani *et al.*, (2007) and Alkhalf *et al.* (2010) reported that broiler chickens fed a diet supplemented with probiotic had a significant increase in the Newcastle antibody titers than control group. The enhancement of immunity in *S. Enteritidis*-probiotic treated birds was recorded by Wafaa *et al.*, (2006) and Revollo *et al.*, (2009). The positive effect of feeding diet containing probiotic on the immune response indicates the enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes. The direct effect might be related to stimulate the lymphatic tissue (Kabir *et al.*, 2004), whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract or through the reduction of *S. Enteritidis* colonization. Shoeib *et al.*, (1997) reported that the bursa of probiotic-treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. Christensen *et al.*, (2002) suggested that some of these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. On the other hand, Okamoto *et al.*, (2007) demonstrated that this type of treatment had few beneficial effects for chicks, particularly during the first days of life.

4. Conclusions

From this study, it could be concluded that both the locally prepared autogenous *S. Enteritidis* bacterin in double doses and the probiotic preparation are effective and safe methods for prevention of *S. Enteritidis* infection in broiler chickens. It should be taken in consideration that the vaccine or the probiotic must be go parallel to bio-security measures and good management practices to eradicate *S. Enteritidis* infection in poultry flocks.

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Table 1. The protection rate of the locally prepared inactivated *S. Enteritidis* bacterin and the probiotic treatment against *S. Enteritidis* infection in broiler chickens

Groups	Total No. of birds	No. of dead birds/week post challenge			Total No. of dead birds	Mortality rate	Total No. of survived birds	Protection %
		1 st week	2 nd week	3 rd week				
Blank control	75	0	0	0	0	0	75	100 ^a
Infected, non-treated	75	17	5	1	23	30.67	52	0 ^d
Vaccinated, Infected	75	3	1	0	4	5.33	71	82.61 ^b
Probiotic treated, Infected	75	6	3	0	9	12	66	60.87 ^c

$$Protection\ rate = \frac{Survived\ test - survived\ control\ positive}{Dead\ control\ positive} \%$$

*Different letters within the same column were significantly difference at (P≤0.05).

Table 2. The faecal shedding rate of *S. Enteritidis* in broiler chickens after vaccination with the locally prepared bacterin and treatment with probiotic

Treatment group	Weeks post challenge						+Ve/Total	%
	1 st wk		2 st wk		3 st wk			
	+Ve/Total	%	+Ve/Total	%	+Ve/Total	%		
Blank control	0/75	0 ^{Ad}	0/75	0 ^{Ad}	0/75	0 ^{Ad}	0	0 ^d
Infected, non-treated	30/58	51.72 ^{Aa}	16/43	37.2 ^{Ba}	8/32	25 ^{Ca}	54/133	40.6 ^a
Vaccinated, Infected	13/72	18.06 ^{Ac}	4/61	6.56 ^{Bc}	0/51	0 ^{Cc}	17/184	9.24 ^c
Probiotic treated, Infected	18/69	26.08 ^{Ab}	10/56	17.86 ^{Bb}	2/46	4.35 ^{Cb}	30/171	17.5 ^b

+Ve= Positive birds

Different small letters within the same column were significantly difference at (P≤0.05).

Different capital letters within the same row were significantly difference at (P≤0.05).

Table 3. The re-isolation rate of *S. Enteritidis* from different organs of sacrificed broiler chickens after vaccination with the locally prepared bacterin and treatment with probiotic

Group	Re-isolation rate															Total
	At the end of 1 st Wk post challenge					At the end of 2 nd Wk post challenge					At the end of 3 rd Wk post challenge					
	Examined Organs															
	Liver	Heart	Spleen	Cecum	Total	Liver	Heart	Spleen	Cecum	Total	Liver	Heart	Spleen	Cecum	Total	
Blank control	0	0	0	0	0% ^d	0	0	0	0	0% ^d	0	0	0	0	0% ^d	0% ^d
Infected, non-treated	7/10	5/10	6/10	8/10	26/40 65% ^{Aa}	4/10	4/10	5/10	6/10	19/40 47.5% ^{Ba}	2/10	1/10	4/10	4/10	11/40 27.5% ^{Ca}	56/120 46.67% ^d
Vaccinated, Infected group	2/10	2/10	2/10	3/10	9/40 22.5% ^{Ac}	1/10	0/10	1/10	2/10	4/40 10% ^{Bc}	0/10	0/10	0/10	1/10	1/40 2.5% ^{Cc}	14/120 11.67% ^e
Probiotic treated, Infected group	3/10	2/10	3/10	4/10	12/40 30% ^{Ab}	3/10	2/10	2/10	3/10	10/40 25% ^{Ab}	1/10	0/10	1/10	2/10	4/40 10% ^{Bc}	26/120 21.67% ^b

Different small letters within the same column were significantly difference at ($P \leq 0.05$)

Different capital letters within the same row were significantly difference at ($P \leq 0.05$)

Table 4. Average body weight, cumulative feed conversion and European Production Efficiency Factor in broiler chickens after vaccination with the locally prepared *S. Enteritidis* bacterin and treatment with probiotic

Treatment group	Average body weight/g						CFC	EPEF**
	Age/week							
	Before challenge			After challenge				
	1	2	3	4	5	6		
Blank control	133.40±6.30 ^a	291.1±5.21 ^a	563.75±11.86 ^b	744.12±24.77 ^b	984.1±42.21 ^b	1580.2±65.22 ^b	2.03	189.12
Infected, non-treated	128.11±5.31 ^a	290.1±8.679 ^a	501.1±18.0 ^c	699.22±19.63 ^c	801.50±13.50 ^c	1355.1±54.10 ^c	2.43	148.25
probiotic treated, Infected.	130.43±3.62 ^a	288.2±9.90 ^a	599.1±21.8 ^a	810.8±21.3 ^a	1100.1±55.3 ^a	1705±21.50 ^a	1.78	213.44
Vaccinated, Infected.	134.73±2.77 ^a	293.1±8.7 ^a	589.1±18.21 ^{ab}	799.90±12.10 ^{ab}	1005.0±71.00 ^{ab}	1640.1±55.91 ^{ab}	1.89	205.44
LSD	16.45	25.2	30.1	63.48	115.80	119.75		

CFC= Cumulative feed conversion

EPEF**= European Production Efficiency Factor. The higher the value, the better the performance

LSD= Least significant difference as determined by Fisher's protected LSD procedures

*Means within the column with different letters are significantly different ($p \leq 0.05$)

Table 5. Microagglutination antibody titers in the sera of broiler chickens after vaccination with locally prepared *S. Enteritidis* bacterin and treatment with probiotic

Age/day	Interval	Group	No. of tested samples	Antibody titers of chicken at different serial dilutions								GMT	
				0	20	40	80	160	320	640	1280		
1	Before 1 st vaccination dose	G1	10	10	0	0	0	0	0	0	0	0	0
		G2	10	10	0	0	0	0	0	0	0	0	0
		G3	10	10	0	0	0	0	0	0	0	0	0
		G4	10	10	0	0	0	0	0	0	0	0	0
10	Before booster vaccination dose	G1	10	10	0	0	0	0	0	0	0	0	0
		G2	10	10	0	0	0	0	0	0	0	0	0
		G3	10	0	0	3	7	0	0	0	0	0	65
		G4	10	0	0	4	6	0	0	0	0	0	60.6
20	Before challenge	G1	10	10	0	0	0	0	0	0	0	0	0
		G2	10	10	0	0	0	0	0	0	0	0	0
		G3	10	0	0	0	8	1	1	0	0	0	98
		G4	10	0	0	2	7	1	0	0	0	0	74.5
27	1 st week post challenge	G1	10	10	0	0	0	0	0	0	0	0	0
		G2	10	0	0	4	6	0	0	0	0	0	60.6
		G3	10	0	0	0	0	7	2	1	0	0	211.1
		G4	10	0	0	2	1	7	0	0	0	0	113.1
34	2 nd week post challenge	G1	10	10	0	0	0	0	0	0	0	0	0
		G2	10	0	0	0	3	7	0	0	0	0	130
		G3	10	0	0	0	0	6	3	1	0	0	226.2
		G4	10	0	0	0	2	3	5	0	0	0	197
41	3 rd week post challenge	G1	10	10	0	0	0	0	0	0	0	0	0
		G2	10	0	0	0	2	8	0	0	0	0	139.3
		G3	10	0	0	0	0	6	2	2	0	0	242.5
		G4	10	0	0	0	3	3	4	0	0	0	171

GMT: Geometric mean titer. G1: Blank control. G2: Infected non treated group. G3: Vaccinated infected group. G4: Probiotic treated infected group.

Table 6. ELISA mean optical densities of antibodies in sera of broiler chickens after vaccination with the locally prepared *S. Enteritidis* bacterin and treatment with probiotic

Age/day	Intervals	Blank control group	Infected, non-treated group	Vaccinated, infected group	Probiotic treated, infected group	LSD
1	Before 1 st vaccination dose	0.234±0.064 ^{Aa}	0.234±0.052 ^{Ca}	0.234±0.032 ^{Ca}	0.234±0.039 ^{Da}	0.044
10	Before booster vaccination dose	0.212±0.031 ^{Ac}	0.212±0.027 ^{Cc}	1.614±0.249 ^{Ba}	0.561±0.034 ^{Cb}	0.115
20	Before challenge	0.245±0.035 ^{Ac}	0.245±0.024 ^{Cc}	2.543±0.427 ^{Aa}	0.953±0.078 ^{Bb}	0.198
27	1 st week post challenge	0.232±0.042 ^{Ac}	1.781±0.396 ^{Ab}	2.281±0.366 ^{Aa}	1.574±0.375 ^{Ab}	0.298
34	2 nd week post challenge	0.231±0.050 ^{Ac}	1.457±0.346 ^{Bb}	2.340±0.294 ^{Aa}	1.845±0.452 ^{Aa}	0.291
41	3 rd week post challenge	0.241±0.033 ^{Ad}	1.274±0.339 ^{Bc}	2.486±0.444 ^{Aa}	1.779±0.458 ^{Ab}	0.328
LSD		0.039	0.230	0.298	0.274	0.274

Different small letters within the same row were significantly difference at (P≤0.05).

Different capital letters within the same column were significantly difference at (P≤0.05).

+ve result = > 0.65

-ve result < 0.65