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COMPARATIVE STUDIES ON THE EFFECT OF PROBIOTIC AND AUTOGENOUS BACTERIN ON SALMONELLA TYPHIMURIUM INFECTION IN CHICKEN

BY

Nagah Arafat[1] and Reham El-Shafii[2]

[1]Department of poultry diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura

[2]Department of pharmacology, Faculty of Veterinary Medicine, Mansoura University, Mansoura

ABSTRACT

This study was carried out to investigate and compare the efficacy of the locally prepared autogenous *Salmonella typhimurium* (*St*) bacterin as well as the commercial probiotic in protecting male layer type chicks from *Salmonella typhimurium* infection. A total of three hundred and eighty, one day-old chicks were used. At day old, twenty chicks were sacrificed and examined bacteriologically to ensure their free from *Salmonella typhimurium* infection. Three hundred and sixty chicks were randomly divided into six equal groups with two replicates for each group (30 chicks per replicate). Chicks in group (1) were kept as negative control (non challenged-non treated birds), while those of group (2) were positive control (challenged-non treated Chicks). Group (3) was single dosed subcutaneously vaccinated by autogenous bacterin at the first day of age in a dose of 0.2 ml/bird. Group (4) was double dosed subcutaneously vaccinated by autogenous bacterin at the first day of age in a dose of 0.2 ml/bird and boostered as a second dose at 14 days of age in a dose 0.5 ml/bird, however, group (5) was given a commercial probiotic preparation (PrimaLac®) as 12gm/ 100 liter of the drinking water from the first day of age and continued for 10 successive days, while, group (6) was given a commercial probiotic preparation (PrimaLac®) as 12gm/ 100 liter of the drinking water from the first day of age and continued throughout the entire study. All birds in groups 2, 3, 4, 5 and 6 were challenged orally by 1 ml containing 6 x 10^8 CFU *S. typhimurium* at 28 days of age. All the groups were kept under complete observation three weeks post challenge for recording signs, moralities, gross lesions, shedding rate of *S.*

typhimurium, the growth performance, re-isolation of the organism as well as detection of the titer of antibodies serologically using microagglutination test. The results showed that both a single or double doses and the probiotic administration either a specific period or continuous are significantly effective in reducing the signs, the mortalities, the gross lesions, the shedding rate and the re-isolation of *S. typhimurium* and also the increasing in the performance of chickens when compared with the challenged-non treated chicken. Moreover, the serological investigation revealed significantly improvement in the titer of antibodies in two doses of vaccination and continuous probiotic treatment birds. In conclusion, the locally prepared autogenous *S. typhimurium* bacterin both a single or double doses and the probiotic preparation either a specific period or continuous were effective with superiority of two doses of vaccination and continuous probiotic treatment.

INTRODUCTION

Salmonella enterica remains a major cause of food-borne gastroenteritis throughout the world. Poultry are a major recognized source of infection (**EFSA, 2007**). Therefore, a reduction in *Salmonella* infection in chicken will reduce public health risks associated with poultry products and will also likely improve growth of chickens **(Snoeyenbos *et al.*, 1979)**. Therefore, control programs are being currently looked for ways to reduce the amount of *Salmonella* in commercial poultry. These *Salmonella* intervention strategies include biosecurity, therapeutic antibiotics, probiotics and competitive exclusion products, organic acids and vaccination (**White *et al.*, 2007**). Vaccination of poultry becomes one of the most important control measures, because of the cost and impracticability of improvements in hygiene and the increasing antibiotic resistance of bacteria (**Zhang-Barber *et al.*, 1999)**. A decreased incidence of human *S. enteritidis* infections in the United Kingdom followed the widespread vaccination of egg-laying hens **(Cogan and Humphrey, 2003)**. Competitive exclusion (CE) cultures and probiotic cultures consisting of live beneficial bacteria have been used to reduce levels of *Salmonella* in live poultry, with positive results (**Waters *et al.*, 2005**). CE cultures have been shown to reduce or eliminate *S. infantis* (**Schneitz *et al.*, 1991**) *S. typhimurium* (**Hoszowski *et al.*, 1996**) and *S. enteritidis* (**Corrier *et al.*, 1993**) from the gastrointestinal tracts of poultry when used prophylactically. The aim of this study was designed to study and compare the effect of two programs of autogenous bacterin and two

programs of probiotic on mortality, fecal shedding, growth performance and organ colonization (liver, spleen and cecum), as well as serological responses of chicken inoculated with *S. typhimurium* at 28 days of age.

MATERIALS AND METHODS

2.1 Experimental Chickens. A total of three hundred and eighty, day-old male, white layer type chicks that obtained from Misr Company for poultry production were used. The chicks were kept under complete observation for seven weeks (experimental period) in a wire bottom cages, housed in well ventilated disinfected room and were provided with unmedicated and *Salmonella* free commercial starter ration and water ad-libitum under 24-h lighting. All chicks were vaccinated against Newcastle disease, infectious bronchitis disease, avian influenza disease and infectious bursal disease.

2.2 The challenge inoculum. Broth culture of *S. typhimurium* field strain was centrifuged at 3000 rpm for 10 min. Sediment was diluted with sterile buffer saline, adjusted using MacFerland matching tube to contain 6 x 10^8 CFU/ml. At 28 days of age, each bird in the experimentally infected groups was inoculated orally with 1 ml containing 6 x 10^8 CFU *S. typhimurium*.

2.3 Probiotic (water-soluble PrimaLac® Star-Labs, USA). A commercial preparation consisted of *Lactobacillus acidophilus*, *L. casei*, *Bifido bacterium bifidium thermophilum*, *Streptococcus faecium*, Starch, Dextrose and Citric acid was administered in a dose of 12 g/100 liter dinking water in two programs. The first program was administrated for the first ten days of age. The second program was administrated continuously from first day of age until seven weeks.

2.4 Preparation of Local *S. typhimurium* Bacterin. The bacterin was prepared from *S. typhimurium* field strain according to **Timms et al. (1990)**. The prepared *S. typhimurium* bacterin contain 10^{11} CFU/ ml and tested for purity, complete inactivation, sterility and safety according to the Standard International Protocols as described by the **British Veterinary Codes (1970)**. 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. typhimurium* bacterin was given for the experimental chicken in two programs. The first program, it

was given in a single dose at the first day of age in a dose of 0.2 ml/bird. The second program, it was given in two doses at the first day of age in a dose of 0.2 ml/bird and boostered as a second dose at 14 days of age in a dose of 0.5 ml/bird. The bacterin was given by S/C route in the neck.

2.5 Preparation of *S. typhimurium* antigen: The *Salmonella* antigen was prepared according to the methods of **Williams and whittemore (1973)**.

2.6 Experimental Design. Three hundred and eighty, day-old chicks were used. At arrive randomly cloacal swabs were taken to assess they were free from salmonellae and randomly 20 chicks were sacrificed and cultured for salmonellae. All chicks were negative for salmonellae by culture. A completely randomized design was used as two replicates, each consists of 30 chicks and the groups were divided into six groups as the followings; **Group (1):** Negative control (non challenged-non treated birds). **Group (2):** Positive control (challenged-non treated birds). **Group (3):** A single dose vaccinated and *S. typhimurium* challenged birds. **Group (4):** Two doses vaccinated and *S. typhimurium* challenged birds. **Group (5):** First program PrimaLac® treated and *S. typhimurium* challenged birds. **Group (6):** Second program PrimaLac® treated and *S. typhimurium* challenged birds.

2.7 The measured Parameters

2.7.1 Clinical Signs, Mortalities and Gross Lesions. The birds were observed twice daily for three weeks post challenge till the end of the study for clinical signs of illness and mortality, and dead chicks were removed from units and necropsied for gross lesions and their organs cultured.

2.7.2 Monitoring of *ST* fecal shedding. Cloacal swabs were taken from birds in each group just before experimental infection (at 28 days of age) to ensure that the birds free from *S. typhimurium* infection and weekly after the challenge up to 7 weeks of age.

2.7.3 Evaluation of growth performance. During the experiment, birds were weighed and feed intake per each group was recorded weekly. Feed intake was determined for each group as the difference between the amount of feed supplied and the remaining feed at the end of each week. Body weight gain was calculated as the difference between the final and the initial bird weight. Feed conversion ratio (g food intake / g weight gain)

was calculated by dividing the amount of feed consumed (g) during the week by the gain in weight (g) during the same week (**Smith, 1999**).

2.7.4 Re-isolation of *S. typhimurium* from internal organs. Eight birds from each group post challenge were weekly randomly selected, sacrificed and the liver, spleen and caecum were collected for *S. S. typhimurium* re-isolation.

2.7.5 Serological test. Randomly twelve serum samples were collected just before first vaccination (at zero day), then were collected weekly up to 7 weeks of age. The separated sera were stored at -20°C till used. Serum samples were two fold serially diluted in the test. The antibody titer against *S. typhimurium* was determined using Micro agglutination test (MAT) according to **Williams and Whittemore (1971)**.

2.8 Statistical analysis: Mean values, standard errors and the degree of significance were calculated for the obtained data and by applying F-test using the SPSS computer program. Values have been calculated according to **Snedecor and Cochran (1989)**.

RESULTS AND DISCUSSION

The results of the present investigation revealed that there is a significant ($P \leq 0.05$) difference in the mortality rate between the challenged-non treated group and the vaccinated and probiotic treated groups as shown in table (1). The mortality rate was significantly higher in the challenged-non treated birds (13.3%) than other treated and control groups. No mortality was seen in chicks of groups (6) treated continuously with probiotic and negative control chicken. While, the mortality rate was (1.6, 5 and 6.6%) in double dose vaccinated, single dose vaccinated and ten days treated probiotic chicken, respectively. These results of vaccinated groups agreed with **Timms *et al.* (1990)** who subcutaneously administrated inactivated *S. enteritidis* PT4 bacterin at 3 weeks of age or at 3 and 6 weeks of age to specific pathogen free (SPF) chickens and they found that the vaccination protect the chickens against the massive challenges at either age. **Ghosh (1989)** reported that vaccination of broilers with *S. virchow* formalin killed bacterin reduced mortalities from 85 to 0%.

The protective efficacy of the probiotic against *S. enteritidis* infection was evaluated by **Wafaa et al. (2006 and 2012)** and **Nagah (2012)** they detected a significant decrease in mortality in *S. enteritidis* infected chicken and treated with probiotic than infected ones.

According to **Fuller (1997)** young chicks were protected by *Lactobacillus reuteri* against death associated with exposure to challenge with *S. typhimurium*. Also inoculation of *Enterococcus* spp. protected chicken against *Salmonella* challenge, due to the combined effects of lactic acid production and bacteriocins (**Audisio *et al.*, 2000**).

The frequency of fecal shedding of *S. typhimurium* from different treated groups was illustrated in table (2). The results declare that there are significant differences between the treated groups and the challenged- non treated one along three weeks post challenge. A gradual decrease in the shedding rate is observed within each group until reached the last week of observation period. The frequency of fecal shedding of *S. typhimurium* was significantly reduced from (69.6%) in group (2) of positive control chicken to 22.4 %, 33.9 %, 49.3 and 50% in continuously treated probiotic, double dose vaccinated, single dose vaccinated and ten days treated probiotic chicken, respectively. Moreover, the two programs of probiotic treatment reduce the fecal shedding with superiority of continuous treatment. These results are in agreement with **Deruyttere *et al*. (1997)** who reported that 24% of the control flocks were *Salmonella*-positive compared with none recovered from competitive exclusion treated flocks. Similarly, **Line *et al*. (1998)** reported a 50% reduction in yeast-treated birds compared with the positive control. Although the results indicated that the double vaccination were more protective than a single ones, yet **Liu et al. (2001)** and **Holt et al. (2003)** found that a single dose of *Salmonella* vaccine were significantly protective against *Salmonella* challenge. So, it was concluded that the two schedule of vaccination significantly diminish the incidence of fecal shedding of the challenge organism in comparison to the non vaccinated challenge group. **Gast et al. (1993)** and **Woodward et al. (2002)** concluded that *S. enteritidis* vaccine application diminish the incidence and duration of *Salmonella* shedding. Reduce fecal shedding will markedly reduce the overall level of environmental contamination and horizontal transmission of *S. typhimurium* within and between flocks.

The results of mean body weights (MBG), average feed intake (AFI) and feed conversion ratio (FCR) of the different treatment groups challenged with *St* at 28 days of age were presented in table (3). The measured parameters show significant improvement in the two programs vaccinated and two programs probiotic treated groups than challenged-non treated one along three weeks post challenge. The best MBG, AFI were observed in negative

control (115.1±2.3 and 498.3±25.5, respectively) and continuously treated probiotic chicken (115.8±1.2 and 501.4±27.4, respectively), while the worst one was in the challenged non treated chicken (94.1±0.89 and 447.9±15.4, respectively). Generally, FCR one week before challenge and three weeks post challenge was significantly improved from (4.76±0.74) in the challenged- non treated chicken to (4.38±0.76, 4.37±0.54, 4.35±0.78, 4.33±0.65 and 4.33±0.77) in ten days treated probiotic, single dose vaccinated, double dose vaccinated, negative control and continuously treated probiotic chicken, respectively.

These results are in agreement with that **Wafaa et al. (2012),** both vaccination of chicks with local prepared *S. enteritidis* vaccine or probiotic treated chicks significantly improve average body weight and cumulative feed conversion ration than infected-non treated or vaccinated chicks. In another study, **Mohrah and Zaki (1995)** demonstrated that vaccination of chickens with *Salmonella gallinarum pullorum* bacterin induced significant increase in the body weight of birds.

The role of probiotic in improvement of the growth performance was discussed previously by **Yang *et al*. (2009)** indicated that treatment of broilers, both challenged and non-challenged, with probiotic in combination with a prebiotic improved the performance parameters of the birds. Moreover, **Wafaa et al. (2006)** and **Rahimi et al. (2007)** demonstrated that probiotic enhanced the bird performance and relieve the growth depressing effect caused by *S. enteritidis* infection. Probiotics deliver many lactic acid bacteria into the GIT upon consumption. Enzymes and other beneficial substances are delivered into the intestines by these microorganisms which modifies the intestinal ileum (**Lutful Kabir, 2009**). Probiotic microbes and pathogenic bacteria start competing for nutrients. The growth of pathogenic microorganisms in the intestines is suppressed on the one hand and on the other the bioavailability to dietary minerals, growth rate and feed efficiency is increased. Lactobacilli bacteria ferment lactose to lactic acid which reduces the pH to a level that harmful bacterial cannot tolerate which favors increased activity for intestinal enzymes and digestibility of nutrients (**Choudhari et al., 2008**).

The results of the re-isolation rate of *S. typhimurium* from different treated groups were shown in table (4). These results indicate that along the whole three weeks post challenge, the highest and significant re-isolation rate was in the challenged non treated chicken (73.6%), while this rate was significantly lower in the ten days treated probiotic (50%) and the single

dose vaccinated chicken (41.7%) until it reaches its lowest and significant level in the double dose vaccinated (18.1%) and continuously treated probiotic chicken (9.7%). The results of **Bolder and Palmu (1995)** proved the possibility of *S. enteritidis* to become extra-intestinal and invade the liver one week post infection. Cecum was more frequently colonized by *S. typhimurium* than were other organs. These findings are in agreement with **Gast and Beard (1990)** they found that higher frequencies of S. *enteritidis* contamination occurred in gastrointestinal sites than nonintestinal sites. It was clear that chicken received two doses of *Salmonella* vaccines gave good protection against colonization of the challenge *S. typhimurium* in the internal organs compared to chicken received one dose of *Salmonella* vaccines. Both programs of vaccination gave good protection against colonization of the challenge *S. typhimurium* in the internal organs in comparison to nonvaccinated-challenged group, these results agree with (**Gast et al., 1992; Liu et al., 2001 and Woodward et al., 2002)**. But this was disagreed with **Clifton-Hadley et al. (2002)** they mentioned that no effect of vaccination upon colonization of internal organs after either high or low oral challenge by *S. Typhimurium*.

It was clear that the two programs of probiotic administration reduced the colonization of *S. typhimurium* in internal organs with superiority of continuously probiotic treated chicken. These are in consistent with **Nisbet *et al.* (1998)** they found that commercial-defined CE culture reduce cecal colonization by *S. gallinarum* also, **Vicente *et al.* (2008)** reported that the administration of either a liquid or lyophilized Lactobacillus based probiotic (FM-B11TM) in the drinking water may significantly reduced cecal colonization by *Salmonella enteritidis*. On the other hand, **Seuna et al. (1980)** found that supplementation of the birds with normal avian gut microflora didn't prevent or only partially prevent *Salmonella* colonization.

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 (**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**).

Results listed in table (5) show the titer of antibodies of probiotic treated, vaccinated and non-treated challenged chicken using microagglutination test at weekly interval from zero day until the end of experiment. The antibody titers in the first 4 weeks were recorded only in group 3 (one dosed vaccinated) and group 4 (double dosed vaccinated), while antibody titers were recorded in other probiotics and challenged groups after challenge. In vaccinated groups, the antibody titers rose quickly to reach peak levels at 2 weeks post vaccination, single dosed vaccinated group (5.9±0.22) and double dosed vaccinated group (5.3±0.65). By 4 weeks post first vaccination immediately before challenge, antibody titers of single dosed vaccinated group decreased to level (3.7±0.40) while, it increased in double dosed vaccinated group to level (6.3±0.33). A week after *S. typhimurium* challenge, antibody titers increases to reach (3.2±0.30, 5.7±0.51, 6.7±0.43, 4.5±0.24 and 6.4±0.28) in the challenged-non treated, single dosed vaccinated, double dosed vaccinated, ten days probiotic and continuously probiotic treated chicken, respectively. Moreover, two weeks post challenge; antibody titers were significantly higher in double dosed vaccinated (5.7±0.21) and continuously probiotic treated birds (6.9±0.26) than challenged-non treated (3.5±0.27). Vaccination against *S. enteritidis* provides protection in chickens by stimulating production of very high levels of humoral antibodies that reduce colonization of internal organs by *S. enteritidis*, (**Miyamoto *et al.*, 1999**). **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. However, despite the rapid production and secretion of *S. enteritidis* specific antibodies in the serum after vaccination, complete clearance of *S. enteritidis* from the internal organs and eggs was not observed (**Liu *et al.*, 2001**). Therefore, we considered that these produced antibodies could not completely prevent the colonization or dissemination of *S. typhimurium*. Humoral immunity alone was unlikely to protect fully against *S. typhimurium*. Total protection against *S. typhimurium* requires the induction of humoral and cellular immunity as well as other nonspecific immunities (**Babu *et al.*, 2003**).

The results concerning the immunopotantiation caused by probiotic administration especially continuous administration concur with **Kabir et al. (2004)** reported a significant higher antibody production in experimental broilers as compared to control one, which also assisted by **Koenen et al. (2004)** they found that probiotics and acidifier have appositive

effect on humoral and cellular immune responses in layer and meat type chicken species. On the other hand **Talebi et al. (2008)** found that inspite of probiotic improve the antibody responses to Newcastle disease virus and Infectious bursal disease vaccination but the antibody titers of the probiotic treated group were not significantly different from those not receiving probiotic. The positive effect of probiotic on the immune response indicates the enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes. The effect might be related to stimulate the lymphatic tissue (**Kabir et al., 2004**).

CONCLUSION

From this study, it could be concluded that both the locally prepared autogenous *S. typhimurium* bacterin either a single or double doses and the probiotic preparation either a specific period or continuous are effective and safe methods for prevention of *S. typhimurium* infection in broiler chicken and subsequently reduction in the incidence of meat contamination with *Salmonella* resulting in a reduction of the human health hazard. It is also clear that two doses of vaccination and continuous probiotic treatment are better than one dose of vaccine and a specific period of probiotic treatment. Good cleaning and disinfection are also required because there is still some contamination risk associated with the presence of *Salmonella* in infected flocks.

Table (1): Mortality rate of different treatment groups orally challenged with *St* at 28 days of age.

Groups	Total No. of birds	No. of dead birds /weeks post challenge			Total No. of dead birds	Mortality rate
		1st week	2nd week	3rd week		
(1) Negative control	60	0	0	0	0	0.0%[a]
(2) Positive control	60	5	3	0	8	13.3%[b]
(3) Single dose vaccinated challenged	60	2	1	0	3	5%[ac]
(4) Double dose vaccinated challenged	60	1	0	0	1	1.6%[ac]
(5) Ten days probiotic administrated-challenged	60	2	2	0	4	6.6%[c]
(6) Continuous probiotic administrated- challenged	60	0	0	0	0	0.0%[a]

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

Table (2): Recovery of *St* from cloacal swabs of different treatment groups orally challenged with *St* at 28 days of age.

Groups	No. of positive birds/Total No. of live birds						+ve/ Total	%
	Weeks post challenge							
	1st week		2nd week		3rd week			
	+ve/ Total	%	+ve/ Total	%	+ve/ Total	%		
(1) Negative control	0/60	0.0^{a}	0/52	0.0^{a}	0/44	0.0^{Aa}	0/156	0.0^{a}
(2) Positive control	45/55	81.8^{b}	29/44	65.9^{b}	20/36	55.6^{b}	94/135	69.6^{b}
(3) Single dose vaccinated-challenged	40/58	67^{c}	18/49	36.7^{cd}	15/41	36.6^{c}	73/148	49.3^{c}
(4) Double dose vaccinated-challenged	30/59	50.9^{d}	15/51	29.4^{c}	7/43	16.3^{d}	52/153	33.9^{d}
(5)Ten days probiotic treated-challenged	36/58	62.1^{c}	21/48	43.8^{d}	16/40	40^{c}	73/146	50^{c}
(6) Continuous probiotic treated-challenged	20/60	33.3^{e}	10/52	19.2^{e}	5/44	11.4^{d}	35/156	22.4^{e}

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

Table (3): Mean weight gain, Average feed intake and Feed conversion ratio of different treatment groups challenged with *Salmonella typhimurium* at 28 days of age.

Parameters	Days of age	Groups					
		1	2	3	4	5	6
Mean weight gain (g)	21-28	95.8±1.3^{a}	97.3±0.66^{a}	96.3±0.68^{a}	94.7±045^{a}	100.2±0.89^{a}	101.8±0.48^{a}
	28-35	112.3±1.4^{a}	90.5±1.2^{b}	110.3±1.6^{a}	111.3±1.3^{a}	107.3±0.67^{a}	111.3±1.7^{a}
	35-41	122.8±2.1^{a}	95.8±1.6^{b}	114.2±1.3^{ac}	120.2±1^{a}	107.8±0.94^{c}	121.8±0.68^{a}
	42-49	129.4±2.6^{a}	110.8±1.8^{b}	122.2±1.6^{a}	126.7±1.8^{a}	121.3±2.4^{a}	128.6±2.3^{a}
	21-49	115.1±2.3^{a}	94.1±0.89^{b}	110.7±1.7^{a}	113.2±1.1^{a}	109±1.3^{a}	115.8±1.2^{a}
Average feed intake (g)	21-28	373.6±9.8^{a}	378.4±11^{a}	377.5±8.3^{a}	370.2±9.6^{a}	389.6±10.4^{a}	394.9±10.6^{a}
	28-35	462.7±18.3^{a}	402.7±19.6^{b}	459.9±15.7^{a}	461.8±14.7^{a}	450.6±18.3^{a}	459.6±19.5^{a}
	35-41	556.2±22.1^{a}	456±28.6^{a}	525.3±23^{ac}	549.3±30^{a}	498±26.6^{c}	553±24.6^{a}
	42-49	619.2±22.6^{c}	550.6±32.8^{b}	587.7±38^{ac}	608±42.3^{ac}	585.2±34.5^{c}	613.4±34.2^{a}
	21-49	498.3±25.5^{a}	447.9±15.4^{b}	483±26.8^{ac}	492±19.5^{ac}	475.6±23.3^{c}	501.4±27.4^{a}
Feed conversion ratio (FCR)	21-28	3.9±0.66^{a}	3.89+±0.67^{a}	3.92±0.56^{a}	3.91±0.45^{a}	3.89±0.34^{a}	3.88±0.52^{a}
	28-35	4.12±0.87^{a}	4.45±0.58^{b}	4.17±0.67^{a}	4.15±0.79^{a}	4.2±0.66^{a}	4.13±0.67^{a}
	35-41	4.53±0.77^{a}	4.76±0.89^{b}	4.6±0.79^{a}	4.57±0.92^{a}	4.62±0.87^{a}	4.54±0.66^{a}
	42-49	4.79±0.87^{a}	4.97±0.76^{b}	4.81±0.93^{a}	4.8±0.88^{a}	4.83±0.85^{a}	4.77±0.92^{a}
	21-49	4.33±0.65^{a}	4.76±0.74^{b}	4.37±0.54^{a}	4.35±0.78^{a}	4.38±0.76^{a}	4.33±0.77^{a}

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

Table (4): The re-isolation rate of *Salmonella typhimurium* from internal organs of different treatment groups orally challenged with *Salmonella typhimurium* at 28 days of age.

	Groups	Weeks post challenge												Total (%)
		1st week				2nd week				3rd week				
		Organ culture												
		L	S	C	T (%)	L	S	C	T (%)	L	S	C	T (%)	
1	Negative control	0/8	0/8	0/8	0/24(0.0)^{a}	0/8	0/8	0/8	0/24(0.0)^{a}	0/8	0/8	0/8	0/244 (0.0)^{a}	0/72 (0.0)^{a}
2	Positive control	6/8	6/8	8/8	20/24(83.3)^{b}	6/8	4/8	8/8	18/24(75)^{b}	4/8	4/8	7/8	15/24 (45.8)^{b}	53/72 (73.6)^{b}
3	Single dose vaccinated challenged	5/8	3/8	5/8	13/24(54.2)^{c}	4/8	3/8	3/8	10/24(41.6)^{c}	2/8	2/8	3/8	7/24 (29.2)c	30/72 (41.7)^{c}
4	Double dose vaccinated challenged	3/8	1/8	5/8	9/24(37.5)^{cd}	1/8	0/8	2/8	3/24(12.5)^{d}	0/8	0/8	1/8	1/24(4.2)^{d}	13/72 (18.1)^{d}
5	Ten days probiotic administrated challenged	5/8	4/8	5/8	14/24(58.3)^{c}	5/8	3/8	5/8	13/24(54.2)^{c}	3/8	2/8	4/8	9/24(50)^{b}	36/72 (50)^{c}
6	Continuous probiotic administrated challenged	1/8	1/8	3/8	5/24(20.8)	0/8	0/8	1/8	1/24(4.2)^{d}	0/8	0/8	1/8	1/24(4.2)^{d}	7/72 (9.7)^{d}

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

Liver (L). Spleen (S). Cecum (C).

Table (5): Micro agglutination antibody titers in the sera of different treatment groups orally challenged with St at 28 days of age.

Groups	Interval							
	Before vaccination	Weeks post vaccination				Weeks post challenge		
	Zero day	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week
1	0.0	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
2	0.0	0.0±0.0^{a}	0.1±0.12^{a}	0.0±0.0^{a}	0.0±0.0^{a}	3.2±0.30^{b}	4.6±0.34^{b}	3.5±0.27^{b}
3	0.0	2.7±0.36^{b}	5.9±0.22^{b}	4.5±0.35^{b}	3.7±0.40^{b}	5.7±0.51^{c}	4.9±0.46^{b}	4.5±0.58^{c}
4	0.0	3.1±0.65^{b}	5.3±0.65^{b}	6.4±0.89^{c}	6.3±0.33^{c}	6.7±0.43^{d}	6.4±0.23^{c}	5.7±0.21^{d}
5	0.0	0.0±0.0^{a}	0.33±0.47^{a}	0.0±0.0^{a}	0.0±0.0^{a}	4.5±0.24^{c}	4.3±0.22^{b}	3.7±0.35^{bc}
6	0.0	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	6.4±0.28^{d}	6.9±0.41^{c}	6.9±0.26^{e}

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

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الملخص العربى

دراسات مقارنة على تأثير البروبيوتك و اللقاح الذاتى الخامل على عدوى سالمونيلا تيفوميوريم فى الدجاج

نجاح عرفات[1] و ريهام الشافعى [2]

[1]قسم أمراض الدواجن، كلية الطب البيطرى، جامعة المنصورة

[2]قسم ألأدوية، كلية الطب البيطرى، جامعة المنصورة

لقد أجريت هذه الدراسة لبحث كفاءة ومقارنة كلا من اللقاح الخامل المحضر معمليا من سالمونيلا تيفوميوريم و البروبيوتيك التجارى (بريمالاك) فى حماية الكتاكيت من عدوى السالمونيلاالتيفوميوريمية. تم عمل التجربة على ٣٨٠ كتكوت عمر يوم ، ثم تم ذبح ٢٠ كتكوت لفحصها بكتيريولوجيا لضمان خلوهم من عدوى السالمونيلاالتيفوميوريمية. ثم تم تقسيم ٣٦٠ كتكوت بشكل عشوائى الى ست مجموعات متساوية مع مكرراتان لكل مجموعة (٣٠ كتكوت فى كل تكرار). الكتاكيت فى المجموعة (١) بقيت غير متحداة و غير معالجة (الكتاكيت الضابطة)، فى حين أن المجموعة (٢) كانت متحداة و غير معالجة. المجموعة (٣) تم تحصينها باعطائها جرعة واحدة تحت الجلد فى الرقبة فى اليوم الأول من العمر بجرعة ٢،. مل/ طائر. أما المجموعة (٤) تم تحصينها باعطائها جرعتين الجرعة الأولى فى اليوم الأول من العمر بجرعة ٢،. مل/ طائر و الجرعة الثانية عند عمر ١٤ يوم بجرعة ٥،. مل/ طائر، بينما المجموعة (٥) أعطيت بروبيوتيك تجارى (بريمالاك) لمدة محددة بجرعة ١٢جم/ ١٠٠ لتر فى مياه الشرب من اليوم الأول من العمر ولمدة ١٠ أيام متتالية، فى حين أن المجموعة (٦) أعطيت بريمالاك باستمرار بجرعة ١٢جم/ ١٠٠ لتر فى مياه الشرب من اليوم الأول من العمرحتى ٧ أسابيع. تم عمل عدوى صناعية لمجموعات ٢، ٣، ٤، ٥ و ٦ بواسطة سالمونيلا تيفوميوريم عن طريق الفم فى عمر ٢٨ يوم بجرعة ١ مل تحتوى على ٦ X 10^{8} وحدة مستعمرة سالمونيلا تيفوميوريم. تم ابقاء كل المجموعات تحت الملاحظة لمدة ٣ أسابيع بعد العدوى الصناعية لتسجيل الأعراض، الوفيات، الصفة التشريحية، نسبة الأفراز، معدل أداء النمو وأيضا معدل استعادة عزل الميكروب من الكبد، الطحال و الأعورين وكذلك الكشف عن عيار الأجسام المضادة مصليا باستخدام اختبار التراص المجهرى. أظهرت النتائج أن استخدام كلا من التحصين بجرعة واحدة أو بجرعتين و البربيوتيك لفترة محددة أو مستمرة أدت بشكل كبير للحد من الأعراض، الوفيات، الصفة التشريحية، نسبة الأفراز، معدل استعادة عزل الميكروب من الكبد، الطحال و الأعورين و كذلك زيادة فى أداء الطيور بالمقارنة مع الطيور المتحداة و الغير معالجة. علاوة على ذلك، وجد تحسن ملحوظ فى عيار الأجسام المضادة فى المجموعات المحصنة بجرعتين و المعالجة باستمرار بالبروبيوتك. وفى الختام، تحصين اللقاح الخامل سواء مرة واحدة أو مرتين و أيضا استخدام البروبيوتك سواء لفترة محددة أو باستمرار أعطى كفاءة جيدة مع تفوق التحصين بجرعتين والعلاج المستمر بالبروبيوتك.