Global Animal Science Journal-GASJ 1(4):78-85, 2014; DOI:10.5281/zenodo.26159
Eldemerdash et al 2014; Research Article

STUDY ON THE EFFICACY OF SYNBIOTICS IN THE PREVENTION OF SALMONELLA TYPHIMURIUM IN CHICKENS

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Submitted on 15th January 2014, accepted on 14th April 2014, published on 1st July 2014

Abstract

One hundred and twenty (day-old Cubb) chicks were equally divided into 6 groups (20 chicks per each).Group (1) kept as control negative(non treated), Group (2,5) treated with lactic acid (1% in drinking water in 1st week then decreased to 0.5 % all over the experimental period), Group(3,6) treated with symbiotic ($1x10^8$ CFU in drinking water during entire period of rearing) Group 4, 5 and 6 were orally infected with *S.* Typhimurium (Streptomycin-resistant strain) with infective dose $1X10^8$ CFU at 4th day old. Quantitative and qualitative bacterial colonization were reduced in treated and infected groups. Poultry Star® showed higher reduction colonization rate followed by Lactic acid compared to non-treated group. Hemaglutination-inhibition test (HI) against Newcastle disease (ND) vaccines showed an increase in the antibody titers in Poultry Star® treated groups (3, 6). Furthermore Poultry Star®was capable of enhancing performance, decreasing re-isolation rate of S. Typhimurium either from cloacal swaps and/or from (Liver and spleen). It could be conclude that Synbiotic and organic acid have great value on poultry production as growth promoter by either enhancing performance or reduction the intestinal colonization with S. Typhimurium as a model of pathogenic bacteria and improving the immune response.

Key words: *S.* Typhimurium, Synbiotic, Organic acids, Poultry Star®, broiler production.

Introduction

Salmonella is considered as one of the important causative agents, which infect poultry farms causing a variety of acute and chronic diseases with significant economic losses to poultry producers **Gast (2003).** *Salmonella* typhoid and paratyphoid caused by several species of *Salmonella* which recognized as important health hazard for human. Unfortunately, poultry meat is the major source of food borne paratyphoid infection which recorded by **Mayrhofer et al., (2003) and Murugkar et al., (2005). Dahiya et al., 2006** had created a need to find alternatives to maintain healthy other than using antibiotics in food producing animals. The most alternative additives include probiotics, prebiotics, synbiotics and organic acids **(Doyle 2003)**. Synbiotics are relationship between a prebiotic substance and a probiotic organism suggests synergism allow the balance of the gut micro ecology in favor of beneficial bacteria over other potential pathogens **Schrezenmeir and Vrese (2001)** and **Awad et al.,(2011**). Organic acids found as alternative to antibiotics through acidification of the water, which reduce colonization of *Salmonella* (**Byrd et al., 2001**and **Jarquin et al., 2007**). The study aimed to clarify the role of either Synbiotic or prebiotic as an alternative to antibiotic against S. Typhimurium infection.

Material and Methods

One hundred and twenty (day-old Cubb) chicks were equally divided into 6 groups (20 chicks per each). Group (1) kept as control negative (non treated), Group (2,5) treated withlactic acid (1% in drinking water in 1st week then decreased to 0.5 % all over the experimental period), Group(3,6) treated with synbiotic ($1x10^8$ CFU in drinking waterduring entire period of rearing), Group 4 kept as control positive (infected).Group 4, 5 and 6 were infected orally at 4th day old with 0.1ml containing $1X10^8$ CFU/ bird of *S.* Typhimurium(Streptomycin- resistant strain). All birds were Vaccination via eye

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drop with -HitchnerB1 (8 th day old) -La Sota 18th day old- Gumboro 14th -24th .Vet. Ser. and Vacc. Res. Inst-Cairo- Egypt. Three birds were slaughtered and blood samples collected / group weekly during entire period of rearing.

***Salmonella* Typhimurium** strain (1.4.5.12: i: 1. 2) was kindly obtained from laboratories of the Ministry of health, Cairo. *S.* Typhimurium streptomycin resistant strain **(Saad et al., 1974).** Experimental dose (1 x 10^8 CFU/0.1ml) were inoculated into infected groups (4, 5 and6) at 4th day.

Synbiotic: Poultry Star® (BIOMIN INC.) Containing (1x10^8 CFU/g of *Enterococcus faeciumm, Pediococcusacidilactici, Bifidobacteriumanimalis, Lactobacillus reuteri, Lactobacillus salivarius*) and prebiotic fructo-oligosaccharides (FOS) derived from a natural plant source of the family (Cichoriumintybus).

Lactic acid 88.65% (El Nasr pharmaceutical chemicals Co. abuZaabal)

- Clinical signs, gross lesions, mortalities and performance checked routinely
- Bacteriological studies:

a- Total bacterial count (**Elsayed, 2002**).

b- Re-isolation of *S.*Typhimurium (**USDA/FSIS 2004**).

- Shedding of *Salmonella* Typhimurium resistant strain post-infection (**Bjerrum et al., 2003**)
- Serum antibody titers against NDV were measured by the Hemaglutination inhibition test (HI) according to (**Cunningham 1971**)
- Statistical analysis according to **Snedecor and Cochran (1980)**

Data for all responses variable in the experiment were subjected to analysis of variance (**one-way ANOVA**) using constant statistical software.

Results

Clinical symptoms were observed at the 3rd day PI showed anorexia, general depression, loss of appetite, sleepy appearance, dullness, ruffled feathers, huddle together and white diarrhea and or pasted vent. These findings were clear in infected group (4) followed by Lactic acid infected group (5), while, the least signs were showed in Poultry star® infected group (6). Similar finding were detected in morbidity. At the same time, mortalities was recorded only in group (4) with 5% (1/20 chicks) at the 14th day PI.

Post-mortem (PM) lesions were recorded at 1st week showed congestion in the carcasses and internal organs with engorged blood vessels. Enteritis and ballooning of the intestine were recognized, while at 2nd and 3rd week liver and spleen were severely congested, enlarged, friable texture, enlarged gall bladder and sever pericarditis. Lesions were clear in group (4) compared to group (5), while the least lesions were in group (6).

Performance parameters (mean body weight (MBW) / (gm): Non infected Groups (1, 2, 3) were superior in MBW over the other infected groups (4, 5, 6) during the entire period of the experiment. Group (6) was the superior in MBW over the other infected groups (4 and 5) during the entire period of the experiment, at the same time, it showed insignificant decrease in MBW compared to control negative (group 1) at 7th and 42nd days old (Table 1).

Quantitative and qualitative bacterial colonization through cecal content and organs re-isolation. Group (4) showed significant higher rate of S. Typhimurium re- isolation from cecal content followed by group 5 while group (6) significantly the least rate of isolation (Table 2). While Re-isolation rate of *S.* Typhimurium resistant strain from liver and spleen detected that group (4) had the highest rate of re-isolation from spleen and liver by 80% , 73.3% respectively, followed by group (5) with 60 % ,53, 3% respectively and the least recovery was recorded for group (6) with 40% and 53.3% respectively.**(Table 3, 4).** Non-Infected groups (1, 2 and 3) showed negative re-isolation from cecal content, liver, spleen and heart. Shedding frequency of *S.* Typhimurium resistant strain post-infection (PI): the shedding of *S.* Typhimurium resistant strain at 3rd day

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post infection were 100% in all infected groups, While in group (6) starting to diminish from 6th day PI till it disappeared completely at 34th day PI. Meanwhile, group (5) began to decrease shedding from 13th day PI until it disappeared completely at 38th day PI. Whilst, the *S.* Typhimurium resistant strain was still continually shedding in group (4) till the end of observation period at day 38th with 25%(Fig 1). The *S.* Typhimurium (resistant strain) shedding percentage were 73%, 52.73% and 40% from groups (4, 5 and 6) respectively, while, no *S.* Typhimurium shedding was recorded in non-infected groups (1, 2 and 3).

HI antibody titer against Newcastle disease vaccine showed insignificant increase in antibody titer against Newcastle disease vaccine in-group (3) compared to groups (1 and 2). While in infected groups, Group (6) showed insignificant increase compared to group (4) followed by group (5) during the entire experimental period. **(Table 5)**

Table (1): Mean body weight of treated groups (lactic acid and poultry star®) and or infected with (1X10^8 CFU) *S.* Typhimurium on 4th day old:

Groups	Age /days					
	7th	14th	21st	28th	35th	42nd
Group 1	126.2^a ±1.78	279.05^b ±7.79	637.57^b±8.4	1081.6^b±33.9	1779.29^b±42.2	2513.50^b ±50.5
Group 2	107^b ±.93	272.23^b ±11.96	637.14^b±7.4	1122.9^b±26.4	1830.5^b±35.7	2593.20^b ±48.3
Group 3	132.4^a ±2.2	316 1^a ±5.6	701.4^a±13.3	1280.1^a±28.6	2030.5^a±40.4	2834.04^a±54.3
Group 4	116.1^b±2.6	226.7^c±6.2	469.9^d±12.6	806.54^d±27.3	1258.25^d±37.5	1879.33^d±45.9
Group 5	87.5^c±2.97	206.9^c±7.2	453.4^d±12.8	824^d±28.8	1306.25^d±34.9	1955.33^d±43.2
Group 6	126.2^a±1.98	255.8^b±10.1	523.1^c±13.8	956.7^c ±30.3	1504.75^c±39.4	2211.59^c±49.2

Group 1 (non-infected), Group 2 (lactic acid treated), Group 3 (poultry star® treated) Group 4(control infected), Group 5(lactic acid treated- infected), Group 6 (poultry star® treated- infected). [(a- c)]Means within the same column carrying different superscripts are significant at (p≤ 0.05).

Table (2): Total *S.* Typhimurium (resistant strain) count from cecal contents of treated and/ or infected with (1X10^8 CFU) *S.* Typhimurium on 4th day old: (x 10^6 /gm cecal contents).

Group	Age/days				
	7th	14th	21st	28th	35th
Group1	0	0	0	0	0
Group 2	0	0	0	0	0
Group 3	0	0	0	0	0
Group 4	20^a ± 2.88	2^a± .577	15^a ± 1.73	0.15^a ± 0.011	0.08^a ± 0.0057
Group 5	8^b± 1.154	1^b ± 0.288	5^b ± 0.11	0.06^b ± 0.005	0.05^b ± 0.0057
Group 6	0.8^c ± 0.057	0.2^c ± 0.059	0.5 ± 0.078	0.01^c ± 0.007	0.008^c ± .00045

Group 1 (non-infected), Group 2 (lactic acid treated), Group 3 (poultry star® treated) Group 4(control infected), Group 5(lactic acid treated- infected), Group 6 (poultry star® treated- infected). [(a- c)]Means within the same column carrying different superscripts are significant at (p≤ 0.05)

Table (3):Re-isolation of *S.*Typhimurium from liver and spleen of treated and infected groups (PI):

Groups	Age/days													
	1st		7th		14th		21st		28th		35th		Total	
	N0.	%	N0.	%	N0.	%	N0.	%	N0.	%	N0.	%	N0.	%
Group 1	0/5	0 %	0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/15	0%
Group 2			0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/15	0%
Group 3			0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/15	0%
Group 4			3/3	100%	3/3	100%	2/3	66.6%	2/3	66.6%	1/3	33.3%	11/15	73.3%
Group 5			3/3	100%	2/3	66.6%	2/3	66.6%	1/3	33.3%	0/3	0%	8/15	53.3%
Group 6			2/3	66.6%	2/3	66.6%	1/3	33.3%	1/3	33.3%	0/3	0%	6/15	40%

Group 1 (control non infected), Group 2 (lactic acid treated- non infected), Group 3 (poultry star® treated- non infected) Group 4 (control infected), Group 5 (lactic acid treated- infected), Group 6 (poultry star® treated- infected).

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Table (4): Re-isolation of *S.*Typhimurium from spleen of non-infectedand infected treated groups post infection:

Groups	Age/days													
	1st		7th		14th		21st		28th		35th		Total	
	no	%	N0.	%	N0.	%	N0.	%	N0.	%	N0.	%	N0.	%
Group 1	0/5	0 %	0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/15	0%
Group 2			0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/15	0%
Group 3			0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/3	0%	0/15	0%
Group 4			3/3	100%	3/3	100%	3/3	100%	2/3	66.6%	1/3	33.3%	12/15	80%
Group 5			3/3	100%	3/3	100%	2/3	66.6%	1/3	33.3%	0/3	0%	9/15	60%
Group 6			3/3	100%	2/3	66.6%	2/3	66.6%	1/3	33.3%	0/3	0%	8/15	53.3%

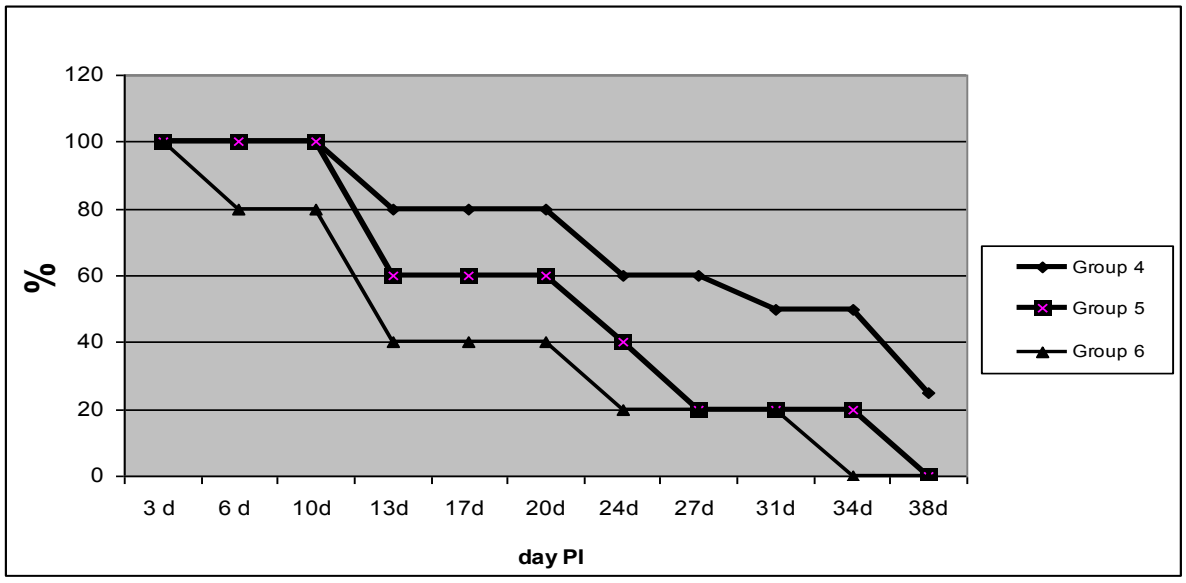
Group 1 (control non infected), Group 2 (lactic acid treated- non infected), Group 3 (poultry star® treated- non infected) Group 4 (control infected), Group 5 (lactic acid treated- infected), Group 6 (poultry star® treated- infected).

Table (5): antibody titer against Newcastle disease vaccine of lactic acid and poultry star® experimentally infectedgroupswith (1X10^8 CFU) *S.* Typhimuriumon 4th day old

	Age/ days					
Groups	0 day	7th day	14th day	21th day	28th day	35th day
Group 1	5± 0.447	3.66^a ± 0.33	2.66^a ± 0.33	3.66^a ± 0.33	4.33^a ± 0.66	4.66^a± 1.2
Group 2		3.33^a± .33	3^a ± 1	4^a± .57	5^a± .881	5.33^a±.33
Group 3		4^a ± .57	3.66^a ± .33	4.33^a ± .66	5.66^a ± .88	5.66^a ± .33
Group 4		3.33^a ± 0.33	2^a ± 0.577	2.6^a± 0.33	3.33^a ± 0.66	3.33^a± 0.33
Group 5		3.66^a ± 1.4	2.66^a ± 1.2	3^a± 0.57	3.66^a ± 0.33	3.66^a± 0.881
Group 6		3.66 ^{a}± 0.66	3 ^{a}± 1	3.6^a ± 0.33	4.3^a± 0.88	4.6^a ± 0.33

Group 1 (control non infected), Group 2 (lactic acid treated- non infected), Group 3 (poultry star® treated- non infected) Group 4(controlinfected), Group 5(lactic acid treated- infected), Group 6 (poultry star® treated- infected). [(a- c)]Means within the same column carrying different superscripts are significant at (p≤ 0.05).

Fig (1): Shedding frequency of *S.* Typhimurium resistant strain of infected groups post infection.



Discussion

Poultry industry has always been confronted with challenges in the form of various diseases, which led to increased use of antibiotics for therapeutic, prophylactic and growth promotion purposes. The presence of antibiotic residues in poultry meat and eggs may have deleterious effects on human consumers. Several alternatives to antibiotic as growth promoters have been proposed for example probiotics (**Corcionivoschi et al., 2010)** and organic acids (**Král et al., 2011**). All chicken groups experimentally infected with *S.*

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Typhimurium showed similar symptoms to those described by (**Marthedal 1977; Barrow et al., 1987 and Gast 2003)**. These findings were clear in infected untreated control (group 4), while it was reduced in lactic acid infected (group 5) and limited in Poultry Star® infected (group 6). These results might attribute to using lactic acid and Poultry Star®, which is able to minimize the drastic effect of experimental infection with *salmonella*. Similar finding were reported by **Zohair (2006**) who found that treated birds with acidifier could minimize symptoms and mortalities as well as reduction in microbial shedding and colonization.

Typical *S.* Typhimurium gross lesions recorded in dead infected and sacrificed chicks were similar to those described by (**Padron 1990; Gast 2003 and Lister and Barrow2008)**. PM lesions were clear in infected untreated control (group 4), less in lactic acid infected (group 5) and limited in Poultry Star® infected (group 6) which prove that synbiotic treatment was the best one for reducing S. Typhimurium infection. The for mentioned results agreed with **Lister (1988)** who reported that the lesions were markedly severe in infected untreated broilers than probiotic treated one which was explained by colonization and proliferation of pathogen in GIT decrease by using probiotics (**Vachkov et al., 2004).** Poultry star® treated (group 3) gave better results of MBW compared with lactic acid (group 2) over untreated control (group 1) along experimental period, these results were in accordance with **Mountzourisetal., 2007; Ashayerizadeh et al., 2009 Mountzourisetal., 2010** and **Taheri et al., 2010)** who detected that the best performance characteristics found in broilers receiving a *Lactobacillus* probiotic during growing period. Orally challenged chicks with *S.*Typhimurium (resistant strain) revealed significant decrease in the MBW in infected untreated (group 4) compared to uninfected untreated (group 1) that attributed to severity of the *S.*Typhimurium infection on growth performance parameters. Our findings were in agreement with **(Hegazy and Adachi 2000; Chalghoumi et al., 2009** and**Vandeplas et al., 2009).** Re-isolation of *S.* Typhimurium from liver and spleen was 0% at day 35th old in chickens supplemented with both Poultry Star®and lactic acid infected groups, while it was 33.3% from liver and spleen in infected untreated control (group 4) which reflected that all treatments able to diminished *Salmonella* colonization in organs, this results agreed with **Gehad el al., (2011**) who re-isolated *S.* Typhimurium organism from the same organs 4 weeks post challenge with (0%) in prebiotic and probiotic treated groups.

*S.*Typhimurium was recovered with highest percentage from spleen & liver in infected untreated (group 4) with 80% and 73.3% respectively, followed by lactic acid infected (group 5) with 60 % and 53, 3% respectively while the least *Salmonella* recovery was recorded in poultry star® infected (group 6) with 40% and 53.3% respectively, This was committed with the results of (**Marcqet al., 2011**) who reported that, using of probiotic and/or prebiotic reduced the percentage of *S.*Typhimurium re-isolation compared with untreated group.

Total *S.* Typhimurium count was significantly lower in Poultry Star® infected (group 6) followed by lactic acid infected (group 5) when compared to infected untreated (group 4).These findings were similar to results obtained by **Fukata et al., (1999)** who reported that, application of FOS alone or in combination with probiotic significantly reduced *S.* enteritidis count compared to control group. **Klose et al., (2006)** stated that**,** poultry star® inhibited *S.* Typhimurium growth in vitro. By adding nutritional substrates known as prebiotics to the selected probiotic strains. These substrates are not digested by poultry and cannot be utilized by pathogenic bacteria but used as a nutritional source for

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production of acids that decrease the luminal pH rendering the environment inhospitable for *Salmonella.* Similar results were detected by **Yan et al., (2011)** who suggested that preibiotic blocking the pathogen binding sites in the gastrointestinal tract.

In Fig (1) showed that shedding of *S.* Typhimurium (resistant strain) was highly diminished in Poultry Star® infected (group 6) starting from 6th day PI till it completely disappeared at 34th day PI. Meanwhile, lactic acid infected (group 5) showed decreased shedding rate starting from 13th day PI till it stopped completely at 38th day PI. While, in infected untreated (group 4) continue shedding (till the end of observation period) nearly similar finding were detected by **Gehad el al., (2011**) who found that synbiotic prevent shedding of the *S.* Typhimurium organism in the first 14 days post infection and gave better results when compared with untreated. **Zohair (2006)** reported that treated birds with acidifier could minimize microbial shedding and colonization could be a result of antimicrobial effect of different acidifiers and beneficial effect on cells of gastrointestinal tract.

Antibody titer against Newcastle disease vaccine showed insignificant increases Poultry star® treated (group 3) in compared to untreated control (group 1) nearly similar results recorded by **Talebi et al.,(2008)** and **Sohail et al., (2010)**. Untreated infected (group 4) showed decreased HI antibody titer against ND vaccine than the untreated uninfected (group 1) these results attributed to drastic effect of *Salmonella* infection on relative weights of immune organs, which decrease immune response against vaccination, similar finding were detected by **Ali et al., (2013).**

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