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PROTECTING NEWLY HATCHED BROILER CHICKS AGAINST INFECTION WITH SALMONELLA TYPHIMURIUM

BY

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ABSTRACT: *This study was conducted to evaluate the protection effectiveness of Lactobacillus and/ or lactose against Salmonella typhimurium (S.typhimurium) colonization in newly hatched chicks. One hundred ninty five Hubbard broiler chicks were divided into five treatment groups (of 3 replicates, 13 chicks each). All groups were inoculated on the 3rd day of age with 10^{6} colony forming unit (cfu) S.typhimurium/ chick, except the first group was a negative control. Group 3 and 5 received 10^{9} cfu lactobacillus/ chick on the first day of age. However, group 4 and 5 drank water contained 2.5% lactose from hatch day till the end of the study.*

Challenge by S.typhimurium resulted in chicks with significant (P<0.05) low market body weight, caecal volatile fatty acids (VFA) and lactic acid concentrations as well as impaired liver and kidney function (as reflected from blood analysis). Infected chicks also recorded significant high caecal count of S.typhimurium and pH value. Chicks recieved lactobacilli and/ or lactose were protected against the pathogenic effect of Salmonella as shown by significant increases in market weight, ceacal VFA and lactic acid values(it was most pronounced in those received lactose)as well as, significant decreases in pH value. Chicks treated with either probiotic or prebiotic or both of them also recorded significantly the lowest levels of blood contentes which indicated the normal liver and kidney functions (such as AST, ALT, uric acid, billirubin, triglyceride, cholesterol levels and H/ L ratio).

In conclusion, these results indicated that using lactobacillus and lactose in broiler flocks was most beneficial in reducing the prevalence of Salmonella by modulating susceptibility of chicks for it , and the consequent elimination and/ or prevention of incidence human Salmonellosis.

INTRODUCTION

Poultry are recognized as one of the primary sources for transmitting Salmonella species to humanbeing, 40% of the clinical cases were attributed to consumption of contaminated poultry products **(Sancheze *et al.*, 2002)**. Approximately, 40,000 cases of human Salmonellosis diagnosed annually in U. S. A, resulted in approximately 600 deaths **(Mead *et al.*, 1999)**. Salmonella has an economic effect due to costs associated with disease investigation, health care, loss of productivity and death in addition costs to the poultry industry by threatening consumer markets and increasing production and processing costs **(McMeekin *et al.*, 1993 and Bender and Mallinson, 1991)**. Implementing strategies to reduce, eliminate or prevent such infection are the key to reducing the prevalence of Salmonellosis **(Ned *et al.*, 2007)**.

Barnes *et al.* (1979) found that broiler chicks are more susceptible to Salmonella than adult chickens. They attributed these finding to the lower value of volatile fatty acids (VFA) as well as to the high pH of the intestine in newly hatched chicks compared to those of adult ones. Recent evidence that various dietary and microbial supplements can influence host immunity against enteric diseases **(Lee *et al.*, 2007)**. Lactobacilli and lactose might be a new hurdle in the strategy to control Salmonella in broiler flocks, make chicks less susceptible to infection with Salmonella **(Heres *et al.*, 2003)**. Either Lactobacilli or lactose exert their effectiveness to hamper the growth of Salmonella through the improvement of VFA activity. Intestinal Lactobacillus of mature chickens produce short chain VFA to provide resistence to gastro intestinal infection by Salmonella. However, lactose lowed the pH of chicks caeca which is more important to increasing the undissociated VFA that have antibacterial activity as well as, lactic acid level that is detrimental for survival of Salmonella and can hinder its multiplication **(Russel and Diez Gonzalez, 1998 and Alakomi *et al.*, 2000)**.

On the other hand, **Miyamoto *et al.*, 2000 and Kizerwetter- Swide and Binek, 2005)** illustrated that lactobacilli protect against the introduction of Salmonella in broiler flocks by blocking the binding sites for Salmonella adhesion to epithelial cells of intestine.

The aim of this study was to evaluate the protective effectiveness of Lactobacilli and/ or lactose in broiler flocks, fed on contaminated diet as reflected by modulating, eliminating or preventing the pathogenic effects of Salmonella typhimuruim on: Market weight, some hematological and physiological traits and caecum contents.

MATERIALS AND METHODS

This study was carried out at Poultry Nutrition Farm, Department of Poultry Production, Faculty of Agriculture, Ain Shams University.

Experimental Flock:

One day- old Hubberd broiler chicks were randomly brooded in brooding batteries as five groups of 39 chicks (3 replicates of 13 chicks each). They were maintained in a 23L: 1D light cycle. Chicks were fed commercial starter diet (days 1- 21) and grower diet (days 22- 50). The experimental diets were planned to meet the nutrient requirements of broilers as recommended by **NRC (1994)**. Feed and water were provided *ad libitum* and weekly body weight was recorded.

Experimental procedure:

Random samples from sources of water, diets and litters, as well as caecal contents of the adult broilers which were taken to be used for isolation of lactobacillus were tested before starting of the study to prove their free from Salmonella.

Salmonella typhimurium used for colonisation of young chicks was obtained from Animal Health Research Center, Giza, Egypt. The suspension was prepared according to the method described by **Bailery *et al*. (1988)**. Challenge dose was adjusted to ensure a final estimat of 10^6 cfu / bird and administered orally on day three of age. Lactobacillus (Native microflora) for S.typhimurium exclusion was isolated from caecal contents of 3 adult broilers according to the method of **Hinton *et al.* (1992)**. A final concentration of 10^9 cfu of lactobacillus / bird was given orally on day one of age. Lactose solution was prepared in drinking water to contain 2.5% .

Treatments:

Chicks randomly treated as follow:

T_1: negative or non infected control was untreated.

T_2: infected control inoculated orally with 10^6 cfu S.typhimurium/ chick at the 3^{rd} day of age.

T_3: inoculated orally with 10^9 cfu Lactobacillus/ chick at the 1^{st} day of age.

T_4: received 2.5% lactose in drinking water from the beginning till the end of the study.

T_5: treated with lactobacillus one time at first day of age as well as lactose solution from day old till the end of the study.Chicks in groups 3, 4 and 5 also were inoculated orally with Salmonella at the 3rd day of age.

Sampling and sample analysis:

Five chicks from each treatment were slaughtered at 3 and 4 weeks of age to determine caecal VFA, lactic acid and pH values at three weeks of age and caecal contents of survival Salmonella at four weeks of age according to the method of **Hinton *et al*. (1992)**. At the end of the study additional 5 chicks from each treatment were slaughtered and blood samples were collected in heparinized tubes,centrifuged at speed 3000 rpm for 15 minutes and the plasma was stored at -20C for later analysis. Hematocrit (Ht) value and hemoglobin (Hb) level were determined. Total plasma protein, albumin, cholesterol, AST, ALT, uric acid, total billirubin and triglyceride were determined by enzymatic method using available commercial kits. Globulin was calculated by substraction of plasma albumin from total protein. For differential counts of 100 leucocytes, blood smears were stained with Wright's stain and H/ L ratio was calculated.

Statistical analysis:

Data were subjected to a one- way analysis of variance **(SAS Institute 1994)** using the following model:

$Yij = \mu + Ti + eij$

Where, Yij = observation for each dependent variable; μ = Overall means; Ti = Treatments effect (i = 1,2,.....,5); eij = remiander error. Differences among means were detected by using Duncan's multiple range test **(Duncan, 1955)**.

RESULTS AND DISCUSSION

In regard to market weight, lactobacillus and/ or lactose enhanced resistence to experimental S.typhimurium infection, was the best exemplified by increasing ($P<0.05$) final body weight as compared with infected control group(1754 and1717 *vs* 1554, respectively) as shwon in Figure 1a. Other attempts postulated that probiotic may be alter the composition of the intestinal microflora by producing substances inhibiting the harmful bacteria, as well as decrease intestinal pH resulted in improvement body weight and feed utilization **(Abdel- Azeem *et al*., 2001; Heres *et al*., 2003 and Abdel Mageed *et al*., 2004)**.

At three weeks of age, caecal VFA and lactic acid values were significantly greater in chicks drank water supplemented with lactose (48.3 µ mol/g VFA and 32.5 µ mol/g lactic acid in T4, 45.1 and 14.3 in T5 *vs* 4.6 and 1.0, 3.9 and 0.55, 6.1 and 1.1 in T1, T2 and T3 respectively) as shown in Figures 1c and 1d. Opposite trend was noted for caecal pH, the infected control chicks recorded the highest ($P<0.05$) pH value (6.2 *vs* 4.5, 3.8 and 3.5 in supplemented groups T3, T4 and T5, respectively) and the non infected control group (T1) was intermediate (pH 5.5, Figure 1b).

This study clearly exhibited that either lactobacillus or lactose lowered caecal pH to maintain in a range unsuitable for pathogenic activity of Salmonella. The present results are consistent with the previous studies investigated that the mechanism that lactobacillus and lactose used to inhibit the growth of Salmonella are primarily through production of VFA and lactic acid to decrease caecal pH. Thus, the acidifying effect has a very high ability to kill most of Salmonella **(Miyamoto *et al*., 2000; Cutler *et al*., 2005 and Lee *et al*., 2007)**.

The total counts of S.typhimurium in caecum of the experimental chicks were determined after 4 weeks of hatching. Data showed that the total count of Salmonella were zero in all contaminated groups, except those infected by Salmonella without receiving lactobacillus and/ or lactose (infected control) the count was $17x10^2$ cfu/ ml. It was clear that under the conditions of the current study either lactobacillus (as a probiotic) or lactose (as a prebiotic) had antiseptic effect against Salmonella. Comparable results were obtained by **Abdel Mageed *et al*. (2004)**. They reported that dry yeast as a probiotic decreased the count of pathogenic organism in intestinal quail chicks. Eliminating of Salmonella colonization from caecal chicks by using lactobacillus and/ or lactose might be primairly attributed to their acidifying effect. These results are in accordance with the results of **Payne *et al*. (2007)** who concluded that Salmonella population can be reduced to below detectable limits by interactive effect of low pH for control of its growth and survival.

With respect to blood analysis, data in Table (1) illustrated that total plasma protein and its fractions were not affected by the different treatments. In this regard, other studies indicated that neither probiotic nor prebiotic exerted any significant effect on total plasma protein or its fractions **(Abdel- Azeem *et al*., 2000, 2004)**. However, **Galal *et al*. (2000)** claimed that Lacto-Sacc supplementation significantly increased total plasma protein. As shown in Table (1) the infected control chicks recorded

lowered (P<0.05) Ht and Hb(27 and 5.6 vs 38 and 8.7 in negative control , respectively)but significantly the highest H/ L ratio ,as a good indicator of stress (0.9 vs 0.3 in negative control, respectively. It is evident that lactobacilli and/ or lactose supplementation to chicks infected by Salmonella was beneficial in reducing the adverse effect of it on these hematological parameters to maintain nearly similar to the normal range(32-33.7 for Ht, 8.1-8.3 for Hb and 0.4 -0.5 for H/L ratio vs 38, 8.7 and 0.3 , respectively in negative control).

On the other hand, the contamination by Salmonella without adding lactobacillus or lactose resulted in chicks with a greater increase in the values of blood constituents which reflecting the symptoms of impaired liver and kidney functions (such as cholesterol, triglyceride, AST, ALT, billirubin and uric acid , see Table 1.). The present data also revealed that, treated the infected chicks by lactobacillus or lactose was more effectiveness in modulating this deleterious pathogenic action of Salmonella on blood components (as shown in Table 1.,the modulation was more pronounced in lactobacillus group). **Abdel-Azeem *et al*. (2004)**. illustrated that both probiotic and prebiotic have the ability to protect blood constituents from the harmful effects of enteric bacterial diseases. Probiotics exert their protection on animal performance through improving the immune status as well as the antibiotic action of them against toxins **(Guerrero and Hoyos, 1990).**

It could be concluded that lactobacillus and/ or lactos seemed to have a protective effectiveness against Salmonella colonization. In generall, it is evident that lactose was more important than lactobacillus in causing a significant increases in caecal VFA and lactic acid levels which are detrimental for survival of Salmonella and can hinder its multiplication. However, protecting blood components from the deleterious effect of Salmonella was most pronounced by using lactobacillus. Finally lactobacillus and lactose might be beneficial for reducing the prevalence of Salmonella among broiler chicks and the consequent incidence of human Salmonellosis that reprent a one of true risk to people.

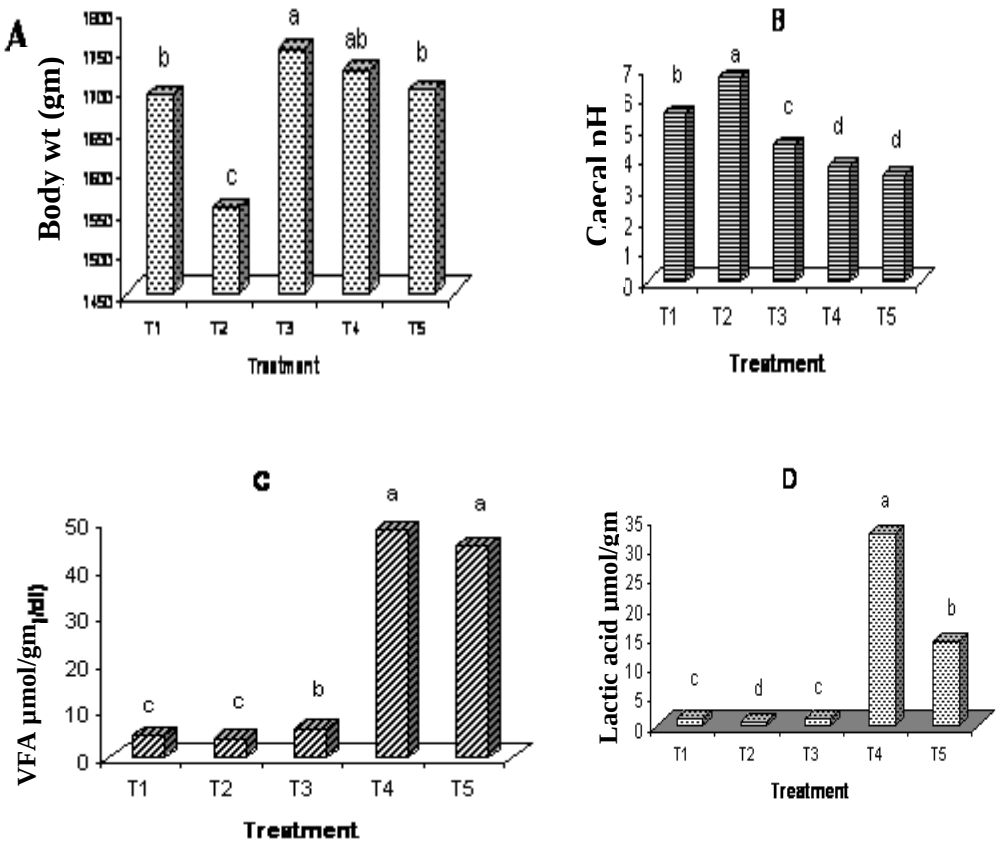

Figure (1): Modulating effects of lactobacillus and/or lactose against the pathogenic action of Salmonella on: **(A)** Market body weight; **(B)** Caecal pH; **(C)** Caecal FVA; **(D)** Lactic Acid.

T1= Untreated chicks (non-infected control); T2= Chicks inoculated with Salmonella; (infected control); T3= Chicks inoculated with Lactobacillus plus Salmonella T4= Chicks received lactose plus Salmonella; T5= Chicks received lactose plus lactobacillus plus Salmonella

Table (1): Effectiveness of lactobacillus and lactose for modulating the pathogenic effect of Salmonella on some blood components in broiler chicks.

	Hematological Traits			Biochemical Traits								
Treatments	Ht %	Hb (g/dL)	H/L Ratio	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	AST (U/L)	ALT (U/L)	Billirubin (mg/dL)	Uric Acid (mg/dL)	Cholesterol (mg/dL)	Triglycerides (M mol/dL)
T1	38.21^{a}	8.74^{a}	0.31^{b}	4.30	1.22	3.08	120.31^{b}	23.04^{b}	0.41^{b}	4.82^{b}	126.70^{b}	82.60^{c}
	±0.25	±0.11	±0.03	±0.01	±0.07	±0.11	±5.62	±2.04	±0.02	±0.07	±7.08	±5.64
T2	27.11^{c}	5.62^{b}	0.92^{a}	3.91	1.15	2.76	141.06^{a}	35.70^{a}	0.77^{a}	6.43^{a}	153..31^{a}	130.62^{a}
	±0.16	±0.04	±0.02	±0.06	±0.05	±0.08	±6.08	±2.95	±0.05	±0.11	±11.05	±11.20
T3	33.71^{ab}	8.12^{a}	0.42^{b}	3.74	1.23	2.51	106.73^{d}	15.62^{c}	0.31^{b}	3.54^{b}	107.20^{d}	83.04^{c}
	±0.14	±0.08	±0.04	±0.07	±0.11	±0.06	±7.32	±1.06	±0.01	±0.15	±9.82	±7.01
T4	32.14^{bc}	8.33^{a}	0.53^{b}	4.33	1.13	3.20	114.65^{bc}	16.41^{c}	0.40^{b}	3.92^{b}	114.43^{c}	109.33^{b}
	±0.21	±0.05	±0.06	±0.10	±0.08	±0.10	±9.12	±1.10	±0.06	±0.07	±10.12	±10.03
T5	33.34^{ab}	8.11^{a}	0.52^{b}	4.01	1.06	2.95	112.71^{cd}	18.09^{c}	0.33^{b}	3.70^{b}	123.05^{b}	112.07^{b}
	±0.19	±0.07	±0.03	±0.08	±0.06	±0.09	±10.21	±1.25	±0.03	±0.13	±9.94	±8.87
Sig.	*	*	***	NS	NS	NS	**	**	*	*	**	**

$^{a-d}$ Means within a column with no common superscript differed significantly. NS = Not significant *= p ≤0.05; **= p ≤ 0.01; ***=p≤0.001.

T_1 = Untreated chicks (non infected control).

T_2 = Chicks inoculated with S.typhimurium on the 3rd day of age (infected control).

T_3 = Chicks inoculated with lactobacillus on the 1st day of age then by S.typhimurium on the 3rd day of age.

T_4 = Chicks drank water contained lactose from hatch till the end of the study + St. infection.

T_5 = Chicks treated as in T_4 plus inoculated with lactobacillus as in T_3.

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الملخص العربي

وقاية كتاكيت اللحم حديثة الفقس ضد التلوث بالسالمونيلا

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2- المعمل المركزي للتغذية والأعلاف- مركز البحوث الزراعية

استخدم في هذا البحث عدد (195) كتكوت لحم من النوع الهابرد عمر يوم تم تقسيمهم عشوائيا إلي خمس مجاميع كل مجموعة تتألف من 39 كتكوت موزعة بالتساوي علي 3 مكررات. ظلت المجموعة الأولي بدون أي معاملة حيث استخدمت كمجموعة المقارنة بينما أعطيت المجوعة الثانية عن طريق الفم ميكروب السالمونيلا بتركيز 10^6 cfu/chick في اليوم الثالث من الفقس. أعطيت كتاكيت المجموعة الثالثة عمر يوم عن طريق الفم مزارع من اللاكتوباسلاس المستخلصة من دجاج اللحم البالغ بتركيز 10^9 cfu/chick. فى حين تناولت كتاكيت المجموعة الرابعة اللاكتوز بتركيز 2.5% عن طريق ماء الشرب بداية من عمر يوم وحتى نهاية التجربة بينما خضعت كتاكيت المجموعة الخامسة لمزيج من المعاملة الثالثة والرابعة معا. كتاكيت المجاميع 3، 4، 5 تم تلقيحها أيضا عند عمر 3 أيام من الفقس بنفس الجرعة من ميكروب السالمونيلا التي أعطيت للمجموعة الثانية.

أهم النتائج المتحصل عليها:-

1- أدي تلوث كتاكيت اللحم حديثة الفقس بميكروب السالمونيلا إلي انخفاض معنوي في وزن الجسم عند التسويق.

2- صاحب الإصابة بميكروب السالمونيلا انخفاض معنوي في محتوي الأعور من الأحماض الدهنية الطيارة وحمض اللاكتيك مع ارتفاع معنوي في رقم الحموضة كما أوضح تحليل الدم وجود بعض القصور في وظائف الكبد والكلي.

3- أظهرت نتائج فحص عينات الأعور المستخرجة من كل المجاميع التجريبية خلوها تماما من ميكروب السالمونيلا ماعدا المجموعة الثانية والتى لوثت بالميكروب دون اعطائها اى إضافات أخرى .

4- على النقيض سجلت الكتاكيت المعاملة بالسالمونيلا بالإضافة إلى اللاكتوباسلاس أو اللاكتوز أو الأثنين معا معنويا اعلي معدل وزن عند التسويق ، أعلى مستوي للأحماض الدهنية الطيارة و حمض اللاكتيك في الأعور (خاصة المجموعة المعاملة باللاكتوز) مع إنخفاض معنوى فى رقم الحموضة .

5- صاحب المعاملة بللاكتوباسلاس أو اللاكتوز تأثير معنوى واضح فى المحافظة على مكونات الدم ضد التأثيرات العكسية لميكروب السا لمونيلا لتظل أقرب لمستواها الطبيعى حيث سجلت كتاكيت هذه المجاميع أقل مستوى في مكونات الدم التى تصاحب وجود أى تقصير فى وظائف الكبد والكلي أو التعرض للإجهاد (مستوى البلازما من الكوليستيرول ، الجلسريدات الثلاثية ، إنزيمات الكبد ، البليروبين ، حمض اليوريك أو معدل H\L) وكانت المجموعة المعاملة باللاكتوباسلاس (المجموعة الثالثة) هي الأفضل في هذا التأثير.

نستخلص من نتائج هذه الدراسة إمكانية استخدام كلأ من مزارع اللاكتوباسلاس أو اللاكتوز في حماية كتاكيت اللحم (أحد المصادر الهامة لإصابة الإنسان بالسالمونيلا) من خطر الإصابة بهذا الميكروب وكذلك إمكانية التصدي له في حالة حدوث الإصابة .