Journal of Animal and Veterinary Advances 11 (7): 955-961, 2012
ISSN: 1680-5593

Effect of a Single Dose of *Lactobacillus salivarius* on Prevention of *Salmonella enteritidis* Infection in Young Broilers

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Abstract: *Lactobacillus salivarius* strain LP 4.2-2 (*L. salivarius* LP 4.2-2) isolated from chicken cecum can strongly inhibit *Salmonella enteritidis* (*S. enteritidis*) *in vitro* but little is known about its effect on prevention of this pathogen in live chickens. Therefore, the main objective of this study was to determine whether a single dose of *L. salivarius* LP 4.2-2 given at low or high dose by oral or cloacal route would prevent *S. enteritidis* infection in young broilers in addition, effects of the experimental treatments on total bacterial count in cecal contents, body weight, organ weight and intestinal length in chicks were determined. In this study, 240 of 1 day old male broiler chicks were randomly assigned to 6 groups of 40 chicks each. Chicks in each group were housed separately in a cage (size 1.5×1.5 m^2). At 1 day of age, each group received none, 10^4 or 10^{10} (cfu/chick) of *L. salivarius* LP 4.2-2 by either oral or cloacal route. At 2 days of age, all chicks except those in one group (a negative control) were challenged orally with 10^4 (cfu/chick) of *S. enteritidis*. At 3 days of age, a half number of chicks in each group (n = 20/group) were randomly selected for the detection of *S. enteritidis* infection in cecal tonsils. Other parameters such as total bacterial count, body weight and intestinal length were also measured. The remaining chicks were allowed to grow until 9 days of age and then the procedures for measuring each parameter were done the same as those described above. The results showed that at 3 days of age, rates of *S. enteritidis* infection were lower in all groups administered with *L. salivarius* LP 4.2-2 than in a positive control group (13/20 or 65%-17/20 or 85% versus 19/20 or 95%). However, at 9 days of age, rates of *S. enteritidis* infection were high in all groups (95-100%), except in a negative control (0%). No significances were seen in total bacterial counts and in body weights between groups either at 3 or 9 days of age. After adjusted for body weight, weights of most internal organs in all groups and total lengths of intestine in most groups did not differ significantly. In conclusion, a single dose of *L. salivarius* cannot prevent *S. enteritidis* infection in all chicks but it can reduce rate of the infection in 3 days old chicks. However, the preventive effect is diminished over time.

Key words: Broiler, *Lactobacillus salivarius*, *Salmonella enteritidis*, body weight, organ weight, intestinal length, prevention

INTRODUCTION

Lactobacillus sp. bacteria normally found in chicken gastrointestinal tract have been under extensive studies both *in vitro* and *in vivo* for their potentials to use as probiotics in poultry industry (Ashraf *et al*., 2009; Ehrmann *et al*., 2002; Koenen *et al*., 2004; Mountzouris *et al*., 2007; Musikasang *et al*., 2009; Taheri *et al*., 2009; Van Coillie *et al*., 2007). In *in vitro* studies, *Lactobacillus* sp. have met important criteria of probiotics properties such as ability to adhere intestinal mucosa (Nouri *et al*., 2010) survival in acidic environment (Ashraf *et al*., 2009) and inhibition of microbial pathogens (Surachon *et al*., 2011; Taheri *et al*., 2009). Use of *Lactobacillus* sp. for control of intestinal pathogens in chickens is of particular interest because chickens are a major source of Salmonella contamination, especially *S. enteritidis* causing salmonellosis in human worldwide (European Food Safety Authority, 2009; Mehrabian and Jaberi, 2007). Many *in vitro* studies (Ehrmann *et al*., 2002; Miyamoto *et al*., 2000; Nouri *et al*., 2010; Surachon *et al*., 2011) have demonstrated the success in use of *Lactobacillus* sp. for growth inhibition of *S. enteritidis*. However, the success in *in vitro* studies does not guarantee the success in *in vivo* studies. For *in vivo* studies, the efficacy of *Lactobacillus* sp. in inhibiting

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S. enteritidis in chickens is associated with many factors including *Lactobacillus* sp. (strains and viability), the application methods (dose, route and frequency of administration), farm management methods (overall diet, overall farm hygiene and environmental stress factors) and chickens (genetics, age, health status) (Chichlowski *et al*., 2007a; Vandeplas *et al*., 2010). Moreover, although many mechanisms of actions of *Lactobacillus* sp. or probiotics against intestinal pathogens have been proposed (e.g., competitive exclusion (Mead, 2000; Zhang *et al*., 2007) antimicrobial substances (Lima *et al*., 2007; Stern *et al*., 2006) and immunomodulation and gut mucosal immunity (Chichlowski *et al*., 2007b; Farnell *et al*., 2006; Koenen *et al*., 2004) the exact mechanisms remain unknown. The combinations of these problems can result in variation of the outcome and indicate that much research needs to be done.

Neonatal chicks with immature immunity are more susceptible to *S. enteritidis* infection. After gastrointestinal tracts of the chicks have been infected (mostly via ingestion of contaminated foods), *S. enteritidis* can invade to internal organs such as spleen and ovary (Gast and Beard, 1990; Gast *et al*., 2004). However, the infected chicks usually do not show any signs of severe illness; instead, they act as reservoirs and discharge *S. enteritidis* via the manure into environment. Therefore, to prevent neonatal chicks from the infection is important. As mentioned before, dose and route of *Lactobacillus* sp. administration are important factors. Single dose of *Lactobacillus* sp. if effective would be easy for application in real use in poultry industry. In previous studies (Higgins *et al*., 2010), application of single dose, muti-strains of Lactobacillus-based probiotics in neonatal chicks experimentally infected with *S. enteritidis* reduced the incidence rate of *S. enteritidis* infection. For administration of *Lactobacillus* sp. in neonatal chicks, two potential routes are possible; oral or cloacal route. In oral route, *Lactobacillus* sp. are taken down from mouth along the length of gastrointestinal tract. It is easy for administration via this route but *Lactobacillus* sp. may be destroyed with acidic environment in the stomach before they reach the lower intestine where intestinal pathogens (e.g., *Salmonella* sp. and *Campylobacter* sp.) are commonly present. On the other hand in cloacal route, *Lactobacillus* sp. are dropped into the vent lip and cloaca and then they taken down into lower intestine via retrograde peristalsis (Corrier *et al*., 1994; Van Der Sluis *et al*., 2009).

In previous study (Surachon *et al*., 2011), researchers isolated *L. salivarius* TP4.2-2 from the cecum of the clinically healthy broiler. This isolate can strongly inhibit the growth of *S. enteritidis in vitro* but little is known about its effect on the prevention of *S. enteritidis* in live chickens. Therefore, the objective of this study was to determine whether a single dose of *L. salivarius* LP 4.2-2 given at low or high dose by oral or cloacal route would prevent *S. enteritidis* infection in young broilers in addition, effects of the experimental treatments on total bacterial count in cecal contents, body weight, organ weight and intestinal length of young broilers were evaluated.

MATERIALS AND METHODS

Preparation of *L. salivarius*: In this study, researchers used *L. Salivarius* TP4.2-2 which was isolated from chicken cecum and strongly inhibited *S. enteritidis in vitro* from the previous study (Surachon *et al*., 2011). *L. salivarius* TP4.2-2 was grown in the tube containing MRS broth (Oxoid, Hampshire, England) and incubated in microaerophilic environment with BD GasPack (Becton, Dickinson and Company, Sparks, MD, USA) at 37°C for 48 h. The culture was diluted to expected concentrations of 10^4 and 10^{10} cfu mL^{-1} for administration in the experimental chicks. Actual colony-forming units given per chick (cfu/chick) in the experiment were determined retrospectively from spread plating on MRS agar (Oxoid, Hampshire, England). The actual cfu/chick is shown in Table 1.

Preparation of *S. enteritidis*: In this study, researchers also used *S. enteritidis* isolated from broiler chicks, the same strain as the previous study (Surachon *et al*., 2011). *S. enteritidis* was grown in tryptic soy broth (Hardy Diagnostics, Santa Maria, CA, USA) at 37°C for 24 h until

Table 1: Experimental design and sample collection

	L. salivarius LP 4.2-2 administrations in 1 day old chicks		Challenge dose of *S. enteritidis*	Sample collection (number of chicks)	
Treatments	Dose	Route	in 2 days old chicks	3 days old chicks	9 days old chicks
1 (negative control)	None	None	None	20	20
2 (positive control)	None	None	10^4 cfu/chick	20	20
3	10^4 cfu/chick	Oral	10^4 cfu/chick	20	20
4	10^{10} cfu/chick	Oral	10^4 cfu/chick	20	20
5	10^4 cfu/chick	Cloacal	10^4 cfu/chick	20	20
6	10^{10} cfu/chick	Cloacal	10^4 cfu/chick	20	20

reached concentration of 1×10^8 cfu mL^{-1} (Equivalent to MacFarland standard No. 0.5) and then diluted to 10^4 cfu mL^{-1} by using 0.1% peptone water for further use. Actual colony-forming units given per chick in the experiment were determined retrospectively from spread plating on Xylose Lysine Deoxycholate (XLD) agar (Difco, Becton Dickinson, Sparks, MD, USA). The actual cfu/chick is shown in Table 1.

Animals and experimental design: Use of animals was approved by Animal Ethic Committee of Khon Kaen University. In this study, 240 of 1 day old male broiler chicks were randomly assigned to 6 groups of 40 chicks each. Chicks in each group were housed separately in a cage (size 1.5×1.5 m^2) with unlimited access to food and water. Experimental design was shown in Table 1. At 1 day of age, a chick in each group received different doses (cfu/chick) of *L. salivarius* LP 4.2-2 and different routes of administration as follows: treatment 1 and 2 (negative and positive controls, respectively), each chick receiving no *L. salivarius*; treatment 3 and 4, each chick receiving 10^4 or 10^{10}, respectively by oral gavage; treatment 5 and 6 each chick receiving 10^4 or 10^{10}, respectively by cloacal route. The cloacal route was done with the methods described previously (Corrier *et al*., 1994; Higgins *et al*., 2008). Briefly, a chick was inverted gently and the solution containing *L. salivarius* LP 4.2-2 was carefully dropped into the vent lip. The chick was held inverted until the treatment solution was taken into the cloaca. At 2 days of age, except chicks in group 1, all chicks were challenged with 10^4 cfu of *S. enteritidis* by oral gavage. At 3 days of age, a half number of chicks in each group (n = 20/group) were randomly selected for the detection of *S. enteritidis* infection in cecal tonsils for making total bacterial count in the cecal contents and for measuring of body weight and intestinal length. The remaining chicks (n = 20/group) were allowed to grow until 9 days of age and then the procedures for measuring each parameter were done the same as those described.

Detection of *S. enteritidis* infection: At 3 days of age, a half number of chicks in each group (n = 20/group) were randomly selected for the detection of *S. enteritidis* infection in cecal tonsils. The selected chicks were killed by cervical dislocation then their cecal tonsils were aseptically removed and placed in sterile tubes containing 10 mL of tetrathionate broth (Becton Dickinson, Sparks, MD, USA). The samples were incubated at 37°C for 18 h and then streaked for isolation on Xylose Lysine Deoxycholate (XLD) agar plates. Plates were incubated at 37°C for 18 h and then observed for the presence or absence of characteristic Salmonella colonies which are black on this media. The remaining chicks (n = 20/group) were determined for *S. enteritidis* infection at 9 days of age.

Total bacterial count: Five broilers per treatment at 3 and 9 days of age were randomly sampled for determination of total bacterial count in cecal contents. After chicks were killed, ceca were excised and removed. The cecal contents were aseptically transferred into a sterile bag. Then, a sample of the cecal contents (1 g) was homogenized in 9 mL of 0.1% peptone water and 10-fold serial dilutions were prepared. The serial dilutions were then plated on plate count agar (Difco, Becton Dickinson and Company Sparks, USA) and incubated at 37°C for 24 h. Colonies were counted and expressed as $\log_{10}$ cfu g^{-1} of cecal contents.

Evaluation of body weight, organ weight and intestinal length: For body weight, all chicks were weighted individually. For organ weight, after chicks were killed and their abdomens were exposed, internal organs comprising cloacal bursa (bursa of Fabricius), gizzard, heart, liver and spleen were removed and weighted individually. Organ weight was done only in 9 days old chicks. For intestinal length, the whole intestine ranging from gizzard to cloaca was removed. Then, the intestine was measured in segments defined as duodenum (from gizzard to entry of the bile and pancreatic ducts, covering the length of duodenal loop), jejunum (from entry of the ducts to yolk stalk (Meckel's diverticulum)), ileum (from yolk stalk to ileocecal junction) and rectum (from ileocecal junction to cloaca). Total length of intestine was calculated from combining of the lengths of duodenum, jejunum, ileum and rectum. In addition, length of cecum (from ileocecal junction to the apex) was measured from both right and left sides.

Statistical analysis: The incidence of *S. enteritidis* infection was compared using Fisher's exact test to determine significant differences between experimental groups. Data of continuous variables (e.g., body weight, organ weight and length of intestinal segments) were tested for normal distribution by use of the Kolmogorov-Smirnov test. One-way Analysis of Variance (ANOVA) was used to compare means of total bacterial counts ($\log_{10}$ cfu g^{-1} of cecal contents) or means of body weights among groups. Analysis of Covariance (ANCOVA) was used to compare means of organ weights or means of intestinal lengths among groups with use of the chick's body weight as a covariate. Whenever ANOVA or ANCOVA resulted in significant F-values, Tukey's HSD was used for post hoc multiple comparisons. Significance was set at $p<0.05$. All statistical analyses were done by using SPSS Version 17 (SPSS Inc., Chicago).

RESULTS AND DISCUSSION

Detection of *S. enteritidis* infection: The results showed that at 3 days of age rates of *S. enteritidis* infection were lower in all groups administered with *L. salivarius* LP 4.2-2 than in a positive control group (13/20 or 65%-17/20 or 85% vs. 19/20 or 95%) (Table 2). However, at 9 days of age rates of *S. enteritidis* infection were high in all groups (95-100%), except in a negative control (0%) (Table 2).

Total bacterial count in cecal contents: There was no significant difference in total bacterial count between groups either in 3 days old chicks or in 9 days old chicks. Means (cfu g^{-1} of cecal contents) of total bacteria ranged from 9.09±0.34 (SD) to 9.89±0.66 in 3 days old chicks and from 9.06±0.68-9.83±0.46 in 9 days old chicks (Table 3).

Evaluation of body weight, organ weight and intestinal length: Body weights of chicks did not differ significantly between groups either at 3 or 9 days of age (Table 4). Means (g) of body weight ranged from 62.50±7.69 (SD) to 68.50±8.75 in 3 days old chicks and from 205.75±25.66-218.00±17.65 in 9 days old chicks (Table 4). After adjusted for body weight, weights of most internal organs (cloacal bursa, gizzard and liver) in 9 days old chicks did not differ significantly between groups (Table 4). After adjusted for body weight, total lengths of intestine in most groups did not differ significantly but lengths of intestinal segments tended to vary between groups either at 3 or 9 days of age (Table 5 and 6).

This study was aimed in evaluating the effect of a single dose of *L. salivarius* LP 4.2-2 given at either low or high dose by either oral or cloacal route would prevent *S. enteritidis* infection in young broilers. The results show that a single dose of *L. salivarius* cannot prevent *S. enteritidis* infection in all chicks but it can reduce rate of the infection in 3 days old chicks. These findings were supported by the results that at 3 days of age, rates of *S. enteritidis* infection in the cecal tonsils were lower in all groups administered with *L. salivarius* LP 4.2-2 than in a positive control group (Table 2).These findings also confirmed and extended those of previous studies (Higgins *et al*., 2010, 2008; Kizerwetter-Swida and Binek, 2009; Pascual *et al*., 1999) that administration of a single dose of single strain or multi-strains of Lactobacillus-based probiotics can reduce the rate of *S. enteritidis* infection in chicks. However, rates of the reduction vary between studies; these variations may be due to the variations in experimental conditions and settings. It is interesting that application of *L. salivarius* LP 4.2-2 via cloacal route results in reduction of *S. enteritidis* infection. These findings are similar to those of the previous study (Higgins *et al*., 2008) and could be explained that after *Lactobacillus* sp. have been dropped

Table 2: Effect of *L. salivarius* on *S. enteritidis* recovered from cecal tonsils of 3 or 9 days old broiler chicks

	No. *S. enteritidis* positive/total samples (%)	
Treatments[1]	3 days old chicks	9 days old chicks
1	0/20 (0)[a]	0/20 (0)[a]
2	19/20 (95)[b]	20/20 (100)[b]
3	13/20 (65)[c]	20/20 (100)[b]
4	16/20 (80)[b]	19/20 (95)[b]
5	17/20 (85)[b]	20/20 (100)[b]
6	16/20 (80)[b]	19/20 (95)[b]

[1]Treatment assignment (Treatment 1 = Negative control; Treatment 2 = Positive control; Treatment 3 = Chicks receiving *L. salivarius* LP 4.2-2 10^4 cfu/chick by oral route; Treatment 4 = Chicks receiving *L. salivarius* LP 4.2-2 10^{10} cfu/chick by oral route; Treatment 5 = Chicks receiving *L. salivarius* LP 4.2-2 10^4 cfu/chick by cloacal route; Treatment 6 = Chicks receiving *L. salivarius* LP 4.2-2 10^{10} cfu/chick by cloacal route), [a-b]Different superscripts within each column indicate significant differences ($p<0.05$)

Table 3: Total bacterial counts in cecal digesta of chicks in each group

	Total bacterial count (log_{10} cfu g^{-1} of cecal content)	
Treatments[1]	3 days old chicks[2]	9 days old chicks[2]
1	9.47±0.47	9.83±0.46
2	9.09±0.34	9.06±0.68
3	9.60±0.56	9.38±0.83
4	9.70±0.43	9.27±0.57
5	9.59±0.44	9.13±0.48
6	9.89±0.66	9.53±0.67

[1]Treatment assignment (Treatment 1 = Negative control; Treatment 2 = Positive control; Treatment 3 = Chicks receiving *L. salivarius* LP 4.2-2 10^4 cfu/chick by oral route; Treatment 4 = Chicks receiving *L. salivarius* LP 4.2-2 10^{10} cfu/chick by oral route; Treatment 5 = Chicks receiving *L. salivarius* LP 4.2-2 10^4 cfu/chick by cloacal route; Treatment 6 = Chicks receiving *L. salivarius* LP 4.2-2 10^{10} cfu/chick by cloacal route), [2]No. of chicks in each group (n = 5)

Table 4: Effect of treatments on body and organ weights

	Body weights (g)		Organ weights[2] (g)				
Treatments[1]	3 days old chicks[3]	9 days old chicks[3]	Bursa	Gizzard	Heart	Liver	Spleen
1	62.75±7.69	218.00±17.65	0.43±0.19	11.06±1.45	1.79±0.41[a]	11.71±1.08	0.20±0.06[a]
2	66.00±7.18	208.25±27.11	0.43±0.11	10.41±1.30	2.07±0.53[ab]	11.99±1.85	0.25±0.05[ab]
3	62.50±7.69	205.75±25.66	0.38±0.09	9.94±2.04	2.79±0.53[b]	12.23±2.20	0.26±0.06[ab]
4	68.50±8.75	208.50±19.54	0.39±0.13	10.16±1.46	2.16±0.67[ab]	12.13±2.24	0.23±0.05[a]
5	64.75±4.99	207.25±20.23	0.45±0.09	10.39±1.05	2.36±0.65[ab]	12.10±0.92	0.32±0.08[b]
6	63.25±5.68	208.00±21.48	0.41±0.10	10.27±1.66	2.10±0.43[ab]	11.51±1.48	0.23±0.06[a]

[1]Treatment assignment (Treatment 1 = Negative control; Treatment 2 = Positive control; Treatment 3 = Chicks receiving *L. salivarius* LP 4.2-2 10^4 cfu/chick by oral route; Treatment 4 = Chicks receiving *L. salivarius* LP 4.2-2 10^{10} cfu/chick by oral route; Treatment 5 = Chicks receiving *L. salivarius* LP 4.2-2 10^4 cfu/chick by cloacal route; Treatment 6 = Chicks receiving *L. salivarius* LP 4.2-2 10^{10} cfu/chick by cloacal route); [2]Organ weights were measured only in 9 days old chicks; [3]Number of chicks in each group (n = 20); [a-b]Different superscripts within each column indicate significant differences ($p<0.05$)

Table 5: Effect of treatments on intestinal lengths of 3 days old chicks

	Length of intestinal segments (cm)					
Treatments[1]	Duodenum	Jejunum	Ileum	Rectum	Cecum[2]	Total[3]
1	11.25±1.12	26.98±1.61^{ab}	25.83±0.49^{ab}	4.63±0.63^{a}	5.85±0.63	68.68±3.21^{a}
2	11.00±0.92	25.33±1.81^{a}	23.85±0.52^{bc}	3.23±0.47^{b}	5.43±0.41	63.40±4.30^{b}
3	10.85±0.75	25.35±1.81^{a}	23.60±0.43^{c}	3.35±0.37^{b}	5.43±0.49	63.15±3.89^{b}
4	10.70±0.92	26.70±2.46^{ab}	24.85±0.50^{abc}	3.60±0.42^{bc}	5.80±0.57	65.85±4.74^{b}
5	11.45±0.69	28.25±2.90^{b}	26.55±0.68^{a}	3.45±0.28^{b}	5.53±0.60	69.70±5.80^{a}
6	11.30±0.92	26.73±2.82^{ab}	24.53±0.52^{abc}	3.90±0.50^{c}	5.88±0.58	66.45±4.41^{ab}

Table 6: Effect of treatments on intestinal lengths of 9 days old chicks

	Length of intestinal segments (cm)					
Treatments[1]	Duodenum	Jejunum	Ileum	Rectum	Cecum[2]	Total[3]
1	15.55±1.23^{ab}	37.40±3.96	35.70±2.76^{a}	5.38±0.48^{abc}	7.93±0.71	94.03±6.14^{a}
2	15.40±1.19^{ab}	35.65±5.97	33.60±2.89^{a}	4.80±0.66^{a}	8.40±0.72	89.45±8.02^{a}
3	14.30±1.13^{a}	37.10±4.91	25.73±7.85^{b}	5.65±0.90^{c}	8.25±1.23	82.78±7.82^{b}
4	15.65±1.81^{b}	36.50±3.80	35.78±3.64^{a}	4.90±0.55^{ab}	8.10±0.79	92.83±7.79^{a}
5	14.85±1.27^{ab}	36.23±3.57	36.23±3.57^{a}	5.23±0.41^{abc}	8.38±0.67	92.53±7.54^{a}
6	15.55±1.50^{ab}	37.03±3.88	35.94±3.75^{a}	5.40±0.68^{bc}	8.38±0.58	93.92±6.42^{a}

[1]Treatment assignment (Treatment 1 = Negative control; Treatment 2 = Positive control; Treatment 3 = Chicks receiving *L. salivarius* LP 4.2-2 10^{4} cfu/chick by oral route; Treatment 4 = Chicks receiving *L. salivarius* LP 4.2-2 10^{10} cfu/chick by oral route; Treatment 5 = Chicks receiving *L. salivarius* LP 4.2-2 10^{4} cfu/chick by cloacal route; Treatment 6 = Chicks receiving *L. salivarius* LP 4.2-2 10^{10} cfu/chick by cloacal route), [2]Cecal length in each chick was calculated from the average of right and left cecal lengths; [3]Total length was calculated from combination of all intestinal segments except the cecum; [a-c]Different superscripts within each column indicate significant differences ($p<0.05$)

into the vent lip and cloaca then they are taken down into lower intestine via retrograde peristalsis (Corrier *et al*., 1994; Van Der Sluis *et al*., 2009). However, much research needs to be done to understand the mechanism of retrograde peristalsis in a distal portion of the chicken intestinal tract.

However, the preventive effect of *L. salivarius* LP 4.2-2 on *S. enteritidis* infection has diminished over time. These findings indicated the temporal effect of *L. salivarius* LP 4.2-2 and were supported by the results that, at 9 days of age, rates of *S. enteritidis* infection in the cecal tonsils were high in all groups (95-100%) except in a negative control (0%). The exact mechanisms why *L. salivarius* LP 4.2-2 has a temporally preventive effect on *S. enteritidis* infection are unknown but may be associated with survival of *L. salivarius* LP 4.2-2 in chick gastrointestinal tract or with other factors. Therefore although, *L. salivarius* LP 4.2-2 can strongly inhibit *S. enteritidis in vitro* (Surachon *et al*., 2011) the *in vitro* effect does not warrant the *in vivo* effect because many factors are associated with *in vivo* effect (Vandeplas *et al*., 2010). Results of the study reported here indicate that concentrations of total bacterial count in cecal contents do not differ significantly. These findings were similar to those of the previous study (Jin *et al*., 1998). However, the study has some limitations; that is cecal microflora composition did not determine. In the previous studies (Mountzouris *et al*., 2007, 2010) administration of probiotics has an effect on cecal microflora composition.

In this study, body weights of chicks in all groups treated with *L. salivarius* TP 4.2-2 did not differ significantly from those in a positive control group either at 3 or 9 days of age. These results indicate that administration of *L. salivarius* does not improve the weight gain in young broilers. These results are similar to those of the previous study (Mountzouris *et al*., 2010) that body weights of chicks in starter phase (1-14 days of age) do not differ between groups treated with probiotics and a control. However, body weights of chickens at marketable age in groups treated with probiotics or *Lactobacillus* sp. are higher than those in a control (Angelakis and Raoult, 2010; Mountzouris *et al*., 2010) indicating a long term effect. For organ weights, after adjusted for body weight, weights of most internal organs in 9 days old chicks do not differ significantly between groups. These results are in agreement with those of the previous study (Awad *et al*., 2009). After adjusted for body weight although, lengths of intestinal segments significantly vary between groups either at 3 or 9 days of age, total intestinal lengths in most groups do not differ significantly. Overall, these results suggest that administration of a single dose of *L. salivarius* LP 4.2-2 has little effect on growth parameters in young broilers.

CONCLUSION

A single dose of *L. salivarius* TP 4.2-2 given at low or high dose by oral or cloacal route cannot prevent *S. enteritidis* infection in all chicks but it can reduce rate of the infection in 3 days old chicks however, this preventive effect has diminished over time. In addition, administration of a single dose of *L. salivarius* LP 4.2-2 has almost no effect on growth parameters in young broilers.

ACKNOWLEDGEMENT

This study was supported by Research Fund of the Faculty of Veterinary Medicine, Khon Kaen University, Thailand (Project No.: KKUVet 200904).

REFERENCES

Angelakis, E. and D. Raoult, 2010. The increase of *Lactobacillus* species in the gut flora of newborn broiler chicks and ducks is associated with weight gain. PLoS One, 5: e10463-e10463.

Ashraf, M., M. Arshad, M. Siddique and G. Muhammad, 2009. *In vitro* screening of locally isolated lactobacillus species for probiotic properties. Pak. Vet. J., 29: 186-190.

Awad, W.A., K. Ghareeb, S. Abdel-Raheem and J. Bohm, 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens. Poult. Sci., 88: 49-56.

Chichlowski, M., J. Croom, B.W. McBride, G.B. Havenstein and M.D. Koci, 2007a. Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: A brief review of current knowledge. Int. J. Poult. Sci., 6: 694-704.

Chichlowski, M., J. Croom, B.W. McBride, L. Daniel, G. Davis and M.D. Koci, 2007b. Direct-fed Microbial primalac and salinomycin modulate whole-body and intestinal oxygen consumption and intestinal mucosal cytokine production in the broiler chick. Poult. Sci., 86: 1100-1106.

Corrier, D.E., D.J. Nisbet, A.G. Hollister, R.C. Beier, C.M. Scanlan, B.M. Hargis and J.R. Deloach, 1994. Resistance against Salmonella enteritidis cecal colonization in Leghorn chicks by vent lip application of cecal bacteria culture. Poult. Sci., 73: 648-652.

Ehrmann, M.A., P. Kurzak, J. Baue and R.F. Vogel, 2002. Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. J. Applied Microbiol., 92: 966-975.

European Food Safety Authority, 2009. The community summary report on trends and sources of Zoonoses and Zoonotic agents in the European union in 2007. EFSA J., No. 223. http://www.efsa.europa.eu/en/scdocs/scdoc/223r.htm.

Farnell, M.B., A.M. Donoghue, F.S. de los Santos, P.J. Blore, B.M. Hargis, G. Tellez and D.J. Donoghue, 2006. Upregulation of oxidative burst and degranulation in chicken heterophils stimulated with probiotic bacteria. Poult. Sci., 85: 1900-1906.

Gast, R.K. and C.W. Beard, 1990. Isolation of *Salmonella enteritidis* from internal organs of experimentally infected hens. Avian Dis., 34: 991-993.

Gast, R.K., J. Guard-Bouldin and P.S. Holt, 2004. Colonization of reproductive organs and internal contamination of eggs after experimental infection of laying hens with *Salmonella heidelberg* and *Salmonella enteritidis*. Avian Dis., 48: 863-869.

Higgins, J.P., S.E. Higgins, A.D. Wolfenden, S.N. Henderson and A. Torres-Rodriguez *et al*., 2010. Effect of lactic acid bacteria probiotic culture treatment timing on *Salmonella enteritidis* in neonatal broilers. Poult. Sci., 89: 243-247.

Higgins, S.E., J.P. Higgins, A.D. Wolfenden, S.N. Henderson, A. Torres-Rodriguez, G. Tellez and B. Hargis, 2008. Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella* enteritidis in neonatal broiler chicks. Poult. Sci., 87: 27-31.

Jin, L.Z., Y.W. Ho, N. Abdullah and S. Jalaludin, 1998. Growth performance, intestinal microbial populations and serum cholesterol of broilers fed diets containing *Lactobacillus cultures*. Poult. Sci., 77: 1259-1265.

Kizerwetter-Swida, M. and M. Binek, 2009. Protective effect of potentially probiotic Lactobacillus strain on infection with pathogenic bacteria in chickens. Pol. J. Vet. Sci., 12: 15-20.

Koenen, M.E., J. Karmer, R.van der Hulst, L. Heres and S.H.M. Jeurissen and W.J.A. Boersma, 2004. Immunomodulation by probiotic lactobacilli in layer and meat-type chickens. Br. Poult. Sci., 45: 355-366.

Lima, E.T., R.L. Andreatti Filho, A.S. Okamoto, J.C. Noujaim, M.R. Barros and A.J. Crocci, 2007. Evaluation *in vitro* of the antagonistic substances produced by *Lactobacillus* spp. isolated from chickens. Can. J. Vet. Res., 71: 103-107.

Mead, G.C., 2000. Prospects for competitive exclusion treatment to control salmonellas and other foodborne pathogens in poultry. Vet. J., 159: 111-123.

Mehrabian, S. and E. Jaberi, 2007. Isolasion, identification and antimicrobial resistance patterns of salmonella from meat products in Tehran. Pak. J. Biol. Sci., 10: 122-126.

Miyamoto, T., T. Horie, T. Fujiwara, T. Fukata, K. Sasai and E. Baba, 2000. *Lactobacillus* flora in the cloaca and vagina of hens and its inhibitory activity against *Salmonella enteritidis in vitro*. Poult. Sci., 79: 7-11.

Mountzouris, K.C., P. Tsirtsikos, E. Kalamara, S. Nitsch, G. Schatzmayr and K. Fegeros, 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult. Sci., 86: 309-317.

Mountzouris, K.C., P. Tsitrsikos, I. Palamidi, A. Arvaniti, M. Mohnl, G. Schatzmayr and K. Fegeros, 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins and cecal microflora composition. Poult. Sci., 89: 58-67.

Musikasang, H., A. Tani, A. H-kittikun and S. Maneerat, 2009. Probiotic potential of lactic acid bacteria isolated from chicken gastrointestinal digestive tract. World J. Microbiol. Biotechnol., 25: 1337-1345.

Nouri, M., F. Rahbarizadeh, D. Ahmadvand, F. Moosakhani, E. Sadeqzadeh, S. Lavasani and V.K. Vishteh, 2010. Inhibitory effects of *Lactobacillus salivarius* and *Lactobacillus crispatus* isolated from chicken gastrointestinal tract on *Salmonella enteritidis* and *Escherichia coli* growth. Iranian J. Biotechnol., 8: 32-37.

Pascual, M., M. Hugas, J.I. Badiola, J.M. Monfort and M. Garrgia, 1999. *Lactabacillus Salivarius* CTC2197 prevents *Salmonella enteritidis* colonization in chickens. Applied Environ. Microbiol., 65: 4981-4986.

Stern, N.J., E.A. Svetoch, B.V. Eruslanov, V.V. Perelygin and E.V. Mitsevich *et al*., 2006. Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. Antimicrob Agents Chemother., 50: 3111-3116.

Surachon, P., P. Sukon, P. Chaveerach, P. Waewdee and C. Soikum, 2011. Screening of lactic acid bacteria isolated from chicken ceca for *in vitro* growth inhibition of *Salmonella enteritica* Serovar enteritidis. J. Anim. Vet. Adv., 10: 939-944.

Taheri, H.R., H. Moravej, F. Tabandeh, M. Zaghari and M. Shivazad, 2009. Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. Poult. Sci., 88: 1586-1593.

Van Coillie, E., J. Goris, I. Cleenwerck, K. Grijspeerdt and N. Botteldoorn *et al*., 2007. Identification of lactobacilli isolated from the cloaca and vagina of laying hens and characterization for potential use as probiotics to control *Salmonella* enteritidis. J. Applied Microbiol., 102: 1095-1106.

Van Der Sluis, H.J., R.M. Dwars, J.C.M. Vernooij and W.J.M. Landman, 2009. Cloacal reflexes and uptake of fluorescein-labeled polystyrene beads in broiler chickens. Poult. Sci., 88: 1242-1249.

Vandeplas, S., R.D. Dauphin, Y. Beckers, P. Thonart and A. Thewis, 2010. *Salmonella* in chicken: Current and developing strategies to reduce contamination at farm level. J. Food Prot., 73: 774-785.

Zhang, G., L. Ma and M.P. Doyle, 2007. Salmonellae reduction in poultry by competitive exclusion bacteria *Lactobacillus salivarius* and *Streptococcus cristatus*. J. Food Prot., 70: 874-878.