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Impact of two probiotic *Lactobacillus* strains feeding on fecal lactobacilli and weight gains in chicken

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Two probiotic strains, *Lactobacillus agilis* JCM 1048 and *L. salivarius* subsp. *salicinius* JCM 1230 isolated from chicken intestine, exhibited probiotic characteristics that can be applied for chicken production. After 7 days of probiotic feeding (FD7), the count of intestinal lactobacilli in the probiotic group (group P, n=10) was significantly (p<0.05) higher than that in the control group (group C, n=9). After 40 days of probiotic feeding (FD40), the lactobacilli and enterococci counts were stable but the *Enterobacteriaceae* number was significantly reduced (p<0.05). A total of 163 isolated lactobacilli were identified as the *L. acidophilus/gallinarum* group (49.7%), *L. agilis* (30.7%), *L. salivarius* (9.2%), *L. reuteri* (9.2%), and *Lactobacillus* spp. (1.2%). The probiotic lactobacilli positively affected the *Lactobacillus* biota in chickens at FD7, with a significant increase in the number (p<0.05) of *L. agilis* and group P. The viable counts of each *Lactobacillus* species at FD40, however, showed no differences between two groups. An increasing incidence of *L. agilis* was also noted with probiotic feeding. The probiotic effect of two strains resulted in significantly increased weight gains (10.7%) of group P in comparison with group C at FD40 (p<0.01).

Key Words——chicken; lactobacilli; probiotics; weight gains

Introduction

The use of probiotics to promote health and nutrition has been attracting a great deal of attention for a long time (Gilliland, 1990). The term "probiotic" is defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). The use of probiotics in farm animals has resulted in faster weight gain for the same amount of food consumed (growth promotion and feed efficiency) (Tannock, 1997). The composition of intestinal microbiota remains stable in healthy animals. This stability, however, may be destroyed by various factors such as drastic changes in the animal itself, food products contaminated with pathogenic bacteria or antibiotic administration. A new definition of probiotics was proposed as active microorganisms and microbial cell components that promote beneficial effects on the host animal (Salminen et al., 1999).

Probiotics can be applied to animals in various ways. They can either be included in the pelletted feed or produced in the form of capsules, paste, powder, or granules which can be given to the animals directly or with their food. Most probiotics contain single or multiple strains of lactic acid bacteria. The effect of probiotics on animal production has been widely studied, but the results are often contradictory (Jin et al., 1997).

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Some strains of *Lactobacillus acidophilus* isolated from chicken intestine resulted in an improvement of intestinal microbiota and production in chickens (Miles et al., 1981; Tortuero, 1973). However, other *Lactobacillus* species originating from chickens were not suitable for development as probiotic strains.

In the present study, we attempted to apply beneficial lactobacilli isolated from chicken intestine as probiotic strains for chicken production.

Materials and Methods

Strains used. Two strains of *L. agilis* JCM 1048 and *L. salivarius* subsp. *salicinius* JCM 1230 isolated from chicken intestine were studied for probiotic use. Two strains of the same species as the probiotic strains derived from other sources were used as the respective controls.

Probiotic characteristics. Acid production was tested on MRS agar supplemented with 0.5% $CaCO_3$. The clear zone around the colony after 48 h of incubation at 30°C demonstrated acid production. Growth at different temperatures was observed in MRS broth after incubation at 10, 15, 20, 25, 30, 35, 40, 45, and 50°C for 7 days. Resistance to salt was determined by growing the test strains on MRS broth containing NaCl at 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10%. Bile tolerance was tested using MRS broth containing 10, 20, 30, and 40% bile. The growth of tested strains at pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 was determined in MRS broth after incubation at 30°C for 7 days.

Detection of antagonistic activity. An agar spot test and a well diffusion assay were used for detection of antagonistic activity (Schillinger and Lücke, 1989). In chickens, campylobacter, coliforms and salmonella are the main pathogenic microorganisms in the intestinal tract (Garriga et al., 1998). Therefore, the indicator strains used for this study were *Salmonella enteritidis* NIHE 642, *S. typhimurium* NIHE 689, *Escherichia coli* NIHE 105, and *Campylobacter jejuni* NIHE 972, obtained from the National Institute of Hygiene and Epidemiology, Vietnam.

Resistance to feed additives. Commercial feed products for chickens available in Vietnam markets such as HIGRO (Vietnam-Thailand joint venture) and PROCONCO (Vietnam-France joint venture) may contain some antibiotics. Thus, sensitivity to additives in different feed mixtures was also tested for probiotic strains. A solution of homogenized feed in distilled water (ratio 1 : 10 w/v) was obtained by centrifugation and filtration through a 0.22 μm-pore size cellulose acetate filter. The supernatant was adjusted to pH 6.5 and subjected to several doubling dilutions: 1, 1/2, 1/4, 1/8, 1/16, and 1/32. Fifty microliters of each diluent was added to each 5 mm-diameter well of an agar plate inoculated with 0.5 ml of an overnight culture of the tested strain (Schillinger and Lücke, 1989). The plates were incubated anaerobically for 24 h at 30°C and were subsequently examined for the zone of inhibition.

Preparation of inoculant and supplemented feed. Two probiotic strains were cultured in MRS broth aerobically at 30°C for 16 h. Bacterial cells were harvested by centrifugation at 3,000 rpm for 20 min at 40°C, washed with sterile saline solution and then resuspended in 10% skimmed milk solution. The cell suspension was freeze-dried for 48 h. For each strain, the dried cells obtained were mixed with commercial chicken feed to achieve the desired count of 10^6 CFU per gram of feed. The commercial feed used in this study was HIGRO containing 21% protein, 2% fat, 4% fiber, 14% moisture, 0.7% calcium, 0.5% phosphorus, and 0.3% NaCl.

Chicken feeding trial. Forty chicks (White Leghorn, 15 days after birth) were divided randomly into two groups (groups C and P). In group C, all chicks were bred conventionally, and in group P, the chicks were fed probiotic supplemented feed. The feed consumed by chicks in the two groups was recorded. The experiment was performed at a chicken farm in Hanoi, Vietnam.

Fecal sampling. Fresh chicken feces (0.3 g) was immediately collected, weighed and homogenized with 2.7 ml of an anaerobic diluent according to method of Mitsuoka et al. (1965). Sampling was performed at two stages, FD7 and FD40. Ten samples from each group were used for bacterial examination.

Bacterial analysis. Samples were serially diluted at 10^{-1} to 10^{-7} with the anaerobic diluents and 0.05 ml of each dilution was spread with a L-shaped glass rod on BL agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 5% horse blood as a non-selective medium for lactic acid bacteria, coliform and enterococci, and DHL (Eiken Chemical Co., Ltd., Tokyo, Japan) as a selective medium for enterobacteria. The BL agar plates for anaerobic bacteria were incubated at 37°C for 48 h in anaerobic steel-wool jars filled with 100% CO_2. The DHL agar plates were incubated aero-

bically at 37°C for 24 h. After incubation, colonies formed on the respective media were carefully observed, and the number of colonies was counted. For each colony on BL agar plates, a part of the colony was stained by Gram's staining for microscopic observation, another part was streaked on a BL agar plate for anaerobic growth and the rest was used for aerobic growth examination. For the bacterial species identified, the bacterial number per gram of wet feces was counted and converted into a logarithmic equivalent. The total bacterial count was calculated from the sum of the counts of each bacterial group.

Identification of lactic acid bacteria isolated from chicken intestine. Gram stains and morphology of isolated lactic acid bacteria were examined after 24 h of incubation on MRS agar. Catalase activity, nitrite reduction and gas production from glucose were determined following the method of Kozaki et al. (1992). Arginine hydrolysis, 0.04% tellurite tolerance, motility, pigment production, pyruvate utilization and bile-esculin hydrolysis tests for enterococci were examined according to Jean et al. (1980). The primary inoculant was prepared for the sugar fermentation test. The strains were cultivated on 5 ml of MRS broth at 30°C for 24 h, and cells harvested by centrifugation were resuspended in 2.5 ml of sterile saline solution (0.1% L-cysteine·HCl·H_2O, 0.1% sodium thioglycolate, 0.85% NaCl in 1 L of distilled water) for sugar test inoculation. Sugar fermentation patterns were examined using a semiautomatic system for bacterial identification (Benno, 1996). Twenty-two sugars (L-arabinose, D-xylose, rhamnose, sorbose, ribose, glucose, mannose, fructose, galactose, sucrose, maltose, cellobiose, lactose, trehalose, melibiose, raffinose, melezitose, starch, mannitol, sorbitol, esculin, and amygdalin) were subjected to a fermentation test in LB basal medium containing 0.1% (w/v) sugar. The LB basal medium was composed of 1,000 ml of 0.55% Bacto Liver (Difco Lab., Detroit, MI, USA) solution, 10 g of Proteose Peptone 3 (Difco Lab.), 5 g of Trypticase (BBL, Becton Dickinson Microbiol. Syst., Cockeysville, MD, USA), 3 g of yeast extract (Difco Lab.), 1 g of Tween 80, 5 ml of salt solution described bellow, and 0.2 g of L-cysteine HCl·H_2O. After the dissolving of all components, the pH of the liquid medium was adjusted to 6.5. The salt solution contained 10 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of $FeSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.3 g of $MnSO_4$, and 250 ml of distilled water.

Body weights. The chickens were weighed at intervals of ten days from the first probiotic feeding day (FD0) to the final probiotic feeding day (FD40).

Statistical analysis. Differences in bacterial counts and weight gain between the group P and group C were determined by Student's *t*-test (Microsoft Excel 97, Microsoft Corp., Redmond, Wash., USA). The same test was used to compare mean values for the samples (FD7 and FD40) and body weights (FD0, FD10, FD20, FD30, and FD40).

Results and Discussion

Studies on microbiota of the alimentary tract in animals have shown the complex of bacteria. Base on their roles, the intestinal bacteria may be divided into two groups: lactic acid bacteria and putrefactive bacteria. Lactic acid bacteria are evaluated as beneficial bacteria by their product of acids (particularly lactic acid), bacteriocin-like substances (Strus et al., 2001) or bacteriocins (Juven et al., 1992). They are not capable of decomposing proteins to cause putrefaction. Putrefactive bacteria are regarded as harmful bacteria in that they decompose proteins, produce foul-smelling substances and some cause diarrhea or enteritis or produce toxins (Mitsuoka, 1978). For these reasons, lactic acid bacteria are paid a great attention to be used as probiotics for animal productions.

For the chicken, the intestinal lactic acid bacteria are mainly *Lactobacillus* and *Enterococcus*. Among the lactobacilli, the *L. acidophilus* group (mainly *L. crispatus*, *L. gallinarum* and *L. johnsonii*), *L. agilis*, *L. salivarius*, and *L. reuteri* are commonly present in the chicken (Mitsuoka, 2002).

Probiotic characteristics of L. agilis JCM 1048 and L. salivarius subsp. salicinius JCM 1230 for chicken production

Probiotics often belong to the genera *Lactobacillus* spp. or *Enterococcus* spp. A good probiotic must fulfill some selection criteria (Nousiainen and Setälä, 1993) such as membership among normal intestinal microbiota, acid and bile tolerance, gut colonization, production of antimicrobial substances or bacteriocin. Then, it must easily to survive growth on a large scale, retain its viability under storage and field conditions, and be cost-effective to use for farm animals.

In this study, we examined *Lactobacillus* strains isolated from the chicken intestine, which are preserved in the Japan Collection of Microorganisms, RIKEN,

Table 1. Probiotic characteristics of *Lactobacillus agilis* JCM 1048 and *Lactobacillus salivarius* subsp. *salicinius* JCM 1230.

Species	*L. agilis*		*L. salivarius* subsp. *salicinius*	
JCM No.	1048	1049	1230	1047
Use as	Probiotic	Control	Probiotic	Control
Isolation source	Chicken intestine	Swine	Chicken intestine	Swine
Growth at 15°C	+	−	+	−
Growth at 45°C	+	w	+	−
Growth at initial pH 4.0	+	−	+	−
Tolerant to 6.5% NaCl	+	w	+	−
Tolerant to 40% bile	+	−	+	−
Resistant to feed additives (antibiotics)	+	+	+	+

Symbol: w, weak reaction.

Wako, Saitama, Japan, and finally selected two strains with excellent probiotic characteristics shown in Table 1. Two strains of *L. agilis* JCM 1048 and *L. salivarius* subsp. *salicinius* JCM 1230 were able to growth at 15 and 45°C, showed tolerance to 40% bile and 6.5% NaCl, grew at initial pH 4.0, and were resistant to feed additives. Salminen et al. (1999) commented that bile resistance and acid tolerance were important factors for candidate probiotic bacteria.

Mitsuoka (1978) revealed the difference in types of the bacteria in different animal species. Table 1 shows the differences in probiotic characteristics of *Lactobacillus* strains within the same species but of different origin. It demonstrates that probiotic strains for chicken must originate from the chicken.

Although *L. agilis* strain was first isolated from municipal sewage (Weiss et al., 1981), some strains of "*L. plantarum* var. *mobilis*" isolated from turkey feces (Harrison and Hansen, 1950) were only tentatively named. According to the original description and later investigations (Sharpe et al., 1973), this organism belonged to *L. agilis*. In our study, the probiotic *L. agilis* strain for chicken production originated from chicken intestine.

Two selected probiotic strains inhibited growth of *Salmonella* spp. (with an inhibition zone 16–18 mm in diameter) and *Campylobacter jejuni* (13–14 mm), but were less effective for *Escherichia coli* (7–8 mm) in the agar spot test . Gilliland and Spect (1977) reported that the antibacterial action produced by *Lactobacillus* was probably due to a combination of factors including acids, hydrogen peroxide and other inhibitory substances such as bacteriocins. However, Jin et al. (1996) claimed that the inhibitory activities of *Lactobacillus* spp. isolated from chicken intestine against salmonella and *E. coli* were not due to the production of hydrogen peroxide or bacteriocins, but probably due to the production of organic acids. In this study, we need more research to confirm whether antagonistic activities of our probiotic *Lactobacillus* strains are due to organic acids or any other bacteriocins which they may produce during their metabolization.

The importance of intestinal microbiota in resistance to salmonella colonization was confirmed by Nurmi and Rantala (1973). They showed that resistance could be restored by dosing newly hatched chicks with intestinal contents from healthy adult birds. Mead and Impey (1987) concluded that the lactobacilli (*L. acidophilus*, *L. fermentum*, and *L. salivarius*) and certain Gram-positive cocci were the most active organisms in the protection of chicks.

Effect of two probiotic Lactobacillus strains on the intestinal microbiota of chickens

The number of lactobacilli at feeding day seven (FD7) in group P was significantly higher ($p<0.05$) than that in control group C and the total bacterial counts in probiotic group P also increased as shown in Table 2. At feeding day forty (FD40), no differences between groups P and C were recorded for lactobacilli and enterococci. The enterobacterial count in group P was significantly reduced ($p<0.05$) in comparison with that in group C.

Probiotic lactobacilli had positive effects on the lactobacilli composition of young chickens in the first

Table 2. Effect of two probiotic strains on fecal microbiota in chickens.

Experimental group	Group C (control)		Group P (probiotic)	
Feeding days	FD7	FD40	FD7	FD40
Total counts	8.1±0.3[a] (9/9[b])	8.8±0.2 (9/9)	8.6±0.5[c] (9/9)	8.7±0.3 (10/10)
Lactobacilli	8.0±0.4 (9/9)	8.7±0.3 (9/9)	8.5±0.5[c] (9/9)	8.6±0.3 (10/10)
Enterococci	7.8±0.7 (9/9)	7.8±0.6 (6/9)	8.0±0.4 (9/9)	7.9±0.4 (7/10)
Enterobacteriaceae	7.2±0.9 (9/9)	7.9±0.2 (10/10)	7.5±0.2 (9/9)	7.5±0.2[d] (10/10)

[a] Data are expressed as means±SD of $\log_{10}$ bacterial counts per gram wet weight of feces.
[b] Numerators show numbers of chickens with microorganisms detected. Denominators show numbers of chickens examined.
[c] Statistically significant at the $p<0.05$ level when compared with the values obtained in group C at FD7.
[d] Statistically significant at the $p<0.05$ level when compared with the numbers obtained in group C at FD40.

stage of feeding (FD7): the number of lactobacilli in group P differed significantly ($p<0.05$) from that in group C as indicated in Table 2. When the chickens matured, the bacterial number reached the saturation level. The enterobacterial count was lower in group P than that in group C at FD40 because of the effect of probiotic strains on them due to their exclusive competition for essential nutrients and the antimicrobial substances produced. The enterococci count showed no significant differences between two groups at FD7 and FD40. The probiotic *Lactobacillus* strains appeared to have no effect on the enterococcal biota of the chicken intestine in this study.

Effect of two probiotic lactobacilli on the composition of Lactobacillus species

One hundred and sixty-three strains of lactobacilli were further characterized based on phenotypic characteristics following the method of Mitsuoka (1969); they were divided into the five groups (1–5) shown in Table 3. According to the sugar fermentation patterns, group 1 (30.7% of total lactobacilli isolated) consisted of four homo-fermentative subgroups (1a, 1b, 1c, 1d) exhibiting ability to ferment mannitol, which were identified as *L. agilis*. Group 2 (9.2%) was composed of two subgroups (2a, 2b) utilizing mannitol and sorbitol but not cellobiose, which were assumed to be *L. salivarius*. Group 3 (49.7%) consisting of a large number of isolates was able to metabolize esculin, salicin, and amygdalin and was divided into four subgroups (3a, 3b, 3c, 3d). Since *L. acidophilus* could not be differentiated from *L. gallinarum* on the basis of phenotypic characteristics (Fujisawa et al., 1992), they were designated as the *L. acidophilus/gallinarum* group in this study. Subgroup 1c showed the same characteristics as the probiotic strain of *L. agilis* and subgroup 2a resembled the probiotic strain of *L. salivarius* subsp. *salicinius* in phenotypic characteristics. Group 4 (9.2%) was able to ferment L-arabinose and produce gas from glucose, and was classified as *L. reuteri* and group 5 (1.2%) was assumed to be *Lactobacillus* spp.

Garriga et al. (1998) reported *L. salivarius* as the predominant species among intestinal microbiota of young chickens after isolation on Rogosa agar in the first step. On the other hand, Kawaguchi et al. (1990) determined that most isolates from chicken intestines belonged to the *L. acidophilus* group and *L. reuteri*. In this study, however, the *L. agilis* strain was found in more isolates than *L. salivarius*. The *L. acidophilus/gallinarum* group was the most common among the isolates, which was consistent with previous data (Kawaguchi et al., 1990).

The effects of the probiotic strains *L. agilis* JCM 1048 and *L. salivarius* subsp. *salicinius* JCM 1230 were investigated based on changes of the lactobacilli composition in treated chicken intestine. The results in Table 4 indicate that after 7 days of feeding trials the lactobacilli count markedly increased for all *Lactobacillus* species, and the counts of *L. agilis* in group P differed significantly ($p<0.05$) from those in group C.

After 40 days of feeding trials the lactobacilli counts showed no significant differences between two groups for any *Lactobacillus* species except that *L. reuteri* was not detected in group C. As mentioned above, the lactobacilli composition in the intestine of mature chickens may reach their highest numbers so that the probiotic strains might contribute to their presence in intestinal microbiota. An increasing incidence of *L. agilis* in

Table 3. Phenotypic characteristics of lactobacilli isolated from chicken intestine.

Lactobacillus group[a]	1				2		3				4			5	Probiotic strains[b]	
Subgroup	1a	1b	1c	1d	2a	2b	3a	3b	3c	3d	4a	4b	4c	5	P1	P2
Gas from glucose	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−
Acid from:																
L-Arabinose	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−
D-Xylose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Rhamnose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ribose	−	−	+	+	−	+	−	±	+	−	+	+	+	−	+	−
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Saccharose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	−	−	+	+	−	−	+	+	+	+	−	+	±	+	+	−
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	−	+	+	+	+	+	−	+	−	−	−	−	+	−	+	+
Melibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−
Starch	−	−	−	−	−	−	−	±	−	−	−	−	+	−	−	−
Mannitol	+	+	+	+	+	+	−	−	−	−	−	+	−	−	+	+
Sorbitol	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	+
Esculin	−	−	+	−	−	−	w	w	+	+	−	−	±	+	+	−
Salicin	−	−	+	+	−	−	+	+	+	+	−	−	+	−	+	−
Amygdalin	−	−	±	−	−	−	−	+	+	+	−	−	+	−	−	−

Symbol: −, no reaction, terminal pH 6.0–7.0; +, positive reaction, terminal pH <4.5; w, weak reaction, terminal pH 4.5–5.0; ±, negative reactions of few strains.

[a] Group 1 closed to species *L. agilis*; group 2 closed to *L. salivarius*; group 3 closed to *L. acidophilus/gallinarum*; group 4 closed to *L. reuteri*; group 5 supposed to be *Lactobacillus* ssp.

[b] P1, *L. agilis* JCM 1048; P2, *L. salivarius* subsp. *salicinius* JCM 1230.

Table 4. Effect of two probiotic strains on *Lactobacillus* distribution in chickens.

Species	Group C		Group P	
	FD7	FD40	FD7	FD40
L. agilis	7.0±0.5[a] (2/9[b])	8.2±0.4 (6/9)	7.8±0.4[c] (5/10)	8.0±0.6 (9/10)
L. salivarius	6.3 (1/9)	8.0±0.2 (6/9)	7.3±0.1 (3/10)	8.0±0.3 (2/10)
L. acidophilus/gallinarum group	7.5±0.3 (5/9)	8.0±0.6 (8/9)	7.9±0.5 (9/10)	8.0±0.3 (8/10)
L. reuteri	6.7±0.5 (2/9)	ND	7.6±0.5[c] (5/10)	7.8±0.7 (2/10)
Lactobacillus spp.	ND	8.0 (1/9)	ND	7.3 (1/10)

[a] Data are expressed as means±SD of $\log_{10}$ bacterial counts per gram wet weight of feces.

[b] Numerators show numbers of chickens with microorganisms detected. Denominators show numbers of chickens examined.

[c] Statistically significant at the $p<0.05$ level when compared with the values obtained in group C at FD7.

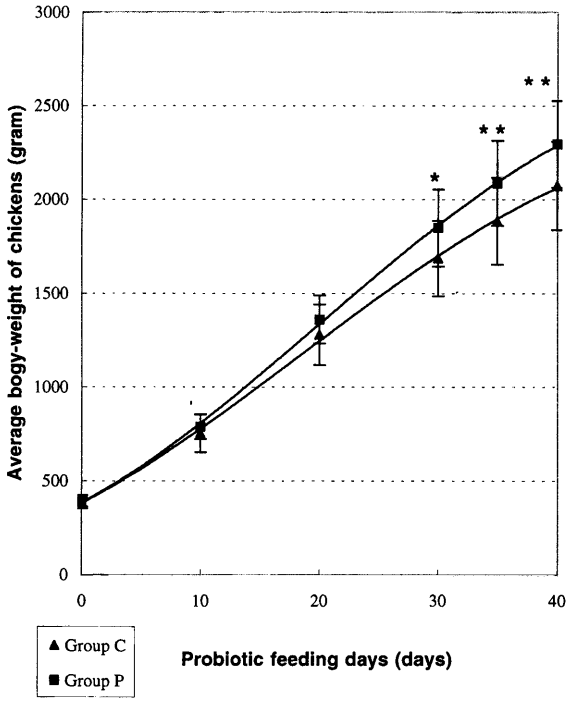

Fig. 1. Body-weight gains of chickens in the probiotic group P (■) and control group C (▲).

Each point represents the mean body weight of 20 chickens± SD. Differences in body weight are significant according to student's *t*-test (* $p<0.05$; ** $p<0.01$).

group P was observed in this study.

Effect of two probiotic lactobacilli on chicken production

The effects of two probiotic strains on chicken production were evaluated by weighing the chickens at intervals of 10 days. Some studies have indicated that there were no beneficial effects of probiotics on animals (Watkins and Kratzer, 1983, 1984), but in this study the body weights of probiotic-administered chickens were significantly increased ($p<0.05$) at FD30 and ($p<0.01$) at FD35 to FD40, in comparison with those of chickens fed a normal diet as shown in Fig. 1. The weight gain of group P at FD40 reached 10.7% compared with that of group C. Figure 1 expresses the weight increment of chickens from 15 days old (FD 0) to 55 days old (FD40). The average weights of chicken in group P (Y_p) can be represented by the following equation: $Y_p=-0.0117\times X^3+1.0261\times X^2+33.745\times X+383.3$, with the minimum square deviation $R^2=0.9995$; the average weights of chicken in group C (Y_c) are represented by: $Y_c=-0.0135\times X^3+0.7585\times X^2+33.351\times X+380.68$, with the minimal square deviation $R^2=0.999$, in which X is the number of feeding days.

Watkins and Kratzer (1983) reported that the oral administration of lactobacilli did not have a significant effect on early broiler chick growth and did not affect feed-to-gain ratios. However, the results in this study clearly demonstrated the significant increase in lactobacilli counts in general (Table 2), and the counts for *L. agilis* and *L. salivarius* in particular increased (Table 4) when chickens were administered with probiotic *Lactobacillus* strains mixed in feedstuff, resulting in weight gains recorded for chickens fed probiotics. We note that characteristics for probiotics were their tolerance to low pH and high concentrations of bile acid.

Fuller (1989) commented that probiotics enhance the growth and performance of animals, and the effects of two probiotic *Lactobacillus* strains on chickens were clearly demonstrated by the body weight gain in this study.

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