Improved growth performance due to hypertrophied intestinal absorptive epithelial cells by heat-killed *Lactobacillus sakei* HS-1 in broiler chickens¹

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ABSTRACT: This study investigated the effect of heat-killed *Lactobacillus sakei* HS-1 (HK LS HS-1) on the growth performance and intestinal histology of broilers through 2 feeding trials. In trial 1, 48 male broilers were separated into 3 groups: 1) basal diet (1 to 21 d, including antibiotics; 21 to 49 d, not including antibiotics) supplemented with 0 bacterial cells of HK LS HS-1/g (control); 2) 10⁶ bacterial cells were used; and 3) 10⁸ bacterial cells were used. Trial 2 was carried out in a tropical area of Thailand, where 50 7-d-old male broilers were separated into 2 groups: 1) basal diet (control group); and 2) basal diet supplemented with 10⁷ bacterial cells of HK LS HS-1/g. In trial 1, compared with the control, BW gain (linear; $P = 0.019$) and G:F (linear; $P = 0.032$) linearly increase with increasing HK LS HS-1 supplementation. In addition, 3 males died in the control group, while none died in the experimental group. In trial 2, growth performance was not significantly different between the groups. Observation of the gross anatomical visceral organs and intestinal histological parameters showed no difference among the groups. However, the weight of the ileum, total

small intestine, gizzard, and ceca decreased, while the height of the ileal villus increased with increasing HK LS HS-1 supplementation ($P < 0.05$). On the duodenal villus apical surface, protuberated cells, cells without microvilli, recently exfoliated regions on villus tips, and deeper cells at the sites of these recently exfoliated cells were more frequently observed in the experimental groups compared to the control; therefore, the duodenal epithelial cells of the experimental groups were hypertrophied by rapid cell turnover. On the ileal villus apical surface, morphological changes (i.e., cell shedding) were not observed, but protuberated cells were observed; thus, the ileal epithelial cells of the experimental groups were hypertrophied by prolonging the detention period of cells on the villus tip without shedding into the intestinal lumen. The results indicate that HK LS HS-1 improves the growth performance of broilers due to the presences of hypertrophied intestinal absorptive epithelial cells on the villus apical surface, which induce enhanced durability against environmental stress.

Key words: growth performance, heat-killed *Lactobacillus*, intestinal histology

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INTRODUCTION

The intestinal mucosa is continuously exposed to an immense load of antigens from feed ingested by poultry, potentially leading to the disruption of intestinal balance by infections, poor diet, and stress, which might cause instability in the intestinal microbiota. Viable probiotic supplementation has been reported to enhance the number and

integrity of intestinal microorganisms in poultry (Smirnov et al., 2005). *Lactobacillus sakei* HS-1 (LS HS-1) is isolated from kimchi and is used as a starter culture for Japanese pickles. Live LS HS-1 is difficult to preserve, and its effect is not constant; therefore, attempts have been made to produce heat-killed LS HS-1 (HK LS HS-1). Most studies have reported that heat-killed lactobacillus activates immune function (Erickson and Hubbard, 2000; Hirose et al., 2006; Cheon et al., 2011). However, knowledge about how heat-killed lactobacillus affects the intestinal morphology of poultry remains limited (Khonyoung and Yamauchi, 2012). Therefore, this current study aimed to investigate how HK LS HS-1 affects the growth performance of broilers, by examining intestinal villus histology (including villus height and cell mitosis numbers) and the absorptive epithelial cell morphology on the villus apical surface.

MATERIALS AND METHODS

Preparation of Heat-Killed *L. sakei* HS-1

Heat-killed *L. sakei* HS-1 (HK LS HS-1) was prepared by RYOSHOKU Co. Ltd. (Tokyo, Japan). *Lactobacillus sakei* HS-1 was prepared in a culture medium of Difco Lactobacilli MRS Broth (Becton, Dickinson and Company, USA) (Table 1) at 30 °C for 24 h. Fifty-five grams of Difco Lactobacilli MRS Broth were suspended in 1 L of purified water, thoroughly stirred, and sterilized at 121 °C for 15 min. Then, the culture medium was concentrated by filtering it through a ceramic membrane, and dry-heating it at 105 °C for 30 min to kill the bacteria. The HK LS HS-1 filtrate was freeze-dried and powdered. Furthermore, 10¹² bacterial cells were diluted with dextrin to 10⁹ bacterial cells of HK LS HS-1 per gram.

Table 1. Culture medium composition for heat-killed *Lactobacillus sakei* HS-1 (HK LS HS-1)

Formulated media (g/L)	Weight (g)
Chemical substances	
Proteose Peptone No. 3	10.0
Beef Extract	10.0
Yeast Extract	5.0
Dextrose	20.0
Polysorbate 80	1.0
Ammonium Citrate	2.0
Sodium Acetate	5.0
Magnesium Sulfate	0.10
Manganese Sulfate	0.05
Dipotassium Phosphate	2.0

Birds, Diet, and Housing

The protocol of trial 1 was approved by the Animal Care and Use Committee of Kagawa University, and the birds were handled according to the humane care guidelines provided by Kagawa University.

In trial 1, newly hatched male broilers (Marshall Chunky) vaccinated against Marek's disease and fowl pox were obtained from a commercial hatchery (KYOWA-FURAN Co., Ltd., Mitoyo, Japan). Conventional starter (1 to 21 d, including antibiotics) and finisher (21 to 49 d, not including antibiotics) diets were used as basal diets (Table 2). At 7 d of age, birds with similar weights were allotted to 3 groups: a basal diet group (control group) and 2 groups with basal diets containing 10⁶ or 10⁸ bacterial cells of HK LS HS-1 (from 10⁹ bacterial cells of HK LS HS-1). Each group had 4 replicates of 4 chickens. The chicks were raised in floor pens in a room with a mean temperature of 19.8 ± 4 °C under natural light in the day and continuous fluorescent lighting at night during the experimental period. Feed and water were available ad libitum throughout the experimental period. Feed intake and BW were monitored weekly until 49 d of age. At the end of trial 1, the weight of the gross anatomical visceral

Table 2. Composition of the basal diets

Item	Starter 1–21 d	Finisher 22–49 d
Ingredient, %		
Corn	59.00	42.00
Milo	2.00	22
Corn gluten meal	29.00	-
Soybean meal (45%CP)	-	27.00
Rice bran	2.30	5.20
Fish meal (57%CP)	7.00	3.00
Tallow	0.50	0.60
Vitamin/mineral premix ¹	0.20	0.20
Total	100.00	100.00
Calculated composition		
ME, kcal/kg	3,050.00	3,250.00
CP, %	22.00	18.00
Crude fat, %	4.00	6.00
Crude fiber, %	4.00	4.00
Crude ash, %	7.00	7.00
Ca, %	0.80	0.70
Available Phosphorus, %	0.50	0.45

¹Vitamin and mineral premix include per kilogram of diet: retinyl acetate, 2,880 µg; cholecalciferol, 48 µg; DL-α-tocopherol acetate, 35 mg; menadione, 2.6 mg; thiamine, 5.8 mg; riboflavin, 7.3 mg; pyridoxine, 10.4 mg; cobalamine, 12.6 µg; biotin, 0.2 mg; folic acid, 1.0 mg; pantothenic acid, 16.1 mg; niacin, 69.1 mg; choline, 1,400 mg; manganese, 92.4 mg; zinc, 79.9 mg; copper, 12.8 mg.

organs was determined, along with the intestinal histological parameters.

In trial 2, all experimental and animal management procedures were performed according to the Ethics, Principles and Guidelines for the Use of Animals for Scientific Purposes, National Research Council of Thailand.

In trial 2, the experiment was conducted in a tropical area of Thailand. Fifty 1-d-old newly hatched male broilers were obtained from a commercial hatchery (CP Co., Ltd., Thailand). The chicks were reared in an electrically heated brooder with a commercial starter basal diet. Conventional starter and finisher mash diets were fed to animals ages 7 to 21 and 22 to 49-d old, respectively. The chemical composition of basal diet was 21% CP and 3180 kcal/kg for 7 to 21 d of age, and 19% CP and 3,200 kcal/kg for 22 to 49 d of age. At 7 d of age, each chick was weighed. Then the chicks were separated into 2 groups with 5 replicates of 5 birds. The treatment groups consisted of: 1) a basal diet group (control group), and 2) a basal diet supplemented with 10^7 dietary LS-HS-1. The birds were provided feed and water ad libitum, and were reared on floor pens until 49 d of age during the summer and rainy season.

Tissue Sampling

At 49 d of age, 4 birds that were of average body weight (within ± 100 g of average BW) from each group were decapitated. The entire small intestine was then removed and processed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4). The same fixative solution was also infused in the intestinal lumen at low pressure. The duodenum extends from the gizzard to the pancreatic and bile ducts, the jejunum extends from the bile ducts to Meckel's diverticulum, and the ileum extends from the diverticulum to the ileocecal junction. A 2-cm length of the middle part of each intestinal segment was excised and prepared for light and scanning electron microscopy (SEM). Each segment was gently flushed with 0.1 M phosphate-buffered saline (pH 7.4) to remove the intestinal contents.

Light Microscopy

Light microscopy samples were excised immediately proximal to the block collected for SEM. The tissue samples were kept in Bouin's solution, embedded in paraplast wax after dehydration through varying concentrations of alcohol, and cut

into 4- μ m transverse sections. Every 10th section was collected and stained with hematoxylin-eosin following the method of [Samanya and Yamauchi \(2002\)](#). Eight sections per intestinal segment per bird were used to measure certain parameters using an image analyzer (Nis-Element D, Nikon Co., Tokyo, Japan).

Measurement of Villus Height and Area

Two villi that had a lamina propria were chosen in one section (8 sections per intestinal segment per bird). The villus length was measured from the villus tip to the bottom, not including the intestinal crypt. A total of 16 villi were counted from different sections in each bird. Sixteen villi were measured to obtain the mean villus height for each bird. Finally, the 4 mean villus heights from 4 birds were expressed as the mean villus height for one group. On the villus area, the villus width was also measured at the basal and apical parts of the same villi. The villus area was calculated from the villus height, basal width, and apical width according to the method of [Iji et al. \(2001\)](#). The mean villus area from 4 birds was combined to obtain a mean villus area for one group.

Measurement of Epithelial Cell Area

The middle portion of 2 villi was randomly selected for each cross section. The epithelial cell layer was measured, and then the number of cell nuclei within the measured epithelial cell layer was counted. Finally, the area of the cell layer was divided by the number of cell nuclei to obtain the epithelial cell area following the method described by [Samanya and Yamauchi \(2002\)](#). The area of 16 cells was calculated for each bird and combined to obtain the mean per bird. The means per bird were then combined to obtain a single mean for each group.

Measurement of Cell Mitosis Numbers in the Crypt

Mitotic cells with homogenous, intensely stained basophilic nuclei with hematoxylin were observed in the crypt ([Tarachai and Yamauchi, 2000](#)). For cells in the late stages of division, cell mitosis was counted as one mitotic event. All mitotic cells were counted in one section. Counts were conducted in 4 sections per bird and combined to obtain the mean mitotic cells per bird. The means per bird were then combined to obtain a single mean for each group.

Scanning Electron Microscopy

Samples for SEM were cut lengthwise along the line of the mesentery, opened, and flushed with 0.1 M phosphate-buffered saline (pH 7.4). These samples were pinned flat to prevent curling and were fixed vertically with the mucosal part facing downward in a mixture of 3% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature for 1 h. The tissue block was cut into 5 × 6 mm squares and were fixed for an additional 1 h. The pieces were rinsed with 0.1 M sodium cacodylate buffer (pH 7.4) and postfixed with 1% osmium tetroxide in ice-cold buffer for 2 h. Then, these pieces were washed in deionizing distilled water, stored in 45% ethanol for 24 h, and kept in 70% ethanol. Just before drying, specimens were transferred to 80, 90, and 100% ethanol solution (120, 60, and 15 min, respectively) and submerged in t-butyl alcohol (15 min; 3 times). Afterward, these specimens were freeze-dried (Hitachi ES-2030 freeze dryer, Hitachi Ltd., Tokyo, Japan). The freeze-dried specimens were mounted on aluminum stubs with electrically conducting carbon paste. They were then coated (E-1030 ion sputters, Hitachi Ltd.) and viewed under a scanning electron microscope (Hitachi S-4300SE/N, Hitachi Ltd.) at 8 kV. Morphological differences on the epithelial cells of the villus apical surface were compared.

Gross Anatomical Observations of Visceral Organs

At 56 d of age, 6 birds from each group were used to measure the length and width of the small intestine, in addition to the visceral organ weight.

After decapitation, whole visceral organs were removed. The weights of the heart, liver, proventriculus,

gizzard, and pancreas were measured. The whole small intestine from the gizzard to the large intestine was removed. The weights and lengths of the duodenum, jejunum, and ileum were recorded.

Statistical Analysis

In trial 1, the growth performance, visceral organ, protuberant cell on villus apical surface, and light microscopic parameter data were analyzed as a completely randomized design using the GLM procedures of SPSS 19.0 for Windows (SPSS 2010). Orthogonal contrasts were used to test the control vs. HK LS HS-1 and linear and quadratic equations were used to determine the different levels of HK LS HS-1. The pen was the experimental unit for growth performance.

In trial 2, the growth performance was compared using the Student's *t*-test. Significance level was set at $P < 0.05$, with a 95% confidence interval.

RESULTS

Growth Performance

In trial 1, compared with the controls, the birds in the HK LS HS-1 groups had similar feed intake (Table 3). Body weight gain (linear; $P = 0.019$) and G:F (linear; $P = 0.032$) linearly increased with increasing HK LS HS-1. At 4 and 5 wk of age, respectively, 1 and 2 birds died due to ascites disease in the control group. Three birds died in the control group (18.75%), while no birds died in the experimental groups.

In trial 2, feed intake, BW gain, and G:F were not different between groups. Eight birds died in the control group, while 6 birds died in the 10^7 HK LS HS-1 group (Table 4).

Table 3. Feed intake, BW gain, G:F, and mortality in broilers fed basal diet (control) with 10^6 and 10^8 bacterial cells of heat-killed *Lactobacillus sakei* HS-1 (HK LS HS-1) during 1 to 7 wk of age in trial 1 ($n = 4$)¹

Item	Control	Level of HK LS HS-1,		SE	Contrast ²		
		10^6	10^8		Control vs. HK LS HS-1	L	Q
FI ³ , g	6,337.50	6,591.88	6,492.81	58.77	0.112	0.277	0.163
Initial BW, g	163.00	163.31	163.56	-	-	-	-
Final BW, g	2,954.54	3,283.44	3,361.25	74.63	0.015	0.019	0.334
BW gain, g	2,791.54	3,120.13	3,197.69	74.83	0.016	0.019	0.337
G:F	0.44	0.47	0.49	0.01	0.040	0.032	0.695
Mortality (Number)	3	0	0	-	-	-	-

¹There were 4 pens per group, with 4 birds per pen.

²Orthogonal contrasts were performed to measure the effect of dietary HK LS HS-1. Control vs. 10^6 and 10^8 bacterial cells of HK LS HS-1; L = linear effect of HK LS HS-1 level; Q = quadratic effect of HK LS HS-1 level.

³Feed intake.

Gross Anatomical Observations of Visceral Organs in Trial 1

The relative values for intestinal length and visceral organ weight were determined for the control and HK LS HS-1 groups (Table 5). Dressing carcass percentage per BW tended to decrease with increasing HK LS HS-1 supplementation (linear; $P < 0.07$). The length of each intestinal segment, as well as total intestinal length, was not significantly different (linear or quadratic) among the groups. Although the weight of the duodenum and ileum was not significantly different (linear or quadratic) among the groups, the weight of the ileum (linear; $P < 0.001$), total small intestine (linear; $P < 0.03$), gizzard (linear; $P < 0.03$), and ceca (linear; $P < 0.05$) linearly decreased with increasing HK LS HS-1. The weight of other visceral organs (including the heart, liver, proventriculus, and pancreas) was not significantly altered (linear or quadratic equations).

Light Microscopic Observations of Intestinal Mucosa in Trial 1

Intestinal villus height, villus area, and cell area did not differ among the groups, except that ileal villus height increased in the HK LS HS-1 groups ($P < 0.05$) (Fig. 1).

Scanning Electron Microscopic Observations of Epithelial Cells on the Apical Surface of the Villus in Trial 1

In the duodenum (Fig. 2), protuberated epithelial cells (small arrows) were observed on the villus apical surface in all 3 groups; namely, the control (A), and 10^6 (B) and 10^8 (C) HK LS HS-1. In addition to cells without microvilli, some regions with recent cell exfoliation on the villus tip, as well as deeper cells at the sites of these recently exfoliated cells, were observed around the central sulcus (large arrows) in the experimental groups. In the jejunum (Fig. 3), cells without microvilli and deeper cells at the sites of recently exfoliated cells (large arrows)

were frequently observed, in addition to protuberated cells (small arrows) in all 3 groups [i.e., control (A), 10^6 (B), and 10^8 (C) HK LS HS-1]. The control group had flat cells, resulting in the villi having smooth apical surfaces because of a faint cell outline between each cell. The 10^6 and 10^8 HK LS HS-1 groups had clear cell outlines, due to the protuberated cells (small arrows), resulting in a rough surface. In the ileum (Fig. 4), the control (A) group had many flat cells, but few protuberated cells (small arrows). Most cells of the 10^6 (B) and 10^8 (C) HK LS HS-1 groups were protuberated cells, resulting in a rough surface. Segmented filamentous bacteria were observed in all groups, representing a characteristic feature. Compared with control, the birds in HK LS HS-1 tended to be greater protuberant cells on duodenal and jejunal villus apical surface (Table 6).

DISCUSSION

Growth Performance

Compared with the control, the BW gain and feed efficiency increased in the dietary HK LS HS-1 groups in trial 1. This result was consistent with a previous study that inactive lactobacillus increased BW gain in broiler chickens (Huang et al., 2004). In addition, the mortality of all experimental groups was zero, while that of the control was 3 birds. As the BW of these dead birds was similar to that of the other control birds (including abdominal dropsy, and lack of vaccination), they may have died suddenly due to cold stress. However, none of the birds fed HK LS HS-1 supplemented diets died. Thus, HK LS HS-1 might function to enhance endurance against environmental stress, resulting in improved growth performance due to hypertrophied intestinal epithelial cells. Mortality in trial 2 was high due to heat stress (temperature of around 34 to 37 °C from 11:00 to 16:00). Most birds in feeding trial 2 had died by the last week of experiment (42 to 49 d of age).

Table 4. Feed intake, BW gain, G:F, and mortality in broilers fed 0 (control) and 10^7 dietary bacterial cells of heat-killed *Lactobacillus sakei* HS-1 (HK LS HS-1) during 7 to 49 days old in trial 2 ($n = 5$)¹

Item	Control	10^7 dietary LH-HS-1	P-value
FI ² (g)	3,253.58 ± 100.93	3,428.15 ± 164.43	0.107
BW gain (g)	2,045.85 ± 112.43	2,145.31 ± 135.58	0.278
G:F	0.629 ± 0.027	0.626 ± 0.038	0.916
Mortality (number)	8	6	-

¹There were 5 pens per group, with 5 birds per pen.

²Feed intake.

Table 5. Intestinal length and weight of visceral organs in broilers fed basal diet (control) with 10^6 , and 10^8 bacterial cells of heat-killed *Lactobacillus sakei* HS-1 (HK LS HS-1) at 8 wk of age in trial 1 (per 100 g BW, $n = 6^1$)

Item	Control	Level of HK LS HS-1		SE	Contrast	
		10^6	10^8		L	Q
BW before kill, g	3,698.33	3,927.50	3,898.33	81.72	0.69	0.89
Carcass, %BW	79.91	76.97	76.64	0.57	0.07	0.40
Length, cm/100 g BW						
Duodenum	0.78	0.86	0.82	0.03	0.62	0.36
Jejunum	2.00	1.92	1.91	0.05	0.55	0.79
Ileum	2.07	2.11	1.97	0.06	0.59	0.55
Total small intestine	4.85	4.90	4.71	0.14	0.69	0.70
Weight, g/100 g BW						
Duodenum	0.46	0.49	0.39	0.02	0.18	0.14
Jejunum	0.91	0.86	0.84	0.03	0.48	0.86
Ileum	0.74	0.72	0.52	0.03	0.001	0.75
Total small intestine	2.10	2.07	1.75	0.07	0.03	0.26
Heart	0.62	0.54	0.64	0.02	0.63	0.09
Liver	2.02	2.25	1.95	0.13	0.85	0.39
Proventriculus	0.30	0.28	0.25	0.02	0.29	0.77
Gizzard	1.10	1.02	0.95	0.03	0.03	0.84
Pancreas	0.15	0.15	0.14	0.01	0.66	0.49
Ceca	0.33	0.30	0.26	0.01	0.05	0.93

¹There were 6 birds per group.

²Orthogonal contrasts were performed to measure the effect of dietary HK LS HS-1. Control vs. 10^6 and 10^8 bacterial cells of HK LS HS-1; L = linear effect of HK LS HS-1 level; Q = quadratic effect of HK LS HS-1 level.

Gross Anatomical Observations of Visceral Organs

The weight of the intestines, gizzards, and ceca of chickens fed dietary HK LS HS-1 decreased. Previously, an increase in gizzard weight was observed (Santos et al., 2006), due to the increase in grinding necessary to process the particle size of whole grains (Gabriel et al., 2003) in chickens fed whole grain. In comparison, the HK LS HS-1 used in the current size would not need to activate gizzard, resulting it being small. Previously, low intestinal weight was observed in both chickens fed a high-protein diet (Buwjoom et al., 2010; Incharoen et al., 2010a), chickens fed a dietary, transformed *Lactobacillus* strain (Siew et al., 2005), and chickens fed a dietary antibiotic of virginiamycin (Miles et al., 2006). *Lactobacillus sake* was reported to produce hydrogen peroxide (H_2O_2) in response to pathogenic microorganisms and *Listeria innocua* via a screening test (Ammor et al., 2006). A bacteriocin-producing strain of *L. sake* L45 and 148 has the biochemical characterization of an antibiotic (Champomier-Vergès et al., 2002). Thus, the low intestinal weight documented in our study might be related to the development of digestion, which responded in an adaptive manner due to the ingestion of HK LS HS-1. Detailed observations of each intestinal segment showed that the effect of

dietary protein was more pronounced in the duodenum compared to the jejunum and ileum of the small intestine. Thus, duodenum weight may be important when evaluating BW gain in young broilers (Wijten et al., 2010). A previous study on pigs reported that the proximal small intestine is more dependent on luminal nutrient supply for intestinal protein synthesis compared with the distal small intestine (Stoll et al., 2000). Also, the weight of the duodenum in the 10^6 dietary HK LS HS-1 group of the current study was greater than that of the control (not significantly). In contrast, the ileum was significantly lighter compared to the control. Therefore, microscopic analysis revealed detailed changes of each intestinal segment.

Intestinal Histology

The proximal small intestine is an important site for protein digestion and absorption (Sklan and Hurwitz, 1980). Previously, broiler fed enhanced dietary amino acids improved BW gain due to increased duodenum weight (Wijten et al., 2010). Broiler duodenum has the greatest gene expression of intestinal peptide transporter than any other intestinal region (Chen et al., 1999), and the expression of nutrient transporter mRNA is enhanced after feeding broilers with high-quality

Figure 1. Villus height, villus area, cell area, and cell mitosis of duodenum, jejunum and ileum in broilers fed a basal diet supplemented with 0 (control), 10^6 and 10^8 bacterial cells of heat-killed *Lactobacillus sakei* HS-1 at 7 wk of age in trial 1. Bar represents the mean of 4 birds.

dietary protein (Gilbert et al., 2008). In a previous study by our group, we found that epithelial cells on the duodenal villus apical surface atrophied in chicken fed low-CP diet (9.4% CP), while those in the jejunum and ileum did not change (Incharoen et al., 2010a). Thus, duodenal morphology is sensitive to the type of feed ingredients. In comparison, as the remnant ileal morphology show

dramatic compensatory enlargement of villi after 50 to 80% jejunal resection, ileal villi adapt to activated intestinal absorptive function (Yamauchi et al., 2010a).

Intestinal villi are covered by single columnar epithelial cells. These cells proliferate in the intestinal crypt and migrate upward to the villus tip (Imondi and Bird, 1966), where they are shed into the intestinal lumen within 48 h after generation (Potten, 1998). Therefore, the components of the intestinal villus parameters could be used as indicators to assess villus function. Cell morphology was also used as an indicator to judge feed supplementation. Flat cells are clearly evident when intestinal function is atrophic, whereas protuberated cells are observed when intestinal function is hypertrophic (Yamauchi et al., 2010b). All birds showing an increase in BW gain had clearly protuberated cells after being fed dietary sugar cane extract (Khambualai et al., 2010), dried fermented ginger (Incharoen et al., 2010b), and bamboo charcoal powder, including vinegar liquid (Yamauchi et al., 2010b). The protuberated cells, area cells without microvilli, and deeper cells at sites of recently exfoliated cells were much more apparent in chicken fed a high-protein diet compared to those fed a low-protein diet (Buwjoom et al., 2010). A recent study showed that protuberated cells and deeper cells at the sites of recently exfoliated cells were activated in broilers (Khonyoung et al., 2015) and piglets (Maneewan et al., 2014). Microscopic analysis in the current study showed that high (but not significant) levels of cell mitosis occurred in the duodenum in the HK LS HS-1 groups, but that villus height remained similar among groups. Electron microscopic observations showed that deeper cells at sites with recently exfoliated cells were frequently detected in the HK LS HS-1 groups. Therefore, duodenal epithelial cells might react to activated intestinal function by quick cell turnover. In contrast, lower (but not significant) levels of mitosis occurred in ileal cells; however, villus height increased (linear; $P < 0.05$) in the HK LS HS-1 groups. On the villus apical surface, only protuberated cells were distributed, while recently exfoliated phenomena were not observed. Therefore, ileal epithelial cells probably react to activate intestinal functioning by remaining on the villus tip for a long time. However, it is unclear why this phenomenon was induced, but it is possible that the HK LS HS-1 used in the current study prolongs the retention period of intestinal cells on the villus tip, without their shedding into the intestinal lumen. Probiotic (*Lactobacillus fermentum*) and antibiotic (aureomycin) treatments inhibit cellular

Figure 2. Duodenal villus apical surface of broilers fed a basal diet (control, A) or diets supplemented with 10^6 (B), and 10^8 (C) bacterial cells of heat-killed *Lactobacillus sakei* HS-1 at 7 wk of age in trial 1. Small arrows; protuberated cells, large arrows; cells without microvilli, some recently cell exfoliated regions on the villus tip and deeper cells at the sites of recently exfoliated cells. Bar length represents 50 μm .

Figure 3. Jejunal villus apical surface of broilers fed a basal diet (control, A) or diets supplemented with 10^6 (B), and 10^8 (C) bacterial cells of heat-killed *Lactobacillus sakei* HS-1 at 7 wk of age in trial 1. Small arrows; protuberated cells, large arrows; cells without microvilli, some recently cell exfoliated regions on the villus tip and deeper cells at the sites of recently exfoliated cells. Bar length represents 50 μm .

apoptosis in piglets (Wang et al., 2012). In addition, heat-killed probiotics (*Lactobacillus rhamnosus* GG) have similar advantages to live probiotics in the intestine of infant rats (Li et al., 2009). Thus, HK LS HS-1 might prolong the retention period of intestinal cells on the villus tip.

In conclusion, HK LS HS-1 appears to improve the growth performance of broilers through

the presence of hypertrophied intestinal absorptive epithelial cells on the villus apical surface. This phenomenon is driven by quick cell turnover in the duodenum and by the prolonged retention period of cells on the villus tip of the ileum. As a result of these morphological changes, the resilience of broilers is enhanced against environmental stress.

Figure 4. Ileal villus apical surface of broilers fed a basal diet (control, A) or diets supplemented with 10^6 (B), 10^7 , and 10^8 (C) bacterial cells of heat-killed *Lactobacillus sakei* HS-1 at 7 wk of age in trial 1. Small arrows: protuberated cells. Bar length represents 50 μ m.

Table 6. Protuberant cell on villus apical surface of broilers fed basal diet (control) with 10^6 and 10^8 bacterial cells of heat-killed *Lactobacillus sakei* HS-1 (HK LS HS-1) at 7 wk of age in trial 1 ($n = 4$)¹

		Level of HK LS HS-1			Contrast ²		
Item	Control	10 ⁶	10 ⁸	SE	Control vs. HK LS HS-1	L	Q
Protuberant cell (cell/ μm ²)							
Duodenum	0.017	0.028	0.027	0.003	0.070	0.121	0.282
Jejunum	0.015	0.021	0.023	0.002	0.093	0.107	0.547
Ileum	0.033	0.032	0.035	0.003	0.991	0.895	0.797

¹There were 4 birds per group.

²Orthogonal contrasts were performed to measure the effect of dietary HK LS HS-1. Control vs. 10^6 and 10^8 bacterial cells of HK LS HS-1; L = linear effect of HK LS HS-1 level; Q = quadratic effect of HK LS HS-1 level.

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