Selected microbial groups and short-chain fatty acids profile in a simulated chicken cecum supplemented with two strains of *Lactobacillus*

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ABSTRACT Among the bacterial fermentation end products in the chicken cecum, butyrate is of particular importance because of its nutritional properties for the epithelial cell and pathogen inhibitory effects in the gut. An in vitro experiment, operated with batch bioreactor, was conducted to quantify butyric-producing bacteria in a simulated broiler cecum supplemented with *Lactobacillus salivarius* ssp. *salicinius* JCM 1230 and *Lactobacillus agilis* JCM 1048 during 24 h of incubation. Selected bacterial species were determined by real-time PCR and short-chain fatty acids and lactate concentrations were monitored. The results showed that after 24 h of incubation, *Lactobacillus* supplementation significantly increased the number of lactobacilli, bifidobacteria and *Faecalibacterium prausnitzii* in medium containing cecal content and lactobacilli supplementation (Cc + L) compared with the control (Cc). Addition of lactobacilli did not alter *Escherichia coli* and *Clostridium butyricum*, whereas it significantly ($P < 0.05$) reduced *Salmonella* in treatment Cc + L compared with the Cc treatment. Propionate and butyrate formation were significantly ($P < 0.05$) increased in treatment Cc + L as compared with the Cc treatment. Lactate was only detected in treatment containing 2 *Lactobacillus* strains. After 24 h of incubation, acetate concentration significantly ($P < 0.05$) decreased in all treatments. It was suggested that lactate produced by *Lactobacillus* in the cecal content improved the growth of butyric producers such as *F. prausnitzii,* which significantly increased butyrate accumulation. Additionally, the results showed that butyrate and propionate inhibited *Salmonella* without influencing the *E. coli* profile.

Key words: microbial group, short-chain fatty acid, chicken cecum

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INTRODUCTION

Within the gastrointestinal tract of poultry, cecum is relatively stable and harbors the biggest collection and a wide variety of microbial species (Mead, 1997). Bacterial fermentation in the cecum leads to the formation of short-chain fatty acids, which are necessary for metabolism of the intestinal epithelial cells. Among short-chain fatty acids, increasing attention has been focused on butyrate. Previous study showed the blocking of β-oxidation inhibited butyrate consumption in the colonocytes, which ultimately resulted in ulcerative colitis due to deficiency in butyrate utilization in ileal and colonic mucosa (Roediger and Nance, 1986), whereas glucose and glutamate were detected to be normal in patients with colitis. Butyrate is a major source of energy for enterocytes and colonocytes (Chapman et al., 1995; Ahmad et al., 2000) and has a fundamental role in maintaining a healthy gastrointestinal tract. Short-chain fatty acids are also involved in prevention of diarrhea (water and Na^+ absorption), pH control within the gastrointestinal tract, and defense against pathogens (colonization resistance). It has been shown that short-chain fatty acids inhibited the growth of *Salmonella* (Van Immerseel et al., 2003). Lawhon et al. (2002) reported that butyrate and propionate were more efficient in inhibiting *Salmonella* Typhimurium, whereas other studies observed that acetic acid was more effective (Van der Wielen et al., 2000). Anaerobic bacteria in the cecum play a major role in fermentation and metabolic end products. Random cloning and sequencing of 16S rDNA genes isolated from the chicken cecum revealed that the cecum was dominated by a large group of nonculturable bacteria most closely related to *Faecalibacterium prausnitzii,* a species that produces butyric acid in the human gut (Bjerrum et al., 2006). Previous studies in human such as those conducted by Duncan et al. (2004) reported that bifidobacteria stimulated the

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proliferation of butyric acid-producing bacteria by the mechanism called cross feeding. Manipulation of broiler cecal fermentation, using *Lactobacillus* supplementation, might improve butyric accumulation in the cecum and hence improve gastrointestinal tract development and prevent disease. In the current study, the potential of *Lactobacillus salivarius* ssp. *salicinius* JCM 1230 and *Lactobacillus agilis* JCM 1048 on the proliferation of *F. prausnitzii* and *Clostridium butyricum* as butyric producers were evaluated in a simulated broiler cecum. Furthermore, the inhibitory effect of these 2 lactobacilli on the growth of *Escherichia coli* and *Salmonella* was also investigated.

MATERIALS AND METHODS

Cecal Sample

Ten 42-d-old chickens, which were raised on a litter floor pen and fed antibiotic-free diet, were randomly chosen from the broiler house at the Department of Animal Science, University Putra Malaysia. The chickens were killed by cervical dislocation and the ceca of each bird were collected aseptically, tied from open sides, placed into an empty sterile plastic bag on ice, and immediately transported to the laboratory. To obtain a uniform sample, cecal contents were prepared in an empty preweight sterile bottle and were mixed with 10% (wt/vol) prereduced anaerobic phosphate buffer, following Bryant and Robinson (1961).

Lactobacillus Strains Used as Inoculants and Their Culture Conditions

Lactobacillus salivarius ssp. *salicinius* JCM 1230 and *L. agilis* JCM 1048 of chicken origin were obtained from the Japan Collection of Microorganisms. Their probiotic characteristics and effects on chicken production have been reported previously (Lan et al., 2004). Each strain was inoculated into MRS broth (Oxoid, Basingstoke, UK) and incubated overnight at 37°C.

Batch Culture Fermentations

Six stirred batch culture fermentation vessels were filled with 450 mL (vessels 1 and 2), 430 mL (vessels 3 and 4), and 480 mL (vessels 5 and 6) of a nonselective medium, modified from that of Bryant and Robinson (1961). The vessels were assigned to one of the following treatments; vessels 1 and 2 filled with medium containing cecal content (**Cc**), vessels 3 and 4 filled with medium containing cecal content and lactobacilli supplementation (**Cc + L**), and vessels 5 and 6 filled with medium containing lactobacilli supplementation (**L**). The basal nutrient medium contained the following: rumen fluid, 600 mL/L; glucose, 0.5 g/L; cellubiose, 0.5 g/L; soluble starch, 0.5 g/L; trypticase, 2 g/L; yeast extract, 0.5 g/L; hemin, 0.025 g/L; Tween-80, 2 mL/L; volatile fatty acids mixture (acetic, 17 mL; propionic, 6 mL; butyric, 4 mL; isobutyric, 1 mL; and n-valeric, isovaleric, and DL-α-methylbutyric, 1 mL each), 3.1 mL/L; $Na_2S{\cdot}9H_20$, 0.5 g/L; cysteine HCl, 0.5 g/L; Na_2CO_3, 4 g/L; K_2HPO_4, 0.225 g/L; KH_2PO_4, 0.225 g/L; $(NH_4)_2SO_4$, 0.45 g/L; NaCl, 0.45 g/L; $MgSO_4$, 0.045 g/L; and $CaCl_2{\cdot}6H_2O$, 0.045 g/L. The final volume (1,000 mL) was adjusted with distilled water. Resazurin (0.001 g/L) was added to the growth medium to act as an indicator of anaerobicity and the vessels were autoclaved at 121°C for 15 min. Bioreactor pH was kept at 5.8 by automatic control through 1 *M* HCl and NaOH. The temperature was maintained at 41°C to simulate the actual temperature in broiler cecum. Anaerobic environmental condition was created with oxygen-free nitrogen flushing into the medium (15 mL/min). Treatments Cc and Cc + L were then inoculated with 50 mL of 10% (wt/vol) cecal slurry prepared in the basal nutrient medium. After that, 10 mL of the basal nutrient medium containing 10^7 cfu *L. salivarius* ssp. *salicinius* JCM 1230 and 10 mL of the basal nutrient medium containing 10^7 cfu *L. agilis* JCM 1048 were inoculated into the Cc + L and L treatments. The batch cultures were run for 24 h, and samples were taken at the start and end of the incubations (T_0 and T_{24}) for the bacterial enumeration and short-chain fatty acids analysis.

Short-Chain Fatty Acids and Lactate Analysis

The samples were centrifuged at 24,000 × *g* for 10 min and the supernatant (20 μL) was injected onto an HPLC system attached to a UV detector at 220 nm as described by Liong and Shah (2006). The column was an ion-exclusion Aminex HPX-87H 300 × 7.8 mm i.d. (Bio-Rad, Hercules, CA) maintained at 62°C. The eluent, 0.009 *M* sulfuric acid, was pumped through the column at a flow rate of 0.6 mL/min. The concentration of lactate, acetate, propionate, and butyrate in the sample was determined using external calibration curves.

The net short-chain fatty acids and lactate production (NS_p) during the 24-h fermentation is calculated by the following equation:

$$NS_p = S\ (T_{24}) - S\ (T_0),$$

where S (T_{24}) is the short-chain fatty acids and lactate concentration at 24 h of incubation and S (T_0) is the initial concentration. A negative value indicates a net loss.

Bacterial Composition Analysis

Selected bacterial gene copy numbers in the fermentation samples from different treatment was determined using the molecular method. Duplicate samples were taken at the start (T_0) and the end of incubation (T_{24}). After DNA extraction, 6 genera and species of predominant beneficial, pathogen, and butyric producers in the

chicken cecum (Table 1) were detected and quantified using the quantitative real-time PCR method. The gene copy number concentration in the samples was determined using standard curves generated from known concentrations of plasmid DNA that carried targeted genes for each genus and species isolated from pure bacterial culture.

Bacterial Strains and Culture Conditions

The strains used for the preparation of DNA standard for real-time PCR analysis were obtained from the American Type Culture Collection and culture collection of the Basic Biotechnology Laboratory, Faculty of Biotechnology and molecular science, University Putra Malaysia. The bacteria used were *Bifidobacterium pseudocatenulatum* G4, *E. coli* ATCC 8739, *C. butyricum* ATCC 19398, *F. prausnitzii* ATCC 27768, *L. crispatus* ATCC 33820, and *Salmonella enterica* ssp. *enterica* serovar Enteritidis ATCC 13067.

Twenty-milliliter serum bottles were filled with 10 mL of Wilkins-Chalgren broth (Oxoid). The bottles were flushed with nitrogen gas, immediately closed with a stopper, sealed, and autoclaved at 121°C for 15 min. Under anaerobic condition, 10^7 cfu of each *B. pseudocatenulatum* G4, *C. butyricum,* and *F. prausnitzii* were inoculated separately into the prepared serum bottles. The bottles were then incubated at 37°C for 48 h. Both *Salmonella* and *E. coli* were inoculated into Wilkins-Chalgren broth (Oxoid) and lactobacilli into MRS broth (Oxoid), and all were incubated aerobically overnight at 37°C.

DNA Extraction from Bacterial Cultures

Bacterial cultures (8 mL) were centrifuged at 6,000 × *g* for 10 min and the supernatant was discarded. Bacterial DNA was extracted using Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) used by Ahmed et al. (2008). The DNA yield from pure culture determined by measuring the concentration of DNA in the eluate using a spectrophotometer (U-2800, Hitachi, Tokyo, Japan) was set for the wavelength at 260 nm, and the ratio of absorbance at the wavelength of 260 and 280 nm revealed the purity of the extract. The obtained DNA was stored at −40°C for PCR experiments.

DNA Extraction from Cecal Content

The DNA was extracted from 10 mL of cecal slurry by using a QIAamp DNA Stool Mini Kit (Bartosch et al., 2004) according to the instructions of the manufacturer (Qiagen). Cells collected by centrifugation at 18,000 × *g* (10 min) were resuspended in 1.4 mL of ASL lysis buffer (provided in the kit) and thoroughly homogenized followed by heating at 95°C for 5 min. Samples were vortexed and centrifuged at 18,000 × *g* for 1 min to pellet stool particles. The supernatant was pipetted (1.2 mL) into a new 2-mL microcentrifuge tube and the pellet was discarded. The DNA-damaging substances and PCR inhibitors were removed from the supernatant containing the DNA by using the InhibitEX tablets provided in the kit. Protein digests and DNA purifications were done by using QIAamp spin columns, according to the instructions of the manufacturer. The concentration of DNA was determined by using a spectrophotometer (U-2800, Hitachi) as described previously. The obtained DNA was stored at −40°C until use.

Primers and PCR Amplification

All primer sets used are listed in Table 1. Different 16S rRNA related genera and species primers were used to quantify predominant bacterial groups in a simulated chicken cecum and only the sequence of the 16S/23S rRNA intergenic spacer region described by Dubernet et al. (2002) was used to quantify lactobacilli at the genus level. The specificities of the sequences for the target organisms were checked with an online tool provided by the Ribosomal Database Project (Cole et al., 2003). The primers were supplied by First Base Company (Selangor, Malaysia). Amplification reaction was performed in a total volume of 25 μL containing 5 μL of PCR buffer (1×), 1.5 μL of $MgCl_2$ (1.5 m*M*), 0.5 μL of deoxynucleoside triphosphate (0.2 m*M*), 0.5 μL of

Table 1. Primer sets used in this study

Target organism	Primer set	Sequence (5′ to 3′)	Product size (bp)	Annealing temperature (°C)	Reference or source
Bifidobacterium genus	Bifi F	CTCCTGGAAACGGGTGG	551	55	(Matsuki et al., 2003)
	Bifi R	GGTGTTCTTCCCGATATCTACA			
Faecalibacterium prausnitzii	Fprau223F	GATGGCCTCGCGTCCGATTAG	198	58	(Bartosch et al., 2004)
	Fprau420R	CCGAAGACCTTCTTCCTCC			
Clostridium butyricum	Cbut825F	GTGCCGCCGCTAACGCATTAAGTAT	208	72	(Bartosch et al., 2004)
	Cbut1038R	ACCATGCACCACCTGTCTTCCTGCC			
Lactobacillus genus	LbLMA-rev	CTCAAAACTAAACAAAGTTTC	225	56	(Dubernet et al., 2002)
	R16–1	CTTGTACACACCGCCCGTTCA			
Escherichia coli genus	Eco F	GACCTCGGTTTAGTTCACAGA	585	61	(Candrian et al., 1991)
	Eco R	CACACGCTGACGCTGACCA			
Salmonella genus	Sal201 F	CGGGCCTCTTGCCATCAGGTG	398	62	(Amit-Romach et al., 2004)
	Sal 597 R	CACATCCGACTTGACAGACCG			

Taq polymerase (2.5 U), 0.5 μL of each forward and reverse primer (0.2 μ*M*), 0.5 μL of DNA template, and 16 μL of sterile distilled water. To determine the optimal annealing temperature for each primer set and testing the specificity of each primer set, a Mastercycler gradient PCR machine (Eppendorf, Hamburg, Germany) was applied. The initial denaturation was performed at 95°C for 3 min and the target DNA was amplified in 40 cycles with a DNA denaturation step at 95°C (30 s), followed by an annealing step (55 s) and elongation step at 72°C (1 min). The PCR was completed with a final elongation step at 72°C (5 min).

Cloning the Target Sequence to the Competence Cell

Polymerase chain reaction products of the different primer sets were purified by using a QIAquick Spin PCR Purification Kit (Qiagen) as described by Bartosch et al., 2004. The purified PCR product was cloned into the pDrive Vector by use of a Qiagen PCR cloning kit (Qiagen) by mixing all of the ligation components (1 μL of PDrive cloning vector, 50 ng/μL; 5 μL of Ligation Master Mix, 2×; 3 μL of purified PCR products; and 1 μL of distilled water) into the tube and incubating at 15°C for 1 h. Afterward, the plasmid vector that contained the target gene was transformed into the competence *E. coli* JM109 cells by heat shock at 42°C. White colonies containing the correct plasmid insert were picked using a sterile toothpick and were inoculated in 10 mL of Luria-Bertani broth supplemented with 200 mg/L of ampicillin. The cultures were incubated in a shaker incubator at 37°C for 18 h and stored at 4°C until plasmid DNA extraction.

Plasmid DNA Extraction

Plasmid DNA was purified by using the QIAprep Spin Miniprep Kit (Gabitzsch et al., 2008) according to the instructions of the manufacturer (Qiagen). The plasmid DNA concentration was determined by electrophoresis and a comparison of band strengths against 100 bp of molecular marker DNA ladder (Promega).

Generation of Standard Curves

Standard curves for quantification of bacterial cells in the samples were created using a known concentration of plasmid DNA containing the target gene. The concentration of extracted plasmid DNA (g/μL) was measured using the spectrophotometer method at 260 nm of absorbance. Afterward, the plasmid DNA was 10-fold serially diluted and from each dilution, 5 μL was mixed with PCR mixture. The target genes were amplified in triplicate by real-time PCR using the same primers in the conventional PCR. The concentration of plasmid DNA was converted from grams per microliter in stock solution to copies per microliter in PCR mixture.

Real-Time PCR

Real-time PCR amplification for detection and enumeration of cecal bacterial DNA was performed in a computer-operated Rotorgene 3000 real-time PCR system (Corbett Research, Mortlake, New South Wales, Australia). In real-time PCR assay, SYBR Green Master Mix (Qiagen) was used as a reporter dye. The DNA template that was already extracted from the fermentation sample was mixed with the other real-time PCR components in one 200-μL PCR tube. The PCR mixture (25 μL) contained the following components: sterile distilled water, 8.5 μL; forward and reverse primers, each 1 μL (0.5 μ*M*); SYBR Green PCR Master Mix, 9.5 μL; and DNA template, 5 μL. Amplification reactions were carried out in duplicate with QuantiFast SYBR Green PCR Master Mix containing Hot Star Taq Plus DNA Polymerase, QuantiFast SYBR Green PCR buffer, deoxynucleotide triphosphate mix (deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, deoxythymidine triphosphate), and ROX passive reference dye (Qiagen). The following temperature profiles were used: 1 cycle at 95°C (3 min), 39 cycles of denaturation at 95°C (30 s), primer annealing (30 s), and a final cycle at 95°C (30 s). Analysis melting curves were made by slowly heating the PCR mixtures from 55 to 95°C (0.2°C per second) with simultaneous measurements of the SYBR Green I signal intensities. Quantitation was based on standard curves of known concentrations of plasmid DNA containing the respective amplicon for each set of primers.

Statistical Analysis

Logarithms of the cecal bacteria gene copy numbers were used to achieve normal distribution and the mean ± SD was calculated. The short-chain fatty acids and genes copy number data were analyzed with 1-way ANOVA using Minitab statistical software (Release 14 for Windows, Minitab Inc., State College, PA). The results were considered statistically significant at a *P*-value of less than 0.05.

RESULTS

Bacterial Enumeration

The results of the bacterial genes enumeration in the different treatments including the inoculum, Cc, and Cc + L are presented in Figure 1. The addition of *L. salivarius* ssp. *salicinius* JCM 1230 and *L. agilis* JCM 1048 was accompanied with a significant ($P < 0.05$) increase of *F. prausnitzii*, lactobacilli, and bifidobacteria in the Cc + L treatment compared with the inoculum and Cc. Lactobacilli supplementation caused a significant ($P < 0.05$) reduction in the population of *Salmonella* in the Cc + L treatment as compared with the 2 other groups. After 24 h of incubation, *E. coli* and *C. butyricum* were significantly ($P < 0.05$) increased in both the Cc and

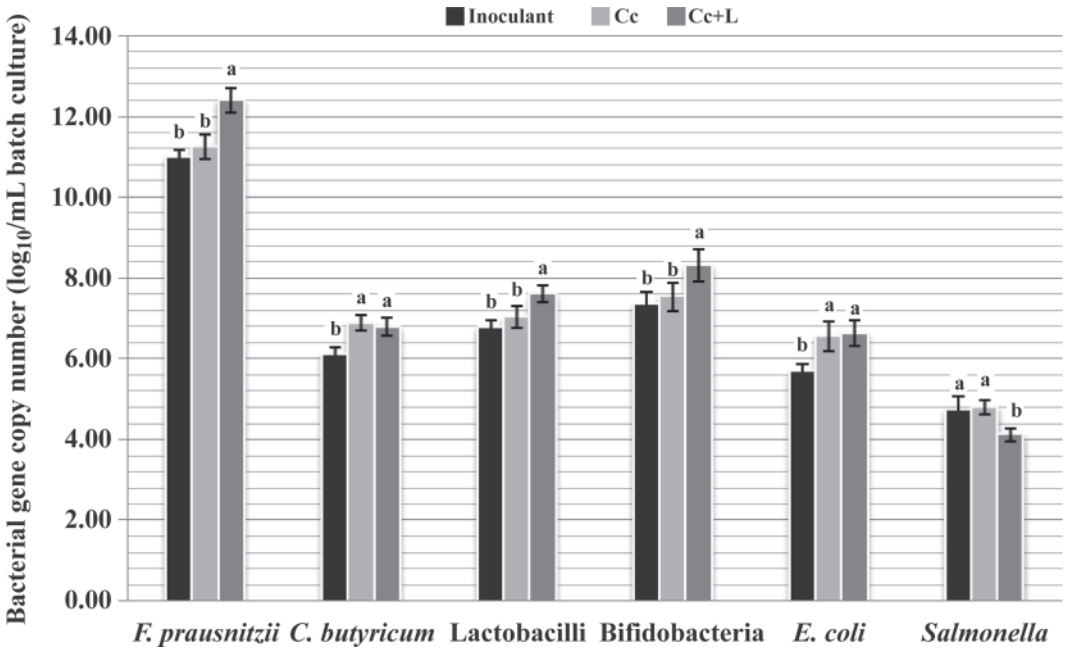

Figure 1. Real-time PCR quantitation of bacterial genes in stirred, pH-controlled batch culture fermentations. Bars are expressed as mean $\log_{10}$ bacterial gene copies (per mL of batch culture) of duplicate determination at 0 and after 24 h of fermentation. Error bars indicate SD of the means. Different letters indicate statistically significant differences ($P < 0.05$). Inoculant = cecal content before fermentation (0 time); Cc = cecal content after 24 h of fermentation; Cc + L = cecal content supplemented with lactobacilli after 24 h of fermentation.

Cc + L treatments compared with the inoculum; however, adding lactobacilli did not change the number of these bacteria in the Cc + L treatment.

Changes in the Short-Chain Fatty Acids and Lactate Concentrations

The concentrations of short-chain fatty acids and lactate, before and postfermentation as well as their net formation are presented in Tables 2 and Figure 2, respectively. Acetate was profoundly reduced in all treatment groups after 24 h of incubation. The concentration of propionate significantly ($P < 0.05$) increased in Cc + L and L treatments, whereas Cc remained unchanged. After 24 h of fermentation, butyrate significantly ($P < 0.05$) increased in the Cc and Cc + L groups (12.81 vs. 34.62%). Lactate was only found in treatment L, which significantly ($P < 0.05$) increased after 24 h of incubation. Comparing the treatments, L significantly decreased acetate compared with Cc and the Cc + L treatment was intermediate. Both Cc + L and L significantly increased propionate compared with Cc. Treatment Cc + L significantly increased butyrate compared with the Cc and L treatments. Butyrate in treatment L was significantly lower compared with treatments Cc and Cc + L and the Cc treatment was intermediate and significantly differed from both Cc + L and L treatments.

DISCUSSION

This study was undertaken to examine the role of *L. salivarius* ssp. *salicinius* JCM 1230 and *L. agilis* JCM 1048 in the fermentation of broiler cecal content.

Table 2. Concentrations of short-chain fatty acids and lactate at 0 and after 24 h of fermentation[1]

	Treatment[2]					
Group	Cc (0)[3]	Cc (24)[4]	Cc + L (0)	Cc + L (24)	L (0)	L (24)
Acetate	72.36 ± 3.90[a]	60.75 ± 4.61[b]	71.39 ± 5.53[a]	56.19 ± 5.05[b]	72.91 ± 3.38[a]	54.65 ± 3.12[b]
Propionate	14.27 ± 2.16[a]	13.95 ± 2.04[a]	15.38 ± 2.36[b]	18.95 ± 1.69[a]	13.62 ± 1.28[b]	16.59 ± 1.89[a]
Butyrate	11.03 ± 0.78[b]	12.65 ± 0.69[a]	11.14 ± 0.85[b]	17.04 ± 0.32[a]	7.58 ± 0.17[a]	7.34 ± 0.14[a]
Lactate	0.05 ± 0.02	ND[5]	0.02 ± 0.02	ND	0.04 ± 0.03[b]	13.14 ± 1.18[a]

[a,b]Means within the same row with different superscripts are significantly different ($P < 0.05$).

[1]Values are (mmol/L) means ± SD of duplicate determinations.

[2]Cc = cecal content; Cc + L = cecal content supplemented with lactobacilli; L = basal medium containing lactobacilli.

[3]Mean concentration at time 0.

[4]Mean concentration at time 24.

[5]ND = not detected.

Although it was not possible to fully characterize the changes that occurred due to the addition of lactobacilli, it was feasible to monitor the populations of selected species believed to be indicative to maintain healthy state of the cecum.

The results clearly showed the butyrogenic effects of *L. agilis* JCM 1048 and *L. salivarius* ssp. *salicinius* JCM 1230 on broiler cecal bacteria (Figures 1 and 2). In the present study, the addition of 2 *Lactobacillus* strains stimulated the proliferation of *F. prausnitzii* coinciding with high butyrate formation, whereas *C. butyricum* did not change. Previous studies on human gut microbial community demonstrated different types of butyric-producing bacteria such as lactate-utilizing butyrate producers (Duncan et al., 2004; Belenguer et al., 2007) or those using acetate (Barcenilla et al., 2000). Bjerrum et al. (2006) showed that *F. prausnitzii* was the predominant butyric-producing bacteria in the chicken cecum and 60% of the cecal clones belonged to this cluster in the conventional broiler chickens, which was associated with high butyric production. *Faecalibacterium prausnitzii* did have the significant ability to use lactate produced by lactobacilli as a substrate for the butyrate production (Belenguer et al., 2007) via cross feeding among cecal microflora. In 24-h batch culture incubation, Belenguer et al. (2007) showed that lactate is rapidly converted to acetate, butyrate, and propionate by the human intestinal microbiota at pH values as low as 5.9. In the present study, the formation of lactate by pure cultures of *Lactobacillus* in treatment L, the lack of lactate in Cc + L and Cc groups, and acetate reduction in all treatment groups (Table 2) clearly showed the mechanism of cross feeding among chicken cecal microbiota, which increased the number of *F. prausnitzii*.

Based on the results of the current study, lactobacilli supplementation enhanced lactobacilli and bifidobacteria population, whereas it inhibited *Salmonella* growth and it did not have any effects on the number of *E. coli*. These results were similar to those observed at pH 5.8 in our previous work using selective media (Meimandipour et al., 2009), although *Salmonella* was not detected. The mechanisms of action by which probiotics inhibit the adhesion and colonization of pathogenic bacteria is different from one type bacteria to another and include competition for nutrients and receptor sites, antimicrobial production such as short-chain fatty acids, and modifications of gut ecology. For example, *Lactobacillus plantarum* stimulated goblet cells to express the mucins MUC2 and MUC3 and by this means inhibited the adherence of enteropathogenic *E. coli* to the intestinal surface (Mack et al., 1999), whereas Duggan et al. (2002) showed that *Lactobacillus* spp. directly impeded the attachment of *E. coli* and *Salmonella*. Furthermore, previous study showed that short-chain fatty acids regulate the invasive phenotype of *Salmonella* by downregulating gene expression responsible for penetration into the host epithelial cells (Thompson et al., 2006). In the current in vitro study, enhanced populations of *F. prausnitzii*, bifidobacteria, and lactobacilli that compete for nutrient exploitation with *Salmonella* and elevated levels of butyrate and propionate by these bacteria could be the mechanisms that reduced the *Salmonella* population after 24 h of incubation. This postulation is support by the findings of Van Immerseel et al. (2003), who demonstrated that butyrate and propi-

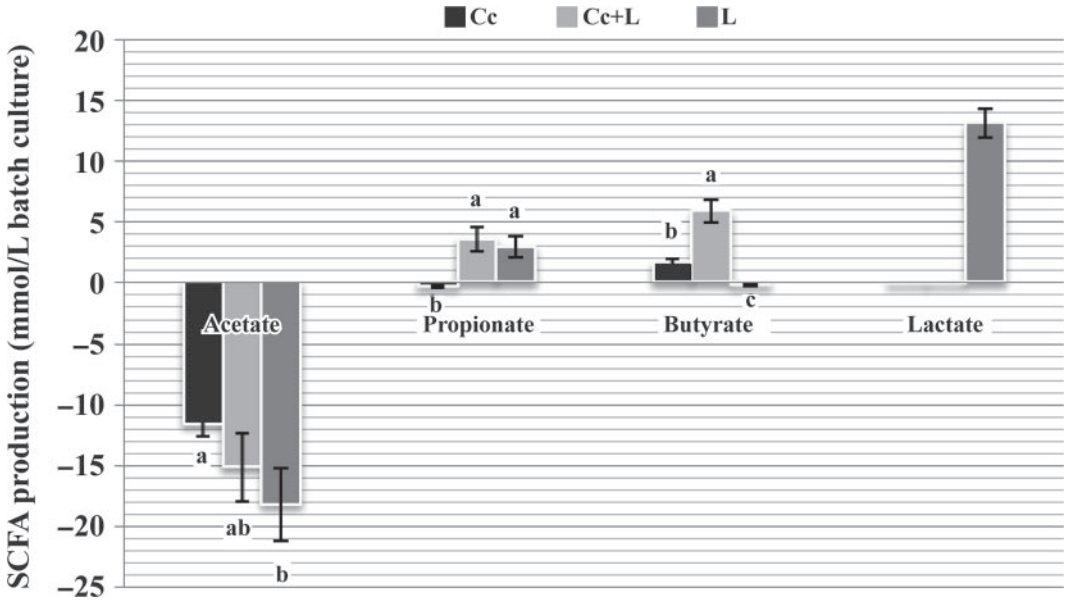

Figure 2. Production of short-chain fatty acids (SCFA) and lactate by cecal microflora of broiler chicken after 24 h of fermentation. Bars are expressed as means of fermentation with 2 replicates and duplicate determinations on the same culture. Error bars indicate SD of the means. Different letters indicate statistically significant differences ($P < 0.05$). Cc = cecal content after 24 h of fermentation; Cc + L = cecal content supplemented with lactobacilli after 24 h of fermentation; L = basal medium containing lactobacilli after 24 h of fermentation.

onate suppressed epithelial cell invasion by *Salmonella* Typhimurium. The lack of any inhibitory effects of 2 probiotic strains on the growth of *E. coli* may support the idea that host-dependant mechanisms such those as mentioned above are very important in reducing the level of coliforms.

The results presented in Table 2 showed that the increase of propionate with supplementation of *Lactobacillus* strains was similar in Cc + L and L treatments. This was in agreement with the results in our previous work (Meimandipour et al., 2009) and demonstrated that lactobacilli did not stimulate the production of propionate by any other bacteria in the cecal content and they were indeed the sole sources of propionate increase in treatment Cc + L.

The present study suggested the potential of *L. agilis* JCM 1048 and *L. salivarius* ssp. *salicinius* JCM 1230 as probiotic bacteria that stimulate the proliferation of *F. prausnitzii* as the predominant butyric-producing bacteria in the chicken cecum. In addition, *L. agilis* JCM 1048 and *L. salivarius* ssp. *salicinius* JCM 1230 had the ability to reestablish proper microbial balance in the gastrointestinal tract. The results clearly demonstrated different mechanisms of control for the growth of *E. coli* and *Salmonella* by lactobacilli. Moreover, short-chain fatty acids appeared to be involved in the growth reduction of *Salmonella*, but this decrease seemed to be dependant on butyrate and propionate but not acetate.

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