



Research Article

Effect of Increasing Dietary Protein from Soybean Meal on Intestinal Microbiota and Their Fatty Acids Production in Broiler Chicken

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ARTICLE HISTORY

Received: 2014-06-20
Revised: 2014-07-10
Accepted: 2014-07-11

Key Words: Broiler chicken, Crude protein, Intestinal microbiota, Essential amino acid

ABSTRACT

To evaluate the effect of increasing dietary protein from soybean meal on chicken growth, three treatments of the control (22% CP), additional crude protein diet (28% CP) and additional essential amino acid diet (22% CP with high dose of EAA) were fed for 900 broiler chickens with an age average of 1–21 days. Twenty birds per treatment were randomly sampling for the effects on intestinal villi, gut microbiota and short chain fatty acid production. Results indicated high protein diet had no effect on chicken growth, however, it decreased the villi height and *Lactobacilli* in jejunum of chicken. Interestingly, the enrichment of CP and EAA supplemented diet promoted especially *Clostridium coccoides-Eubacterium rectal* group for two times of the control in the ileum of chicken. While the suppression of *Campylobacter* in cecum and undetectable *Clostridium perfringens* were observed. There were no significant different for microbial short chain fatty acid production in intestine. The concentrations of lactic acid, propionic acid and acetic acid detected from the control and these two treatments were not significantly different. On the basis of these findings, high protein diet from soybean meal affected broiler gut microbiota especially the increasing of *Clostridium coccoides-Eubacterium rectal* group, but did not support their growth performance and intestinal metabolites.

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ARTICLE CITATION: Nakphaichit M, Phraephaisarn C, Keawsompong S, Sukpiriyagul O, Nitisinprasert S (2014). Effect of increasing dietary protein from soybean meal on intestinal microbiota and their fatty acids production in broiler chicken. *Adv. Anim. Vet. Sci.* 2 (6): 337 – 343.

INTRODUCTION

Crude protein (CP) is a main component for metabolism and has great impact on broiler chicken performance (Malheiros et al., 2003). These amino acids are absorbed and metabolized to body proteins. Most of experiments tried to optimize crude protein and Essential Amino Acid (EAA) concentration for increasing the growth efficiency of broiler chicken which were analyzed in the term of growth rate, body weight gain and feed consumption (Rezaei et al., 2004; Kobayashi et al., 2013). However, these performance data were not enough to determine the effects of crude protein and their concentration on chicken health problem and diseases. Intestinal bacterial community had an enormous impacts on the nutritional and health status of the host. The intestinal microbiota play a major roles on feed digestion, nutrient absorption and pathogenic growth inhibition which directly effect on the health of host (Gong et al., 2007). An important factor influencing on stability of intestinal microorganism is feed composition (Shakouri et al., 2009; Palliyeguru et al., 2010). Dahiya et al., (2005) found that high-protein diet particularly from fish meal contributed incidence of necrotic enteritis (NE) by *Clostridium perfringens* and also decreased the number of

beneficial bacteria of genus *Lactobacillus* in broiler chicken. The great amount of wheat, barley and rye increase intestinal viscosity and influence gut microflora by increasing amount of *C. perfringens* in chicken (Jia et al., 2009). While the prebiotic oligosaccharide supplement in feed could promote the growth of bifidobacteria and lactobacilli which are classified as beneficial bacteria (Kim et al., 2011). Hence both advantage and non-advantage to gut microorganism can be gained due to the composition of feed.

Two sources of CP used in poultry feed are from animal and crop (Drew et al., 2004). Currently meat meal is not allowed to use in Europe because the problem of Bovine Spongiform Encephalopathy (BSE) contamination had occurred (Leeson and Summers, 2009). Therefore, the protein from crop especially soybean meal is frequently used as crude protein in poultry feed formulations (Palliyeguru et al., 2010). Moreover amino acid of lysine, methionine, threonine and tryptophan are likely added more frequently as supplement source in poultry industry to enhance poultry growth and its reproductive performance (Laudadio et al., 2012). Nevertheless, there is still a lack of report linking the effect

of CP from soybean meal and EAA on intestinal microbiota in broiler chicken.

In this study, the authors aimed to demonstrate the effect of additional concentration of crude protein and EAA on poultry production and health by determining the broiler performance, gut microflora and their metabolites.

MATERIALS AND METHODS

Animal, Diet and Feeding Program

Nine hundred 1-day-old male broiler chicken (Ross, Betagro Agro-Group Public Co. Ltd.) were randomly distributed into 30 floor pen of 30 broilers so that ten replicates were available for each of the two treatments (high CP and EAA supplemented groups) and the control group. The commercial soybean meal based diets used in this experiment were suggested by the National Research Council (1994). From 1 to 21 day of age, the chicken of control, high CP and EAA supplemented group were fed with crude protein of 22%, 28% and 22%, respectively. While the diet formula of EAA supplemented group was modified from control by adding methionine, lysine and threonine to final concentration similar to high CP supplemented group as shown in Table 1. All birds had access to feed and water *ad libitum*.

Table 1: Composition of the experimental diets used to feed chicken for the treatment of high crude protein and additional essential amino acids to obtain the final protein concentration of 28%.

Ingredients (g/kg)	Control	High CP	EAA
Corn	61.8	42.4	60.8
Wheat	–	–	–
Palm oil	–	2.58	–
Soybean meal	33.75	50.93	33.75
Salt	0.4	0.4	0.258
Mono dicalcium	1.8	1.66	1.8
Calcium carbonate	1.48	1.42	1.48
DL-Methionine	0.183	0.15	0.242
L-Lysine 98.5%	0.158	0.017	0.6
L-Threonine 98.5%	–	–	0.267
Premix (vitamin/mineral)	0.415	0.415	0.615
Analyzed Composition (%)			
Energy (kcal/kg)	2900	2900.8	2902.7
Crude protein	22.06	28.74	22.64
Fat	2.36	4.6	2.33
Fiber	2.628	2.87	2.61
Calcium	0.947	0.94	0.947
Phosphorus	0.73	0.77	0.73
Salt	0.51	0.458	0.51
Sodium	0.22	0.22	0.22
Total Lysine	1.252	1.598	1.598
Total Methionine	0.50	0.546	0.558
Total Threonine	0.813	1.08	1.072
Total Tryptophan	0.253	0.351	0.253
Linoleic acid	1.31	1.31	1.30

Intestinal Sample Collection

Two birds were randomly selected from each of the 10 pens per treatment (20 birds per treatment) at 21 days post hatch. Jejunum, ileum and cecum of the bird killed by carbon dioxide asphyxiation were collected. The tissues

were sterilized by 70% ethanol, washed twice by sterile water and then kept at -20°C until use.

Genomic DNA Extraction

DNA was extracted from 1 g of the mixture of the digesta and the intestinal wall using the modified method of Nakphaichit et al., (2011). In brief, the bacterial cells were collected from a 10x dilution sample by homogenizing in a stomacher blender (Seward Medical, London) for 2 min followed by centrifugation at 100 xg for 10 min at 4°C to remove the debris. One milliliter of supernatant collected was centrifuged at 15,000 xg for 10 min at 4°C . The cell pellets obtained which mainly composed of bacteria were washed twice with 1 ml PBS (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 , 0.24 g KH_2PO_4 in 1 L at pH 8.0) and then suspended in 900 μl of PBS. The bacterial suspension mixed with 300 μl of phenol-chloroform-isoamyl alcohol (25:24:1) was lysed using a mini-bead beater (Biospec Products Inc., Bartlesville, USA) at 2,700 rpm for 180 sec containing 0.3 g zirconium beads (0.1 mm in diameter, As One Corporation, Osaka, Japan). The cell debris was removed by centrifugation at 20,000 xg for 2 min. The bacterial DNA obtained from upper layer was further purified using the QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to manufacturer's instructions and stored at -20°C until use.

Villi Measurement

The jejunum and ileum samples were cut by the size of $1.5 \times 1.5 \text{ cm}^2$, flushed through with saline solution, fixed on foam using pins and placed into 100 g/l buffered formalin for further analysis. Paraffin-embedded tissues were sectioned to a thickness of 5 μm . Tissue slides were stained using 0.02% toluidine blue for light microscope measurement. The villous height was measured in from the base of the lamina propria to the apex of the villous. The unit of measurement is micrometre (μm) and all reported villi values were an average of 5 measurements per tissue. Assessments were made only on cleanly sectioned, perpendicular villi as previously described by Sun et al., (2005).

Quantitative Real Time PCR

Real time PCR using SYBR technology was used to quantify the target group of microorganism by LightCycler[®] 480 (Roche, Germany). The oligonucleotide primers, optimal annealing temperature and their PCR products size are summarized in Table 2. To obtain a standard curve for absolute quantification, the modified method of Wise and Siragusa (2007) was performed. Standard curves of total bacteria, *Lactobacillus* group, *Campylobacter*, *Acinetobacter*, *Pseudomonas*, *Bifidobacterium*, *Bacteriodes* – *Prevotell* – *Porphyromonas* group, *Clostridium coccoides*–*Enterobacter rectal* group, *C. perfringens* group and *Enterobacteriaceae* were constructed using specific primers to amplify the genomic DNA of *Escherichia coli* TISTR 527, *Lactobacillus salivarius* AC21, *Campylobacter jejuni* ATCC 33291, *Acinetobacter calcoaceticus* TISTR 360, *Pseudomonas sp.* TISTR 1249, *Bifidobacterium bifidum* JCM 1255, *Bacteriodes fragilis* ATCC 25285, *Ruminococcus productus* JGD 07421, *C. perfringens* ATCC 13124 and *Salmonella Typhimurium* TISTR 292, respectively. Each PCR products were cloned into pGEM-T Easy vector

according to manufacturer's instructions (Promega, Madison, WI). Then, the recombinant plasmids were diluted by serial 10-fold dilution until concentration of 10^9 was reached. The set of serial dilution series in each group were used as a template for the standard curve. The standard curves were created by LightCycler® 480 software using second derivative maximum (Roach Applied Science,

Mannheim, Germany). The slope of the standard curve is used to determine reaction efficiency in which accurate quantification require the efficiency of 1.8–2 ($E = 10^{-1/\text{slope}}$). In addition, the error value is evaluated for the accuracy of the quantification result base on the standard curve. An acceptable value should be < 0.2 .

Table 2: The list of primers and their characters used for quantitative real-time PCR

Target	Primer	Seq	Tm	Size	Ref.
Total bacteria	HDA1 HDA2	ACTCCTACGGGAGGCAGCAGT GTATTACCGCGGCTGCTGGCAC	61	200	Tannock et al., 2000
<i>Lactobacillus</i> group	LbF LBR	AGCAGTAGGGAATCTTCCA CACCGCTACACATGGAG	53	341	Walter et al., 2001 Heilig et al., 2002
<i>Campylobacter</i> spp.	CamF CamR	GGATGACACTTTTCGGAG AATCCATCTGCCTCTCC	54	246	Rinttilä et al., 2004
<i>Acinetobacter</i>	AcF AcR	TTTAAGCGAGGAGGAGG ATTCTACCATCCCTCTCC	52	240	Vanbroekhoven et al., 2004
<i>Pseudomonas</i>	PseF PseR	GGCGACGATCCGTAAC CCTTCCTCCCAACTT	57	180	Khan and Yadav et al., 2004
<i>Bifidobacterium</i> spp.	BifF BifR	TCGCGTC(C/T)GGTGTGAAAG CCACATCCAGC(A/G)TCCAC	62	243	Rinttilä et al., 2004
<i>Bacteroid – Prevotell– Porphyromonas</i>	BacPF BacPR	GGTGTCGGCTTAAGTGCCAT CGGA(C/T)GTAAGGGCCGTGC	57	140	Rinttilä et al., 2004
<i>C. coccoides– E. rectal</i> group	ClosF ClosR	CGGTACCTGACTAAGAAGC AGTTT(C/T)ATTCTTGCGAACG	51	429	Rinttilä et al., 2004
<i>C. perfringens</i> group	PerfF PerfR	ATGCAAGTCGAGCGA(G/T)G TATGCGGTATTAATCT(C/T)CCTTT	51	120	Rinttilä et al., 2004
<i>Enterobacteriaceae</i>	EnF EnR	CATTGACGTTACCCGCGAGAAGAAGC CTCTACGAGACTCAAGCTTGC	57	195	Bartosch et al., 2004

The reaction mixture contained 10 µl of 2x SYBR Green I master mix (Roche, Germany), 0.8 µl of 5 pmol forward and reverse primers, 2 µl of DNA template (50–100 ng) and dH₂O added to obtain the final volume of 20 µl. Amplification program included an initial denaturation at 95°C for 5 min, followed by 45 cycles consisting of denaturation at 95°C for 10 sec, annealing temperature according to the Tm of primer for 10 sec and extension at 72°C for 5–18 sec depending on PCR product size. To confirm the specific amplification of the target DNA, dissociation curve was created in the following cycles of a denaturation step at 95 °C for 5 sec, temperature decreasing step to 65°C for 1 min and continuous temperature increasing from 65 °C to 97 °C every 12 sec signal measurement. For statistical analysis, in the case where the incidence of detection within treatment group was less than 100%, the remaining undetection replicates were considered to be at the theoretical limit of detection for purpose of calculating the mean and standard deviation.

Short Chain Fatty Acid Analysis

The concentration of lactic acid, acetic acid, propionic acid and butyric acid were measured by high performance liquid chromatography. Samples were prepared using the modification method of Zeppa et al., (2001). Briefly, one gram of digesta and tissue was diluted by adding 4 ml of 0.008 M H₂SO₄ (mobile phase) and incubated on ice. The cool solution was homogenized every 10 min by vortex mixer for one hour. The supernatant was collected by centrifugation at 15,000 xg for 10 min. The concentration of 0.1%, 0.2%, 0.3%, 0.4% and 0.5% v/v of each acid were

performed for their standard curve. The 20 µl of mixture solution containing 3 volume of analyzed sample or standard solution and 1 volume of internal standard (0.2 % w/v tartaric acid) filtered through 0.2 µm PVDF Syringe filters (Verical, Bangkok, Thailand) was injected into Breeze™ 2 HPLC system (Waters, USA) with Rezex ROR–Organic Acid column (Phenomenex, USA) at 60°C. Mobile phase was run at the flow rate of 0.6 ml/min. The signal was detected by UV detector at 210 nm. All chemical were purchased in HPLC analytical grade.

Statistical Analysis

All results were analyzed by one way ANOVA test (SPSS program version 12, Munich, Germany). The group difference was determined by LSD and Duncan method. Differences were quantified at significant $p \leq 0.05$.

RESULTS

Broiler Performance Response to CP Level

The weekly weight gain, feed consumption and feed conversion ratio of the control and two treatments are represented in Table 3. At the first week, feed consumption of the control was not significantly different from the EAA supplemented group. While feed consumption of the high CP supplemented group was significantly lower than the one of both control and the EAA supplemented group ($p = 0.001$). However, both weight gain and feed conversion ratio at first week were not significant difference ($p > 0.05$).

Villi height of jejunum and ileum were determined at week 3 (Table 3). The villi of jejunal region from two treatments with either high CP or EAA supplemented group were

significantly shorter than the control for 23%–28.7% of the control ($p < 0.05$). Whereas the villi size of ileum from all Table 3: Body weight, feed consumption, feed conversion ratio and villi height observed in broiler chickens fed with high CP and EAA supplemented groups.

treatments were not significantly different ($p \geq 0.05$).

Parameter	Week	Control	High CP	EAA	Pvalue
Body weight gain (g/week)	1	126.11 ± 7.01	118.97 ± 8.09	121.17 ± 12.19	0.236
	2	327.67 ± 14.60	318.50 ± 10.23	321.00 ± 16.85	0.341
	3	529.07 ± 24.14	531.46 ± 24.66	540.69 ± 18.77	0.491
	Overall	982.84	968.93	982.86	0.520
Feed consumption (g/week)	1	169.41 ^a ± 4.82	158.40 ^b ± 7.05	167.58 ^a ± 6.34	0.001
	2	447.43 ± 12.83	461.29 ± 14.98	462.28 ± 24.29	0.140
	3	817.17 ± 61.60	804.28 ± 60.63	827.64 ± 73.87	0.729
	Overall	1448.90	1410	1456.50	0.375
Feed conversion ratio (F/G)	1	1.35 ± 0.06	1.33 ± 0.05	1.39 ± 0.12	0.260
	2	1.41 ± 0.07	1.40 ± 0.04	1.44 ± 0.04	0.370
	3	1.54 ± 0.08	1.52 ± 0.13	1.53 ± 0.10	0.835
	Overall				
Villi size (µm)					
	Jejunum	34.46 ^a ± 5.61	24.56 ^b ± 5.23	26.52 ^b ± 4.60	0.001
	Ileum	28.70 ± 2.96	25.67 ± 4.58	27.42 ± 5.07	0.302

^{a,b}Different letter superscripts indicated a significant difference. ($p \leq 0.05$)

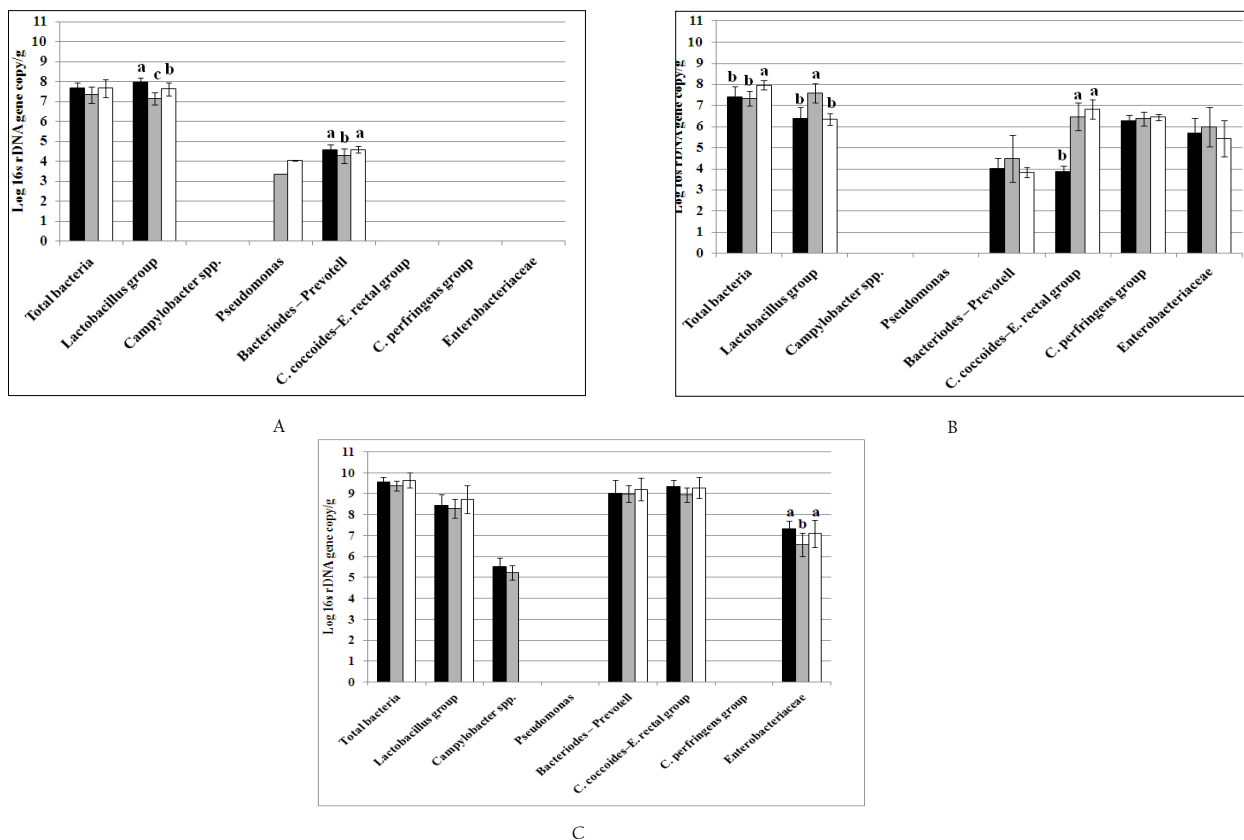


Figure 1: Quantification of target bacteria from chicken's jejunum (a), ileum (b) and cecum (c) of 21 day chicken of the control (black bar), the high CP supplemented group (gray bar) and the EAA supplemented group (white bar). The bacteria and standard deviation value in each group were calculated from the incidence of detection within treatment group: ^{a,b}Different letter superscripts indicated a significant difference. ($p \leq 0.05$)

Effect of CP and EAA on Intestinal Bacteria

Total bacteria and 9 specific bacterial groups from jejunum, ileum and cecum were analyzed by real time PCR according to region of 16s rDNA gene. Considering to jejunal region (Figure 1a), total bacteria of the control and two treatments were 7.33–7.68 log 16s rDNA gene copies which were not

significant difference ($p > 0.05$). Only three bacterial groups of *Bacteroides-Prevotella*, *Lactobacillus* and *Pseudomonas* were observed. The amount of *Bacteroides-Prevotella* from the control and the EAA supplemented group were 4.57 and 4.58 log 16s rDNA gene copies, respectively, which were not

significant difference ($p > 0.05$). However, *Bacteroides-Prevotella* of the control and the EAA supplemented group were significantly higher than high CP supplemented group for 7%. The population of *Lactobacillus* of three treatments were significantly different ($p = 0.001$). The highest concentration of *Lactobacillus* group found in control were 7.98 log 16s rDNA gene copies whereas the lowest one of *Lactobacillus* group found in high CP supplemented group were 7.16 log 16s rDNA gene copies. *Pseudomonas* was not found in control treatment but its low concentration of 3.35–4.03 log 16s rDNA gene copies was detected from 1 chicken of two treatments.

In ileum, the total bacteria (7.96 log 16s rDNA gene copies) were significantly promoted in EAA supplemented group ($p < 0.05$) (Figure 1b). Comparing to jejunum, the microbial community had greater diversity. Additional *C. coccoides-E. rectal* subgroup, *C. perfringens* and *Enterobacteriaceae* were detected while *Pseudomonas* was not detected at the detection limit of 2 log 16s rDNA gene copies. The amount of *Bacteroides-Prevotella*, *C. perfringens* and *Enterobacteriaceae* from the control and the two treatments were 3.82–4.47, 6.27–6.44 and 5.42–5.96 log 16s rDNA gene copies which showed no significant difference. Interestingly, the copy number of *C. coccoides-E. rectal* group from high CP and EAA supplemented group were significantly higher than the control for two times ($p = 0.001$) while the *Lactobacillus* from high CP supplemented group was significantly higher than the control and EAA supplemented group for 15.9 and 16.5%, respectively ($p = 0.001$).

In caecal region, total bacteria from the control and two treatments were 9.37–9.65 log 16s rDNA gene copies which were higher than both jejunum and ileum region as shown in Figure 1c. The detected bacterial groups were similar to ileum region except *C. perfringens*. In addition, low concentrations of *Campylobacter* were also detected from the control and high CP supplemented group (5.51 and 5.23 log 16s rDNA gene copies) from 6 and 3 chickens, respectively. The concentration of *Lactobacillus*, *Bacteroides-Prevotella* group and *C. coccoides-E. rectal* group (8.30–8.74, 9.0–9.21 and 8.96–9.34 log 16s rDNA gene copies) from the control and two treatments were not significantly different, respectively ($p > 0.05$). While caeca condition supported similar growth of *Enterobacteriaceae* from the control and EAA supplemented group but significantly effected to high CP supplemented group with the reduction of 10.0% and 7.91%, respectively.

It was noticed that both *Acinetobacter* and *Bifidobacterium* were not observed under limitation of real time PCR in this study at 10^3 and 10^2 log 16s rDNA gene copies, respectively.

Effect of CP and EAA on Short Chain Fatty Acids (SCFA)

Short chain fatty acids of acetic acid, butyric acid, lactic acid and propionic acid in jejunum, ileum and cecum were analyzed. Butyric acid was not detected. However, lactic acid and propionic acid were detected from all regions of jejunum, ileum and cecum. The concentration of lactic acid from the jejunum of the control, the high CP supplemented group and the EAA supplemented group were 27.3 (SD=3.3), 26.2 (SD=2.64) and 39.3 (SD=3.74) $\mu\text{mol/g}$, respectively which were higher than propionic acid for 2.1, 1.9 and 3.9

times, respectively (Figure 2). While the concentration of lactic acid from ileum of the control, the high CP supplemented group and the EAA supplemented group were higher than the one of propionic acid for 2, 2.0 and 2.4 times, respectively. However, the amount of both acids from all three experiments was not significantly different ($p > 0.05$).

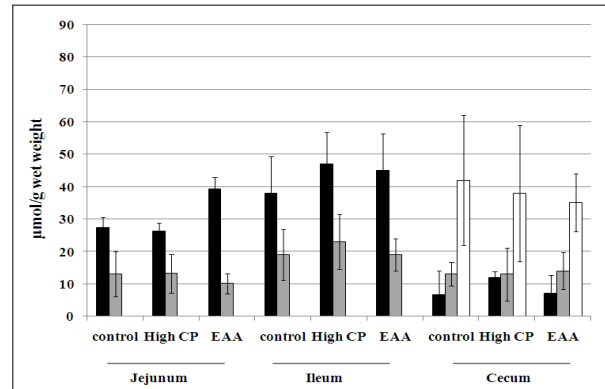


Figure 2: SCFA concentration from digesta of chicken intestine at 21 day. The black bars, gray bars and white bars are represented lactic acid, propionic acid and acetic acid, respectively. The error bars indicate standard deviation from each subject group (n=10); ^{ab}Different letter superscripts indicated a significant difference in each region ($p \leq 0.05$)

In cecum, not only lactic acid and propionic acid but also acetic acid was detected. The concentration of acetic acid in cecum was the highest. However, the amount of these three acids from all three experiments was not significantly different ($p > 0.05$).

DISCUSSIONS

The effect of dietary CP and EAA from soybean meal based diet on broiler production and intestinal bacterial community were investigated. Over-dosage of CP and EAA in both high CP and EAA supplemented group had no effect on chicken growth in the term of weight gain and feed conversion ratio. However, addition of CP and EAA affected the length of jejunal villi but not villi length in ileum that was similar to the results of Yamauchi et al., (1996) reporting that the villi height of both duodenum and jejunum from layer-type hens fasted decreased while the one of ileum had no effect. Swatson et al., (2002) reported that addition of excess dietary protein of 20–40% with both balanced and unbalanced amino acid had no effect on chicken weight gain. However, the heights of the jejunum seem to be lower for birds fed ad libitum on the unbalanced amino acid diet. It was possible that excess protein contents of 28% in the form of either crude protein or amino acid studied in this experiment effected on the jejunal chicken might come from unbalanced amino acid. In addition, the number of *Lactobacillus* expressed as log 16S rDNA gene copies of two treatments from jejuna 21 days were lower than the control. It was possible that the excess unbalanced amino acid would effect to the number of *Lactobacillus* which is an important commensal friendly bacterial group in intestinal gut.

Ten bacterial groups often found in GI-tract of chicken were studied for their diversities of different intestinal region of jejunum, ileum and cecum. The high number of

Lactobacillus producing lactic acid as a major metabolite (Holzapfel et al., 1995) was in parallel with the high concentration of lactic acid detected from jejunum and ileum. However, more microbial diversity occurred at the ileum and cecum. This should be the environmental condition of both ileum and cecum having high pH range of 6.18–6.60 and 5.60–5.83, respectively providing the suitable condition for most bacteria (Gabriel et al., 2006). These caused high number of *Bacteroides-Prevotella* and *C. coccooides-E. rectal* group for more than 9 log 16s rDNA gene copies from cecum which were higher than the ones from both jejunum and ileum. In addition, high concentrations of acetic acid were also detected from only cecum part. *Bacteroides-Prevotella* group is able to metabolize different carbon source to produce acetic acid as a major product while some genus in *C. coccooides-E. rectal* group especially genus *Clostridium* and *Ruminococcus* can utilize lactic acid and produce acetic acid in fermentation process (Hespell, 1987). It was possible that cecum region provided the condition to support the growth of some species of heterofermentative *Bacteroides-Prevotella* and *C. coccooides-E. rectal* group and to produce high acetic acid concentration during their growth.

Addition of either soy CP or EAA clearly supported the growth of *C. coccooides-E. rectal* group in the ileum. It was different from the animal CP source containing high glycine content (Drew et al., 2004) which served as energy or nitrogen source to support the growth of *C. perfringens* causing subclinical necrotic enteritis in chicken (Wilkie et al., 2005; Dahiya et al., 2007). Notably, *Campylobacter* group was not detected in the EAA supplemented group but did 60% and 30% in the control and the high CP supplemented group, respectively. Dahiya et al., (2007) reported that 0.8% methionine supplement could reduce pathogenic bacteria like coliform, *C. perfringens* and *Streptococcus* group D in ileum. In this study, additional EAA also potentially reduced the number of *Campylobacter* group. Actually *Campylobacter* can colonize in chicken intestine without any apparent harmful to the birds. However, its enteritis effect can be observed on human consumers (Bull et al., 2006). Addition of EAA should be therefore realized.

In conclusion, high-dose of CP and EAA in soybean meal based diets had no effect on broiler production. Total viable bacteria and nine specific bacterial groups often found in GI-tract of chicken were studied for their diversities at intestine of jejunum, ileum and cecum. More microbial diversity had occurred at the ileum and cecum region of intestine. Low concentration of *Campylobacter*, *Clostridium perfringens* and *Pseudomonas* were detected from jejunum, ileum and cecum of chicken fed by soybean meal based diet. Addition of high CP and EAA did not effect to those bacterial groups studied except growth promotion of *C. coccooides-E. rectal* group from ileum at 21 day of age. Either the high CP or EAA supplemented group had no effect to the production of those short chain fatty acid of acetic acid, butyric acid, lactic acid and propionic acid. However, high concentrations of lactic acid detected from both jejunum and ileum concerned to high *Lactobacillus* concentration of 7–9 log 16s rDNA gene copies. While the concentration of acetic acid detected from only cecum was in parallel with the high number of *Bacteroides-Prevotella* and *C. coccooides-E. rectal*. These findings provided that high concentration of *C.*

coccooides-E. rectal detected from additional CP or EAA may assist nutrition strategies to develop healthy chicken production with soybean meal based diet in the future.

ACKNOWLEDGEMENTS

This work was supported by a Royal Golden Jubilee (RGJ) grant from Thailand. The authors thanks Matana Nalumpang for animal health contribution.

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