The Thai Journal of Veterinary Medicine

Volume 39
Issue 3 *September, 2009*

Article 7

9-1-2009

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Original Article

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Abstract

Medium chain fatty acids (MCA) are one of new additives used to control Salmonella infection and it is interesting to examine their physiological role in chickens comparing with other additives. The objective of this investigation was to study the effect of MCA, mixed organic acids (ORA) and fructo-oligosaccharide (FOS) on cecal *Salmonella* enterica serovar Enteritidis colonization and physiological changes of intestine in broilers. Six hundred, day old, male and female broiler chicks were allocated into 4 treatments. The treatments were CON: basal corn-soybean meal diet, FOS: basal diet supplemented with 4 g/kg FOS, ORA: basal diet plus water supplemented with ORA at 1:1,000 from day 1 to 45, MCA: basal diet plus water supplemented with MCA at 1:1,000 from day 1 to 35 and 1; 2,000 from day 36 to 42. All chickens were inoculated with 0.3 ml of 10^6 cfu/ml *S.* Enteritidis at day 3 and 1 ml of 10^8 cfu/ml at day 13 post-hatching. At days 21, 35 and 45 post-hatching, body weight and daily feed intake were recorded and averaged to calculate feed conversion ratio (FCR). Cecal samples were examined for *S.* Enteritidis colonization. In situ pH determination in crop small intestine and ceca were measured. Jejunal mucosal samples were collected for the determination of sucrase and maltase activity. Ileal digesta were collected for nutrient digestibility using the indigestible marker technique. Cecal contents were collected for the determination of short chain fatty acids (SCFA) and medium chain fatty acids (MCA). Plasma samples were collected from the portal vein to determine medium chain fatty acids. For the overall period (days 1-45 post-hatching), chicks in the MCA and ORA groups had a significantly ($p<0.05$) higher average daily gain and better feed conversion ratio than the CON group. Chicks in MCA, ORA and FOS groups had significantly ($p<0.05$) higher body weights than the CON group. Chicks in MCA and ORA groups showed a reduction of *S.* Enteritidis in the ceca which was significantly ($p<0.05$) lower than the CON group. Chicks in MCA, ORA and FOS groups had a significantly ($p<0.05$) lower pH of crop and intestines than the CON group. Chicks in MCA group had a significantly ($p<0.05$) higher disaccharidase enzyme, digestibility of nutrients, SCFA (acetic acid and valeric acid), MCA in plasma than the CON group. In conclusion, chicks in the MCA and ORA groups had a better growth performance, better digestibility, less S. Enteritidis colonization and lower pH in the crop and intestines. Chicks in the FOS group tended to have decreased Salmonella colonization in ceca. The chicks in the MCA and FOS groups had improved disaccharidase activity. Medium chain fatty acids had beneficial effects on increased medium chain fatty acid concentrations in the portal vein and SCFA concentrations in ceca. Therefore, MCA is one of the efficient additives appropriate for Salmonella control in broilers.

Keywords : broilers, fructo-oligosaccharide, medium-chain fatty acids, organic acids, *S.* Enteritidis

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Thai J. Vet. Med., 2009. 39(3): 245-258

บทคัดย่อ

ผลของกรดไขมันสายปานกลาง กรดอินทรีย์ และฟรุคโตโอลิโกแซคคาไรด์ต่อการเกาะกลุ่มของเชื้อซัลโมเนลล่า เอนเทอริทิดิสและการเปลี่ยนแปลงของลำไส้ในไก่เนื้อ

สุชีรา โชติกะธรรม[1] อินทิรา กระหม่อมทอง[2] กฤษ อังคนาพร[3*]

กรดไขมันสายปานกลางเป็นหนึ่งในสารเสริมใหม่ที่ช่วยควบคุมการติดเชื้อซัลโมเนลล่า เป็นที่น่าสนใจถึงผลทางสรีรวิทยาของกรดไขมันสายปานกลางในไก่เทียบกับสารเสริมอื่นๆ การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของกรดไขมันสายปานกลาง กรดอินทรีย์รวม ฟรุคโตโอลิโกแซคคาไรด์ ต่อการเกาะกลุ่มของเชื้อซัลโมเนลล่า เอนเทอริทิดิส คุณลักษณะการเจริญเติบโต และการเปลี่ยนแปลงของลำไส้ของไก่เนื้อ โดยในการทดลองใช้ลูกไก่คละเพศ อายุ 1 วัน จำนวน 600 ตัว แบ่งเป็น 4 กลุ่ม กลุ่มที่ 1 ได้รับอาหารพื้นฐานและให้น้ำธรรมดา เป็นกลุ่มควบคุม กลุ่มที่ 2 ได้รับอาหารพื้นฐานที่ผสมฟรุคโตโอลิโกแซคคาไรด์ในระดับ 4 ก.ต่อ กก.อาหาร และให้น้ำธรรมดา กลุ่มที่ 3 ได้รับอาหารพื้นฐานและให้น้ำผสมกรดอินทรีย์ในอัตราส่วน 1:1,000 ผสมน้ำให้กินทุกวัน กลุ่มที่ 4 ได้รับอาหารพื้นฐานและให้น้ำผสมกรดไขมันสายปานกลางในอัตราส่วน 1:1,000 เป็นระยะเวลา 35 วัน หลังจากนั้นให้ในอัตราส่วน1:2,000 ผสมน้ำให้กินทุกวันจนสิ้นสุดการทดลอง วันที่ 3 ทำการป้อนเชื้อ Salmonella ความเข้มข้น 10^6 cfu/มล. จำนวน 0.3 มล. และในวันที่ 13 ทำการป้อนเชื้อ Salmonella ซ้ำที่ความเข้มข้น 10^8 cfu/มล. จำนวน 1 มล. ชั่งน้ำหนักไก่ทดลองทุกกลุ่มและบันทึกปริมาณอาหารที่กินเฉลี่ยเพื่อคำนวณอัตราการแลกเนื้อในวันที่ 21, 35 และ 45 ของการทดลอง ในวันที่ 17, 24 และ 45 ทำการเก็บตัวอย่างไส้ตันเพื่อตรวจการเกาะกลุ่มของเชื้อซัลโมเนลล่า วัดค่าความเป็นกรด-ด่างของกระเพาะพัก ลำไส้และไส้ตัน เก็บตัวอย่างเซลล์เยื่อบุผนังลำไส้เล็กส่วนกลางมาวัดระดับเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่ เก็บตัวอย่างอาหารในลำไส้เล็กส่วนปลายมาตรวจหาค่าการย่อยได้ของโปรตีน พลังงาน และไขมัน เก็บตัวอย่างอุจจาระในไส้ตัน แล้วนำไปวิเคราะห์หากรดไขมันสายสั้น และสายปานกลาง เก็บตัวอย่างเลือดเพื่อใช้ในการวิเคราะห์หากรดไขมันสายปานกลาง การเจริญเติบโตในภาพรวมตลอด 45 วันของการทดลอง พบว่าไก่ที่ได้รับกรดไขมันสายปานกลาง และกรดอินทรีย์ผสมน้ำ การเจริญเติบโตเฉลี่ยต่อวันมากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ (p<0.05) และประสิทธิภาพการเปลี่ยนอาหารเป็นเนื้อดีกว่าไก่ในกลุ่มควบคุมอย่างมีนัยสำคัญ (p<0.05) พบว่าน้ำหนักตัวสุดท้ายของไก่กลุ่มที่เสริมกรดไขมันสายปานกลาง กรดอินทรีย์ ฟรุคโตโอลิโกแซคคาไรด์มากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ (p<0.05) พบว่าผลการเกาะกลุ่มของเชื้อซัลโมเนลล่าในกลุ่มที่ได้รับกรดไขมันสายปานกลางผสมน้ำ และกรดอินทรีย์ผสมน้ำ ลดลงอย่างมีนัยสำคัญทางสถิติ (p<0.05) เมื่อเทียบกับกลุ่มควบคุม และไก่ทดลองที่มีการเสริมกรดไขมันสายปานกลาง กรดอินทรีย์ ฟรุคโตโอลิโกแซคคาไรด์ พบว่าค่าพีเอชในกระเพาะพักและในทางเดินอาหารลดลงแตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญ (p<0.05) นอกจากนี้พบกรดไขมันสายปานกลางในเลือดแตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ (p<0.05) และพบว่าไก่กลุ่มที่ได้รับกรดไขมันสายปานกลางผสมน้ำ มีค่าการย่อยได้ทางโภชนะ ปริมาณเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่และระดับกรดไขมันสายสั้น (กรดอะซิติกและกรดวาเลริค) เพิ่มขึ้น อย่างมีนัยสำคัญ (p<0.05) เมื่อเปรียบเทียบกับกลุ่มควบคุม การศึกษาครั้งนี้แสดงให้เห็นว่า กรดไขมันสายปานกลาง และกรดอินทรีย์ มีผลเพิ่มการเจริญเติบโตและลดการเกาะกลุ่มของเชื้อซัลโมเนลล่า ลดระดับความเป็นกรดในกระเพาะพักและในลำไส้ พบว่ากรดไขมันสายปานกลาง และฟรุคโตโอลิโกแซคคาไรด์ มีผลเพิ่มปริมาณเอนไซม์ซูเครส นอกจากนี้ในไก่กลุ่มที่เสริมกรดไขมันสายปานกลางพบกรดไขมันสายปานกลางในเลือด และพบว่ากรดไขมันสายปานกลางมีผลเพิ่มการย่อยได้ของโภชนะ เพิ่มปริมาณเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่ ในลำไส้เล็กและเพิ่มปริมาณกรดไขมันสายสั้นในไส้ตัน กรดไขมันสายปานกลางเป็นสารเสริมทางเลือกที่มีประสิทธิภาพในการควบคุมเชื้อซัลโมเนลล่าในไก่เนื้อ

คำสำคัญ : ไก่เนื้อ ฟรุคโตโอลิโกแซคคาไรด์ กรดไขมันสายปานกลาง กรดอินทรีย์ ซัลโมเนลล่า เอนเทอริทิดิส

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Introduction

Salmonella spp are recognized in many parts of the world as a major cause of food borne infections in human and the consequent economic loss. In recent years, Salmonella enterica serotype Enteritidis (*S*. Enteritidis) has become the dominant serotype isolated from cases of human food poisoning in many countries, including Thailand. Considering sources of contamination, *S*. Enteritidis, was isolated from 28% of the retail chicken meat, 4.5% of the chicken meat from slaughterhouses, and 6.6% of the excreta from chickens (Boonmar et al., 1998). The US Department of Agriculture Food Safety and Inspection Service (FSIS) data on *S*. Enteritidis in broiler chicken carcass rinses collected from 2000 through 2005 showed the annual number of isolates increased >4-fold and the proportion of establishments with *S*. Enteritidis-positive rinses increased nearly 3-fold (Altekruse et al., 2006) Antibiotic supplementation as a sub-therapeutic growth promoter is widely used in poultry production in the world to control Salmonella (van Immerseel, 2002). However, this practice in animal production is under inspection because it has been implicated as the major cause for the rise in antimicrobial resistance and residues in animal products and environmental contamination has greatly added to the public concern regarding the use of antibiotic in the feed. Since the proposed ban on antibiotic growth promoters in 2006, it is imperative to the feed industry that alternatives to antibiotics in animal feed be searched and tested for ways of efficient animal production. All feed additives have advantages and disadvantages in controlling Salmonella infection and improving feed utilization. Medium chain fatty acids (MCA) are one of new additives used to control Salmonella infection and it is interested to examine their possible role in poultry production when compared with known supplements such as organic acids and prebiotics. MCA is composed of caproic acid (C_6), caprylic acid (C_8) and capric acid (C_{10}) which can reduce pathogenic bacteria (van Immerseel et al., 2004) and provide energy as they can be absorbed directly into the portal vein (Papamandjari et al., 1998) but its effect on physiological changes in the gut has not been elucidated. Changes in pH of gastrointestinal contents, disaccharidase enzyme activities and nutrient digestibility are among the important measures of the gut changes to examine effect of additives in Salmonella inoculated chickens. The objectives of this experiment were to examine the effect of MCA compared with mixed organic acids (ORA) and fructo-oligosaccharide (FOS) on eliminating *S*. Enteritidis colonizing in broiler chickens. Moreover, the effect of these three additives on growth performance, intestinal pH, disaccharidase activities, short chain fatty acids and ileal digestibility of nutrients were investigated.

Materials and Methods

This study was approved by the Institutional Laboratory Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University.

Animals, diets and sample preparation: The experiment was conducted in a closed concrete-floor-pen house with an evaporative cooling facility. The chicks were raised according to routine practice in terms of light and temperature (the light: dark cycle was 24:0 hr in the first three weeks of the trial and the cycle was changed to 16:8 hr in the final three weeks with room temperature recorded and analyzed twice daily). Six hundred, one day old, male and female, Cobb-500 chicks were allocated into 4 groups of 150 chicks (5 replicates of 30 chicks each). The basal diet comprised corn-soybean meal as the major ingredient and the proximate analysis of the diet is demonstrated in Table 1. (starter feed from day 1 to day 21 of the trial and grower-finisher feed from day 22 to 45 of the trial). Chicks in group1 received a basal diet and were given tap water (CON). In group 2, chicks received a basal diet supplemented with 4 g FOS on top of 1 kg feed and were given tap water until the end of the experiment (FOS). Chicks in group 3 were given a basal diet and tap water that was supplemented with mixed organic acids (lactic acid, citric acid, ascorbic acid and propionic acid) (ORA) at 1:1000 until the end of the experiment. Group 4

chicks received a basal diet and were given tap water mixed with medium chain fatty acids (MCA) at 1:1,000 continuously from the start until 35 days and 1:2,000 until the end of the experiment. Feed and water were provided *ad libitum* throughout the experiment. Drinking water was changed daily and water intake was recorded weekly. The chickens were weighed at days 1, 21, 35 and 45. Feed intake was recorded daily during day 0 to 21, day 22 to 35 and day 36 to 45 and average feed intake was calculated in each period. Mortality was recorded daily. At day 3 post-hatching, each chick was inoculated 0.3 ml with tryptone soya broth (TSB) culture of *S*. Enteritidis (nalidixic-resistant strain, nal^r) containing 1 x 10^6 colony forming units (cfu) by oral route using an esophageal tube. At day 13 post-hatching, a 2^{nd} inoculation was given to all chicks with one ml of 1 x 10^8 cfu of the same Salmonella culture. Twenty chickens from each treatment group were randomly selected and slaughtered with an intracardiac injection of pentobarbital sodium (120 mg/kg BW) at days 8, 17, 24 and 45 post-hatching. The abdomen was exposed and the whole intestine from the duodenum to the cloaca was removed. The intestinal section from the entry of pancreatic and bile duct to a section of Meckel's diverticulum was taken as the jejunal (J) part. The ileal (I) part was taken from Meckel's diverticulum to the ileocecal junction. Samples of the intestinal contents were evacuated into containers and the pH was immediately measured using a digital pH meter (ORION, model 420A). The jejunal part was opened longitudinally, rinsed with ice cold saline and placed on a foam pad. Mucosal samples were scraped from the mucosa layer using a glass slide, wrapped with aluminum foil and stored at -70 °C until analysis for disaccharidase activity. A cecum was removed and placed in a plastic bag for bacteriological study. Cecal content from the other cecum was wrapped with aluminum foil and stored at -70 °C until analysis for short-chain fatty acids. In addition, ten chickens from each treatment group at 22 and 43 days post-hatching were randomly selected. These chickens were fed on diets containing celite (20 g/kg in feed) as an indigestible marker for 5 days (days 22-26 and days 43-47). On day 27 and 48 post-hatching, they were sacrificed with an intracardiac injection of pentobarbital sodium (120 mg/kg BW) and the ileum and ceca were removed. The contents in the ileum were collected by gentle squeezing with the thumb and fingers into plastic bottles. The ileal contents from chickens in each replicate were pooled together due to the small amount of contents. The ileal contents were kept frozen at -70 °C until analysis for nutrient digestibility.

Analytical methods: Jejunal mucosal scrapings were analyzed for disaccharidase activity (maltase and sucrase) (Dahlquist, 1968). Total protein concentrations in the jejunal mucosa were determined. (Lowry, 1951). Acid-insoluble ash in the ileal contents and the diets was measured as described by Choct and Annison (1992) and nutrient composition was analyzed using proximate analysis (AOAC, 1995). The energy and protein contents in the feed and ileal digesta were analyzed using Adiabetic Bomb Calorimeter and Modified Kjeldahl methods (AOAC, 1995), respectively. The percentage of ileal digestibility coefficient (IDC) of nutrients (crude protein and energy) was calculated using the following equation:

$$IDC = 1 - \frac{(\text{lleal nutrient (\%)} / \text{lleal acid insoluble ash (\%)})}{(\text{Diet nutrient (\%)} / \text{Diet acid insoluble ash (\%)})} \times 100$$

Cecal short-chain fatty acid concentrations (SCFA) were analyzed using the modified method from Erwin (1961). In brief, frozen intestinal contents were weighed and diluted with an equal volume of distilled water. The solutions were centrifuged at 9,000 rpm for 10 min. The supernatant was separated for SCFA determination. The mixture of four standard SCFA solutions was 70 mM acetic acid, 30 mM propionic acid, 10 mM butyric acid and 2 mM valeric acid. The internal standard used was isocaproic acid. The volume of 0.4 ml working internal standard solution (containing isocaproic acid, formic acid and 25% metaphosphoric acid) was mixed with 0.7 ml of the supernatant or standard solution. The aliquots

were analyzed for SCFA concentration using a gas chromatography equipped with a hydrogen flame ionization detector. The concentration of individual SCFA was expressed as μmole/g cecal content.

Medium chain fatty acid concentration (MCFA) in the plasma and cecal content was analyzed using a method modified from Mingrone et al. (1995). Nonanoic acid (100μg) in 100 μl ethanol was added, as an internal standard, to 0.5 ml of plasma or supernatant acidified to pH 2-3 with 0.15 mol/l HCl , then solutes were extracted by 2 volumes of ethanol kept overnight at -20°C in order to precipitate proteins. The samples were centrifuged at 4,000 g in a refrigerated centrifuge at 4°C for 10 min and the residue was washed twice with ethanol and re-centrifuged. The solutions were reduced to 0.5-1 ml, of which 1 μl was directly injected into a Gas Liquid Chromatograph (GLC) (Hewlett-Packard) equipped with a flame ionization detector (FID). MCA were separated on a 25 mm fused silica capillary column of crosslink methyl siloxane HP-1, 0.32 mm, film thickness 0.17 μm. The concentration of individual MCA was expressed as μg/ml plasma.

Qualitative and quantitative examination of *S.* Enteritidis

Cecal samples were aseptically removed from each chick. The ceca was weighed, chopped and put into buffered peptone water (BPW) (Oxoid, Basingstoke, England) with 2 volumes of weight of ceca, then blended in a stomacher. From the initial 10-1 dilution, 10-fold serial dilutions were made in BPW at dilutions of 1:100, 1:1000 and spread-plated on to XLT4 agar plates plus 25 μg/ml of nalidixic acid. The plates were incubated for 24 h at 37°C and *S.* Enteritidis colonies were identified. The number of colony-forming units of Salmonella was expressed as log10 Salmonella per gram of cecal contents. The cecal sample of 1:10 dilution was also incubated at 37°C for 24 h. Then 100 μl of BPW were inoculated on to a MSRV agar plate and incubated at 42°C for 24-48 h. The suspected colonies in MSRV was cultured on XLT4 agar plates plus 25 μg/ml of nalidixic acid and incubated for 24 h at 37°C. Salmonella suspected colonies from all of the XLT4 agar plates were identified.

Statistical analysis: Data are presented as Mean±Pooled SEM. The effects of treatment were analyzed using One-Way Analysis of Variance (ANOVA). If there were any significant effects, Duncanís New Multiple Range Test was used to compare the individual means. Data, which did not comply with the equal variance and homogeneity tests, were analyzed using non-parametric methods (Kruskral Wallis test). Data on the qualitative *Salmonella* test (positive-negative) before and after treatment were analyzed by Chi-square analysis. The significant level was set at $p<0.05$.

Results

The overall growth performance of chicks is demonstrated in Table 2. Chicks in the ORA and MCA groups had significantly ($p<0.05$) greater final body weights, average daily gain (ADG) than the FOS and CON groups (Table 2). Chicks in the CON group had a significantly ($p<0.05$) lower final body weight than the FOS group. There were no significant differences in daily feed intake (DFI) and the percentage of mortality among groups of chicks. The mortality rate was within normal limits considering the size of the chicken colony and chicks died more in the finisher period than in the starter-grower period, possibly due to heat stress. Chicks in both the ORA and MCA groups had a significantly ($p<0.05$) better feed conversion ratio (FCR) and average daily gain than the CON and FOS groups.

The changes in the pH of the crop and the intestinal content of the jejunum, ileum and ceca are demonstrated in Table 3. At day 24 post-hatching, the jejunum pH values in the FOS (6.46), ORA (6.37) and MCA (6.35) groups were significantly lower ($p<0.05$) than in the CON (6.63) group but there were no differences in the ileal pH and cecal pH among the treatment groups. Likewise, at day 45 post-hatching, the pH of the crop and jejunum decreased significantly ($p<0.05$) in the FOS, ORA and MCA groups, as compared with the CON group. Moreover, the pH in

the crop of MCA group was the lowest (4.86) ($p<0.05$). It is shown that the pH of ileum in the ORA and MCA groups was lower ($p<0.05$) than the FOS and CON groups. There was no difference in the pH of the cecum among groups of chicks.

The mean $\log_{10}$ number of *S*. Enteritidis per gram of cecal content in the chicks challenged with Salmonella was significantly decreased ($p<0.05$) in the MCA group, as compared with the CON group on day 17 post-hatching (Table 4). No difference in the Salmonella numbers in the cecal contents was found between the FOS group and CON group. Qualitative Salmonella percentage was significantly lower in the MCA and ORA groups compared with the CON group on days 45 post-hatching. Furthermore, it is noted that there was no *S*. Enteritidis found in any chicks in the ORA and MCA groups on day 45. Likewise, Salmonella percentage in the FOS group was lower ($p<0.05$) compared with CON group.

Maltase activity in the FOS and MCA groups were significantly higher ($p<0.05$) than the CON group and maltase activity of the MCA group was significantly higher ($p<0.05$) when compared to the ORA group (Table 5). At day 45 post-hatching, there was no significant difference in maltase activity of the jejunal mucosa among experimental groups. There was no significant difference in the sucrase activity of each group on day 24 post-hatching. The sucrase activities of MCA and FOS groups at day 45 post-hatching were significantly greater ($p<0.05$) than the CON and ORA groups.

Cecal concentrations of each short-chain fatty acids at day 27 post-hatching are shown in Table 6. Cecal acetic acid and valeric acid significantly increased ($p<0.05$) in the MCA group, as compared with the CON group. There was no significant difference in the propionic acid and butyric acid concentrations. The level of butyric acid concentration in the CON group was lower ($p>0.05$) compared to other groups. At the finisher period (day 48 post-hatching), the concentrations of acetic acid increased significantly ($p<0.05$) in the MCA group, as compared to the FOS and CON groups. The valeric acid of the MCA group was also significantly ($p<0.05$) higher than other groups. Likewise, on day 27 post-hatching, there was no difference in propionic acid and butyric acid concentrations among groups. The total SCFA concentrations of the MCA group were significantly higher ($p<0.05$) than the CON and FOS groups but not different from the ORA group. Portal plasma concentrations of each medium chain fatty acids at days 21 and 45 post-hatching are shown in Table 6. The caproic (C_6) concentrations were found to be the highest in the MCA group. Moreover, the caprylic acid (C_8) concentrations were found only in the MCA group. For the total MCFA concentration, chicks in the MCA group had the highest MCFA concentrations while there were no MCFA found in the CON group.

The IDC of protein and energy the broilers is demonstrated in Table 7. At day 27 post-hatching, it was found that the IDC of crude protein and energy were not different among groups. However, at day 48 post-hatching, it was found that the IDC of protein in the FOS, ORA and MCA groups were significantly ($p<0.05$) higher than the CON group. Furthermore, it was shown that the broiler chickens in the MCA group had significantly ($p<0.05$) higher IDC of protein than ORA and FOS groups. The IDC of energy in the FOS, ORA and MCA group was significantly ($p<0.05$) higher than the CON group with the MCA > ORA and the ORA > FOS groups ($p<0.05$).

Discussion

The result demonstrated that chicks in the MCA and ORA groups had significantly better growth performance than other groups and this may be due to the antibacterial effect of both fatty acids in controlling Salmonella infection. It was found that chicks in the MCA group had higher short chain fatty acids in the ceca, especially acetate and valerate, compared to other groups. Moreover, concentrations of SCFA similar to those found in the ceca, have been shown to inhibit the growth

Table 1 Composition and nutrient contents of basal diets

Ingredients		Starter	Grower-finisher
Corn		51.80	56.68
Soybean meal		24.06	17.88
Full fat soybean		15.00	15.00
Fat powder		1.50	1.50
Palm oil		2.94	4.13
L-Lysine HCl		0.23	0.26
DL-Methionine		0.40	0.42
L-Threonine		0.09	0.13
Mono-,dicalcium phosphate		1.89	1.95
Limestone		1.32	1.34
Sodium bicarbonate		0.10	0.05
Salt		0.30	0.29
Choline chloride 60%		0.10	0.10
Vitamin/mineral premix*		0.15	0.15
Filler (corn starch)		0.12	0.12
Nutrients (calculated)			
Dry matter	%	89.06	89.13
ME for poultry	Kcal/kg	3,150	3,250
Crude protein	%	21.60	19.02
Crude fat	%	9.42	10.70
Crude fiber	%	3.69	3.36

*Each g of Premix per kg diet comprises Vitamin A 12,000, 10,000 IU. Vitamin D3 3,000, 2,400 IU, Vitamin E 15, 12 mg, Vitamin K3 1.5, 1.2 mg, Vitamin B1 1., 1.2 mg, Vitamin B2 5.5, 4.4 mg, Vitamin B6 2, 1.6 mg, Vitamin B12 0.01, 0.01 mg, nicotinic acid 25, 20 mg, D-calcium pathothenate 12, 10 mg, folic acid 0.5, 0.4 mg, biotin 0.01,0.01 mg , choline chloride 250, 250 mg, Mn 80, 80 mg, Zn 60, 60 mg, Fe 40, 40 mg, Cu 8, 8 mg, I 0.5, 0.5 mg, Co 0.1, 0.1 mg, and Se 0.1, 0.1 mg

Table 2 Effect of treatments on the growth performance of broiler chickens (1-42 day posthatching)

	CON	FOS	ORA	MCA	SEM
Initial weight (g/b)	41.5	40.6	41.7	41.1	1.9
Final weight (g/b)	1,827.7^{c}	1,904.5^{b}	2,004.6^{a}	2,009.23^{a}	40.9
ADG (g/b/d)	39.7^{b}	41.4^{b}	43.6^{a}	43.7^{a}	0.9
DFI (g/b/d)	72.4	74.4	73.5	73.2	3.2
FCR	1.82^{b}	1.79^{b}	1.68^{a}	1.67^{a}	0.06
Mortality (%)	0.67	1.33	0.67	2.00	5.77

^{a,b,c}Means in the same row with unlike superscripts differ significantly ($p<0.05$)

Table 3 Effect of treatments on the pH of the crop and intestinal segments.

Part of GI tract	Treatment				
	CON	FOS	ORA	MCA	SEM
Crop					
Day 45	5.69[a]	5.51[b]	5.17[c]	4.86[d]	0.022
Jejunum					
Day 24	6.63[a]	6.46[b]	6.37[b]	6.35[b]	0.024
Day 45	6.63[a]	6.38[b]	6.10[b]	6.17[b]	0.034
Ileum					
Day 24	7.21	7.18	7.19	7.13	0.024
Day 45	7.10[a]	6.85[b]	6.22[c]	6.18[c]	0.050
Ceca					
Day 24	6.34	6.37	6.23	6.23	0.020
Day 45	6.91	6.90	6.76	6.77	0.037

[a,b,c,d]Means in the same row with unlike superscripts differ significantly ($p<0.05$)

Table 4 Effect of treatments on *Salmonella colonization* in ceca of broilers.

Day	CON		FOS		ORA		MCA	
	% Positive[1]	Salmonella Count[2]	% Positive	Salmonella Count	% Positive	Salmonella Count	% Positive	Salmonella Count
17	100	3.35±0.38[*a]	100	3.26±0.38[a]	90	2.00±0.01[b]	100	2.23±0.12[b]
24	100	2.63±0.68	80	2.23±0.63	90	1.36±0.38	60	1.49±0.49
45	70[a]	ND	20[b]	ND	0[c]	ND	0[c]	ND

[*]Mean ± SE, n = 10,
[1]% of Salmonella positive in cecal content, [2]Salmonella number in cecal content of infected chicken ($\log_{10}$ cfu/g content), ND: not determined
[a,b,c]Means in the same row with unlike superscripts differ significantly ($p<0.05$)

Table 5 Effect of treatments on jejunal disaccharidase activities[1] of broilers

Enzyme	Treatment				SEM
	CON	FOS	ORA	MCA	
Maltase (units/mg protein)					
Day 24	131.43[b]	189.58[a]	142.35[ab]	188.82[a]	30.17
Day 45	95.07	112.91	110.84	141.52	28.33
Sucrase (units/mg protein)					
Day 24	31.32	35.82	30.40	31.52	6.47
Day 45	10.74[b]	20.68[a]	13.08[b]	20.66[a]	3.75

[a,b]Means in the same row with unlike superscripts differ significantly ($p<0.05$)

Table 6 Profile of short-chain fatty acid in cecal contents (mmol/ml) and medium chain fatty acids (mmol/ml) in portal blood of broilers at days 27 and 48 post-hatching

	Treatment				SEM
	CON	FOS	ORA	MCA	
Day 27					
Acetic acid (C_2)	50.82^{b}	65.20^{ab}	65.07^{ab}	73.05^{a}	10.22
Propionic acid (C_3)	14.68	15.98	15.28	18.49	2.86
Butyric acid (C_4)	13.75	19.54	21.29	19.40	7.41
Valeric acid (C_5)	0.92^{b}	1.02^{ab}	1.12^{ab}	1.31^{a}	0.18
Total SCFA	80.38	99.51	102.75	112.25	15.38
Caproic acid (C_6)	0^{c}	8.00^{b}	6.84^{b}	40.43^{a}	11.64
Caprylic acid (C_8)	0^{b}	0^{b}	0^{b}	27.34^{a}	13.02
Day 48					
Acetic acid (C_2)	49.88^{b}	54.55^{b}	73.22^{ab}	93.71^{a}	20.28
Propionic acid (C_3)	16.03	17.11	21.73	25.87	4.88
Butyric acid (C_4)	8.11	9.19	14.57	19.26	6.21
Valeric acid (C_5)	0.66^{b}	0.62^{b}	1.02^{b}	1.51^{a}	0.31
Total SCFA	57.68^{b}	81.47^{b}	110.54^{ab}	140.35^{a}	29.42
Caproic acid (C_6)	0^{c}	13.58^{b}	0^{c}	34.21^{a}	8.37
Caprylic acid (C_8)	0^{b}	0^{b}	0^{b}	37.70^{a}	4.33

^{a,b,c}Means in the same row with unlike superscripts differ significantly (p<0.05)

Table 7 Effect of treatments on percentage of ileal nutrient digestibility coefficient of broilers.

Nutrients		Treatment				SEM
	Day	CON	FOS	ORA	MCA	
Crude Protein	27	0.776	0.772	0.773	0.740	0.004
	48	0.521^{c}	0.675^{b}	0.709^{b}	0.833^{a}	0.003
Energy	27	0.738	0.737	0.735	0.696	0.005
	48	0.599^{d}	0.739^{c}	0.770^{b}	0.885^{a}	0.003

^{a,b,c,d}Means in the same row with unlike superscripts differ significantly (p<0.05)

Salmonella, this inhibition is increased with the reduction in the redox potential of the ceca accompanied by a lower pH of the ceca (McHan and Shotts, 1993). It may be an indication that the undissociated form of volatile fatty acids reduced the numbers of Enterobacteriacae *in vivo* (van der Wielen et al., 2000). It is demonstrated that the pH of crop and small intestines in the MCA, ORA and FOS groups were significantly decreased compared to the CON group. Similarly, the use of acetic, lactic, or formic acid in the drinking water significantly reduced crop pH and decreased the recovery of Salmonella from crop samples (Byrd et al., 2001). In contrast, van Immerseel (2002) reported that there was no effect of FOS on the pH of the crop because oligosaccharides were neither degraded nor hydrolyzed in the upper intestinal tract and reached the ceca. However, this study demonstrated that the pH of the crop in chicks fed on FOS was significantly lower than the CON group at day 45. It might be possible that FOS

was fermented by some Lactobacilli in the crop. Durant et al. (1999) indicated that Lactobacilli are the predominant colonizers of the stratified squamous epithelium of the crop. Moreover, the production of short chain fatty acids by the intestinal flora can be stimulated by adding fermentable prebiotics to the feed (Cumming, 1981). The chicks in the FOS group tended to have higher SCFA in their cecal contents, compared to chicks in the CON group, but these effects were not significant. There were no significant differences in FCR in the FOS group compared to the control. In contrast, Xu et al. (2003) showed that the addition of 4 g/kg FOS significantly increased average daily gain and decrease feed to gain ratio. Moreover, Ammerman et al. (1988) found that addition of 2.5 and 5 g/kg FOS significantly improved feed efficiency over the entire feeding period of 46 days. The results showed that MCA and ORA demonstrated an antibacterial action against Salmonella. The number of Salmonella colonized in ceca of the MCA and ORA groups was significantly lower than the CON group. This may be due to the antibacterial activity of both fatty acids. They can diffuse into the bacterial cells in an undissociated form. Inside the bacterial cell, the acid dissociates, resulting in a reduction of intracellular pH, suppression of cytoplasmatic enzymes and nutrient transport systems and uncouple ATP driven pumps, leading to death (Hsiao and Siebert, 1999). van Immerseel et al. (2004) suggested that all MCA decreased the expression of hilA, a key regulation gene related to the invasive capacity of Salmonella. The bactericidal activity of organic acids is directly associated with increased concentration of undissociated organic acid and the concentration of undissociated acid is dependent on both the total concentration of organic acid and pH (Hinton et al., 1990). It is proposed that the antimicrobial activity of organic acids was dependent on the pKa of the acid, molecular weight (MW) and lipophilic/ hydrophilic character (Dierick et al., 2002). The pKa of MCA was 4.9 (Hsiao and Siebert, 1999) and pKa of SCFA was < 4.8 and the pH in the crop ranged between 4 and 7 (Soerjadi et al., 1982), thus, most of the MCA were in an undissociated form.

Salmonella infection can lead to change in the intestinal mucosa (Suzuki et al., 1992). Changes in intestinal morphology such as shorter villi and deeper crypts have been associated with the toxins, resulting in the reduction of enzyme production (Yason et al., 1987). It is possible that the MCA restored the mucosal cell function as seen in the improvement of brush border disaccharidase enzymes by providing energy to these absorptive cells. MCA also have unique properties in their direct transport via the portal blood to the liver and their preferential oxidation in the mitochondria to provide energy, CO_2 and ketone bodies (Odle, 1999). It is demonstrated that the chicks in the MCA group had high MCA concentrations in portal vein. Odle (1997) showed that medium chain fatty acid had a specialized energy source, and better utilized in neonatal piglets. Kishi et al. (2002) showed that MCA were utilized as an immediate energy source in insufficient fat digestion.

The gastrointestinal tract constitutes the first barrier to nutrient metabolism in animals (Cant et al., 1996). The metabolic activity of the gastrointestinal mucosa can have a tremendous impact on nutrient supply to the animal. The intestinal villi and crypt morphology in chickens has been associated with intestinal function and chicken growth. In the finisher period, the numbers of Salmonella in the CON group were significantly higher than other groups. It is proposed that Salmonella may damage the villi and microvilli of the intestinal mucosa and inhibit the secretion of digestive enzymes. These result in the reduction of the small intestinal absorptive area and the appearance of a less mature enterocyte population. The more immature enterocytes resulted in the reduction of enzyme production. It is demonstrated that chicks in the MCA group had significantly higher maltase activity than the CON group. Guillot et al. (1993) indicated that the liver is the main site of MCA utilization and suggests that a substantial proportion of these acids may also be utilized in the intestinal mucosa. Jorgensen et al. (2001) demonstrated that both C_6 and C_8 fatty acids

seemed to be excellent substrates for colonocyte oxidation in rat. The results agree with a previous study that MCA have a positive effect on epithelial cell membrane bound enzyme activities (Takase and Goda, 1990). Furthermore, MCA improved in intestinal morphology and function, through their positive effects on crypt cell renewal (Jenkins and Thompson, 1993). It is demonstrated that chicks in the FOS group had an increase in sucrase activities in the jejunum and had a slight increase in maltase activity. It is possible that FOS exerted a preferential stimulatory effect on Bifidobacterium and Lactobacillus (Xu et al., 2003), while it suppressed Salmonella in the small intestine. Bifidobacterium readily ferments FOS because of the innate secretion of a β-fructoside enzyme and some other bacteria to produce short chain fatty acids (SCFA) (Gibson, 2004). Sakata (1987) reported that acetate, propionate and butyrate have a dose dependent stimulatory effect on epithelial cell production rates in the jejunum and the distal colon. Moreover, SCFA production from the fermentable fiber may result in a decrease in mucosal atrophy by normalizing cell proliferation in the mucosa (Campbell et al., 1997). *In vitro* studies with rats show the trophic effects of SCFA on epithelial cell proliferation (Frankel et al., 1994). Goldin (1998) indicated that the use of prebiotics can lengthen villi within the gut and also influence the length of the gut. Furthermore, the Bifidobacterium and Lactobacilli spp. can synthesize enzymes, thus increasing the intestinal digestive enzyme activity (Sissons, 1989). The digestive process is highly dependent on endogenous enzyme activity (Pubol, 1991) and enzyme activities increase the availability of nutrients in the small intestine (Sklan, 2001). It is possible that MCA were utilized as immediate energy source and a substantial proportion of these acids may also be utilized in the intestinal mucosa (Guillot et al., 1993). This study showed that MCA (caproic acid and caprylic acid) were found in the portal vein of chicks fed on water supplemented with MCA. It is proposed that MCA can directly be absorbed without hydrolysis and preferentially transported through the portal venous system to the liver (Beerman et al., 2003). This rapid absorption in the portal vein can be explained by 1) a greater solubility of MCA in an aqueous medium which would facilitate their uptake by the intestinal mucosa. 2) a lower affinity of the intestinal fatty acid binding protein (Ockner et al., 1972) and of acyl CoA synthetase (Brindley and Hubscher, 1966) for MCA compared with LCFA. Moreover, it is demonstrated that there were no MCA found in the ceca of MCA group. It is possible that MCA were entirely absorbed in the small intestine or may have been utilized by the colonic mucosa. Jorgensen (2001) indicated that the colonic mucosa can both metabolize and transport MCA. Octanoate and decanoate were oxidized to CO_2 as well as butyrate and thus provided energy to the colonic epithelium (Jorgensen, 2002). This MCA serves as a ready source of energy, with high digestion and oxidation rates (Chiang et al., 1990). The result of this study indicated that chicks in MCA group were supported with rapidly available energy. Furthermore, chicks in the FOS group had significantly higher caproic acid (C_6) in the portal vein, compared to the CON group. It is possible that fermentation of FOS can lead to the production of some C_6. However, it is noted that only one sample from five samples in both the FOS and ORA groups was found. Furthermore, it is demonstrated that chicks in MCA group also had a higher digestibility of nutrients than other groups. It is possible that MCA have an increase in brush border enzyme and they are absorbed more quickly into the intestinal lumen (Papamandjaris, 1998). In addition, MCA are not significantly incorporated into triglycerides and the subsequent chylomicrons as are long chain fatty acids. Therefore, they leave the intestine and enter the portal blood stream and reach the liver directly, providing a supply of energy to this organ (Bach and Babayan, 1982, Decker, 1996). Moreover, Galluser et al. (1993) suggested that a greater solubility of MCA would facilitate uptake by the intestinal mucosa, thus improving intestinal morphology and functions. Furthermore, this study indicated that chicks in the FOS group had higher nutrient digestibility than the CON group. It is possible that FOS supplementation has been shown to increase

numbers of beneficial bacteria such as Bifidobacteria and Lactobacilli. The Bifidobacteria and Lactobacilli colonizing the intestine have been reported to deliver luminal enzymes, thus increasing digestive enzyme activity in the intestines (Sissons, 1989). It is proposed that increased the enzyme activity will affect the efficiency of nutrient digestibility.

In conclusion, the results of this study demonstrate that MCA, ORA and FOS supplemented in chicks were beneficial in ameliorating the adverse effects of Salmonella colonization in broilers. MCA supplementation was equally effective with organic acids in decreasing the levels of colonization in ceca and improved growth performance. Moreover, MCA reduced pH in the crop and small intestine and improved disaccharidase activity and the digestibility of nutrients. In addition, MCA increased cecal SCFA concentrations and MCFA in portal blood. Therefore, MCA is one of the efficient additives appropriate for Salmonella control in broilers.

Acknowledgement

The authors wish to thank to the financial support by the Chulalongkorn University 90th Anniversary, Ratchadaphiseksompoch Research Fund.

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