ผลของกรดไขมันสายปานกลาง กรดอินทรีย์ และฟรุคโตโอลิโกแซคคาไรด์ต่อการเกาะกลุ่มของเชื้อซัลโมเนลล่า เอนเทอริทิดิส การเปลี่ยนแปลงทางกายภาพ และชีวภาพของลำไส้เล็กในไก่เนื้อ

นางสาวสุชีรา โชติกธรรม

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EFFECTS OF MEDIUM CHAIN FATTY ACIDS, ORGANIC ACIDS AND FRUCTOOLIGOSACCHARIDE ON CECAL *Salmonella enterica* Serovar Enteritidis COLONIZATION, PHYSICAL AND BIOLOGICAL CHANGES OF INTESTINE IN BROILER CHICKS

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สุชีรา โชติกธรรม: ผลของกรดไขมันสายปานกลาง กรดอินทรีย์ และฟรุคโตโอลิโกแซคคาไรด์ต่อการเกาะกลุ่มของเชื้อซัลโมเนลล่า เอนเทอริทิดิส การเปลี่ยนแปลงทางกายภาพ และชีวภาพของลำไส้เล็กในไก่เนื้อ (EFFECTS OF MEDIUM CHAIN FATTY ACIDS, ORGANIC ACIDS AND FRUCTOOLIGOSACCHARIDE ON CECAL *Salmonella enterica* Serovar Enteritidis COLONIZATION, PHYSICAL AND BIOLOGICAL CHANGES OF INTESTINE IN BROILER CHICKS) : อ.ที่ปรึกษา: รศ.น.สพ.ดร. กฤษ อังคนาพร, อ. ที่ปรึกษาร่วม: รศ.สพ.ญ อินทิรา กระหม่อมทอง, 91 หน้า, ISBN 974-17-5125-7

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของกรดไขมันสายปานกลาง กรดอินทรีย์ ฟรุคโตโอลิโกแซคคาไรด์ ต่อการเกาะกลุ่มของเชื้อซัลโมเนลล่า เอนเทอริทิดิส ต่อคุณลักษณะการเจริญเติบโต การเปลี่ยนแปลงทางกายภาพ และชีวภาพของลำไส้ของไก่เนื้อ โดยในการทดลองใช้ลูกไก่คละเพศ อายุ 1 วัน จำนวน 600 ตัว แบ่งเป็น 4 กลุ่ม กลุ่มที่ 1 ได้รับอาหารพื้นฐานและให้น้ำธรรมดา เป็นกลุ่มควบคุม กลุ่มที่ 2 ได้รับอาหารพื้นฐานที่ผสมฟรุคโตโอลิโกแซคคาไรด์ใน ระดับ 4 กรัมต่อกิโลกรัมอาหารและให้น้ำธรรมดา 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/ml จำนวน 0.3 ซีซี และในวันที่ 13 ทำการป้อนเชื้อ *Salmonella* ซ้ำที่ความเข้มข้น 10^9 cfu/ml จำนวน 1 ซีซี ชั่งน้ำหนักไก่ทดลองทุกกลุ่มและบันทึกปริมาณอาหารที่กินในวันที่ 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 ฟรุคโตโอลิโกแซคคาไรด์ มากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ ($P<0.05$) พบว่าผลการเกาะกลุ่มของเชื้อซัลโมเนลล่าในกลุ่มที่ได้รับกรดไขมันสายปานกลางผสมน้ำ และกรดอินทรีย์ผสมน้ำ ลดลงอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) เมื่อเทียบกับกลุ่มควบคุม และไก่ทดลองที่มีการเสริมกรดไขมันสายปานกลาง กรดอินทรีย์ ฟรุคโตโอลิโกแซคคาไรด์ พบว่าค่าพีเอชในกระเพาะพักและในทางเดินอาหารลดลงแตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญ ($P<0.05$) นอกจากนี้พบกรดไขมันสายปานกลางในเลือดแตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($P< 0.05$) และพบว่าไก่กลุ่มที่ได้รับกรดไขมันสายปานกลางผสมน้ำ มีค่าการย่อยได้ทางโภชนะ ปริมาณเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่และระดับกรดไขมันสายสั้น(กรดอะซิติกและกรดวาเลลิก) เพิ่มขึ้น อย่างมีนัยสำคัญ ($P<0.05$) เมื่อเปรียบเทียบกับกลุ่มควบคุม

จากการศึกษาครั้งนี้แสดงให้เห็นว่า กรดไขมันสายปานกลาง และกรดอินทรีย์ มีผลเพิ่มการเจริญเติบโตและลดการเกาะกลุ่มของเชื้อซัลโมเนลล่า ลดระดับความเป็นกรด-ด่างในกระเพาะพัก และในทางเดินอาหาร และพบว่ากรดไขมันสายปานกลาง และ ฟรุคโตโอลิโกแซคคาไรด์ มีผลเพิ่มปริมาณเอนไซม์ซูเครส นอกจากนี้ในไก่กลุ่มที่เสริมกรดไขมันสายปานกลางพบกรดไขมันสายปานกลางในเลือดและพบว่ากรดไขมันสายปานกลางมีผลเพิ่มการย่อยได้ของโภชนะ เพิ่มปริมาณเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่ เพิ่มปริมาณกรดไขมันสายสั้น

ภาควิชา สรีรวิทยา
สาขาวิชา สรีรวิทยาการสัตว์
ปีการศึกษา 2548

ลายมือชื่อนิสิต.....สุชีรา โชติกธรรม.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORDS: MEDIUM CHAIN FATTY ACIDS / ORGANIC ACIDS / FRUCTOOLIGOSACCHARIDE / CECAL *Salmonella enterica* Serovar Enteritidis / COLONIZATION/ BROILER

SUCHEERA CHOTIKATUM: EFFECTS OF MEDIUM CHAIN FATTY ACIDS, ORGANIC ACIDS AND FRUCTOOLIGOSACCHARIDE ON CECAL *Salmonella enterica* Serovar Enteritidis COLONIZATION, PHYSICAL AND BIOLOGICAL CHANGES OF INTESTINE IN BROILER CHICKS. THESIS ADVISOR: ASSOC. PROF. KRIS ANGKANAPORN, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. INDHIRA KRAMOMTONG, M.S. (91) pp. ISBN 974-17-5125-7

The objective of this investigation was to study the effect of medium chain fatty acids, organic acids and fructooligosaccharide on cecal *Salmonella enterica* Serovar Enteritidis colonization, physically and biological changes of intestine in broiler chickens. Six hundred day old chicks were allocated into 4 treatments. The treatments were CON: broiler chicks were received basal corn-soybean meal diet, FOS: broiler chicks were received basal diet supplemented 4 g/kg fructooligosacchande, ORA: broiler chicks were received basal diet and given tap water suppiemented with mixed organic acids at 1:1,000 continuously until the end of experiment, MCA: broiler chicks were received basal diet and given tap water diluted with medium chain fatty acids 1:1,000 continuously from start until 35 days and 1: 2,000 until the end of experiment, All chickens were inoculated with *S.*Enteritidis 0.3 ml is culture 10^8 cfu/ml at day 3 and 1 ml of 10^8 cfu/ml at day 13 post-hatching. At days 21, 35 and 45 of age, body weight and feed intake were recorded. Cecal samples were examinated of *S.*Enteritidis colonization. In situ pH determination in crop small intestine and ceca were measured. Jejunal mucosal samples were collected for the determination of disaccharidases. Ileal digesta were collected for nutrients digestibility using the marker technique. Cecal contents were collected for determination of short chain fatty acids (SCFAs) and medium chain fatty acids (MCFAs). Plasma samples were collected to determine medium chain fatty acids.

Forl the overall period (day 1-45 of age), chicks in MCA and ORA groups had significantly ($p<0.05$) higher average daily gain and better feed conversion ratio than CON group. Chicks in MCA, ORA and FOS group had significantly ($p<0.05$) higher body weight than CON group. Chicks in MCA and ORA group showed the reduction of *S.*Enteritidis in the ceca which was significantly ($p<0.05$) lower than CON group. Chicks in MCA, ORA and FOS group had significantly ($p<0.05$) lower pH of crop and intestine than CON group. Chicks in MCA group had significantly ($p<0.05$) higher disaccharidases enzyme, digestibility of nutrients, SCFAs (acetic acid and valeric acid), MCFAs in plasma than CON group.

In conclusion, chicks in MCA and ORA group had better growth performance, less *S.*Enteritidis colonization and pH in crop and intestines. Chicks in FOS group tended to decrease *Salmonella* colonization in ceca. The chicks in MCA and FOS group increased sucrase activity. Moreover, chicks in MCA group were found MCFA concentrations in plasma, increased SCFAs concentrations in ceca and nutrients digestibility.

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Field of study Animal Physiology — Advisor's signature Kris Angk

Academic year 2005 — Co-advisor's signature Indhira Kramomtong

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ABBREVIATION

ADG	=	average daily gain
AIA	=	Acid insoluble ash
ANOVA	=	Analysis of Variance
BPW	=	buffer peptone water
BSA	=	bovine serum albumin
BW	=	body weight
C	=	concentration
CFU	=	colony forming unit
cm	=	centimeter
DFI	=	daily feed intake
FCR	=	feed conversion ratio
FOS	=	fructooligosaccharide
GI	=	gastrointestinal
kilo	=	kilogram
MCA	=	medium chain fatty acid
mg	=	milligram
mg%	=	milligram percent
MIL	=	motility indole lysine medium
min	=	minute
ml	=	milliliter
mM	=	millimolar
mm	=	millimeter
mol	=	mole
MSRV	=	modified semi-rappaport vassiliadis
nmol	=	nanomole
ORA	=	organic acid
rpm	=	round per minute
SCFA	=	short chain fatty acid
SE	=	standard error

TTB	=	tetrathionate broth
TSA	=	tryptic soy agar
TSI	=	Triple sugar iron
U	=	unit
U/mg protein	=	unit per milligram protein
UV	=	ultraviolet
wt	=	weight
w/v	=	weight per volume
XLT_4	=	xylose lysine tergitol4
μg	=	microgram
μl	=	microliter
μmol	=	micromole

CHAPTER I

INTRODUCTION AND AIMS

Salmonella are recognized in many parts of the world as a major cause of foodborne infections in human and consequent economic loss. In recent years, *S.* Enteritidis has become the dominant serotype isolated from cases of human food poisoning in many countries, including Thailand (Bangtrakulnonth, 2004). *Salmonella* are usually associated with food poisoning by virtue of their ability to colonize in the alimentary tracts of livestock, particularly poultry. This results in considerable contamination of carcasses at slaughter, with entry of *Salmonella* into human food.

In Thailand, *S.*Enteritidis (19.9%) was the most serovar among the 14,599 *Salmonella* isolates from chickens (Bangtrakulnonth, 2004). Considering sources of contamination, *S.*Enteritidis, was isolated from 28% of the retail chicken meat, 4.5% of the chicken meat from slaughterhouse, and 6.6% of excreta from chickens (Boonmar et al., 1998).

Contamination of poultry products with *Salmonella* is not only a domestic public health problem but also an international problem, because exporting poultry and related products is one of the major businesses in Thailand. Elimination of *Salmonella* in poultry is difficult because of the numerous sources of *Salmonella* contamination in poultry, including the chicks, feed, rodent, wild birds, insects, vehicles, and the processing plant.

Antibiotic therapy is a simple way to prevent *Salmonella* infection and it has been exploited extensively. Many years ago, antibiotic supplementation is widely used in poultry production in the world to control *Salmonella* (van Immerseel, 2002). The utilization of antimicrobial drug has played an important role in animal husbandry because they are used in sub therapeutic dosage as growth promoters for the chicken industry. However, the practice of using antibiotics as growth promoters 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 product and environment contamination largely added to the public concern regarding the use of antibiotic in the feed. In addition, the use of most antibiotic growth promoters has been banned in the EU and the regulation to prevent use of antibiotic growth promoters will commence in year 2006 (David, 2005). This results in considerable interest to develop an alternative way to reduce using antibiotics in poultry industry.

At present, there are many feed additives in the market which most of then are proposed to replace antibiotic growth promoters. Fructooligosaccharides (FOS) belong to a class of carbohydrate known as prebiotic. It is demonstrated that FOS can be substituted for subtherapeutic levels of antibiotics to enhance growth and production efficiency of broilers. (Ammerman et.al., 1988a,b, 1989). FOS can improve the microbial ecology of the gut and protect against bacterial pathogens, particularly in the large intestine. Therefore, researcher's interest has been focused on FOS substituted antibiotic growth promoters.

Another additive, organic acids; has also been studied for their ability to decrease pathogenic bacteria. Organic acids have been used to control *Salmonella* in feed and in water supplies for livestock and poultry. Organic acids suppress pathogenic bacteria in intestine by providing an unfavorable acidic environment for pathogenic bacteria but favorable for beneficial bacteria (Ricke, 2003). Organic acids are widely utilized in poultry industry.

Medium chain fatty acid (MCFA) has been of interest to reduce *Salmonella* colonization and invasion in chickens. MCFA composes of capronic 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 absorbed directly into portal vein (Papamandjari et al., 1998).

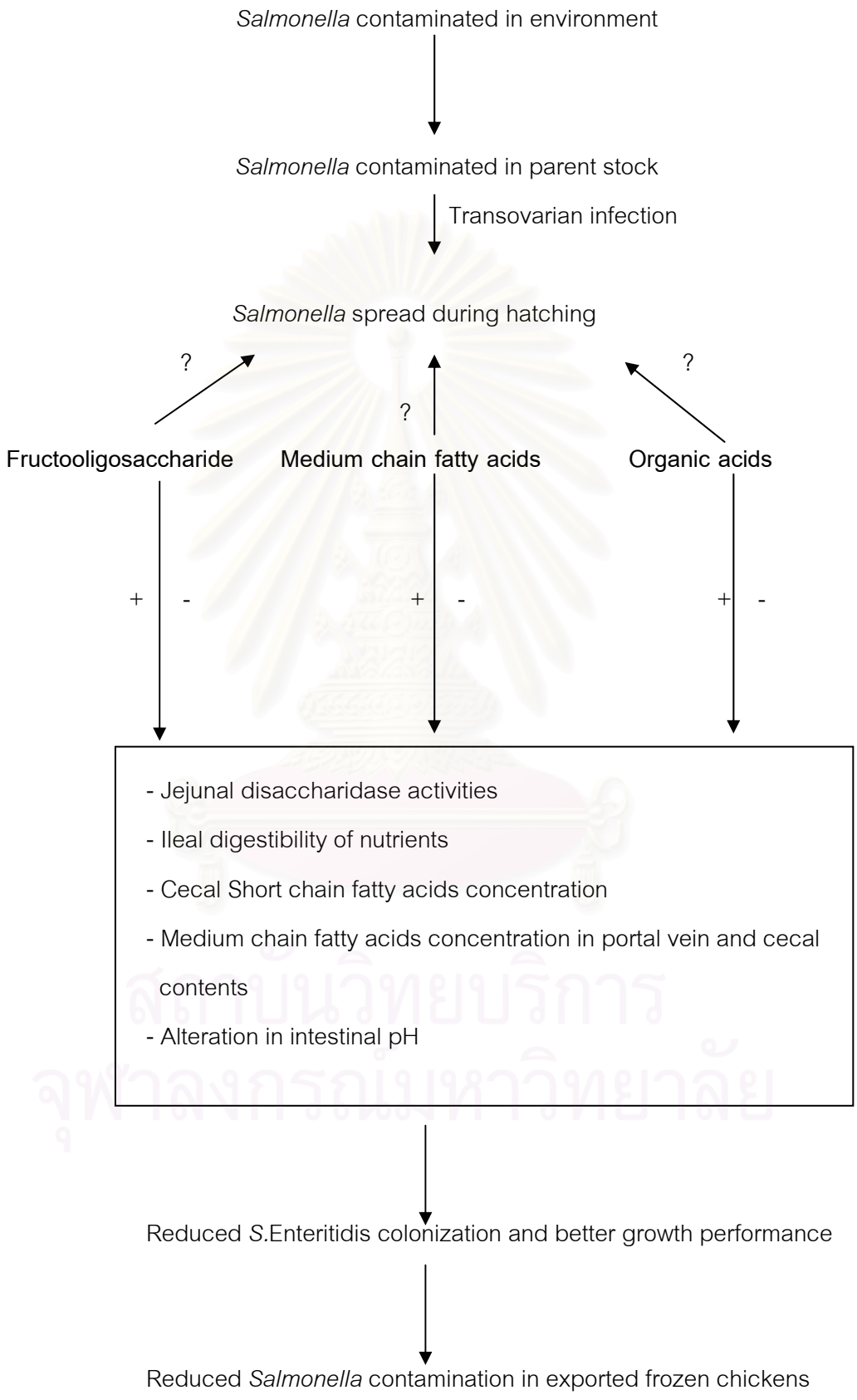
Since the proposed ban of antibiotic growth promoters commence in year 2006, this is the trend that everyone in the animal feed industry is considering and it is imperative that alternatives to antibiotics for use in animal feed being searched and tested for the way of efficient animal production. All feed additives are advantage and disadvantage in controlling *Salmonella* infection and improving feed utilization. Medium chain fatty acids are the new additive used to control *Salmonella* infection and there are few studies examined on their possible role in poultry production.

In this study, medium chain fatty acids are compared with other alternative used in poultry production such as organic acids and fructooligosaccharide (FOS). Therefore, the objectives of this experiment were:

1). to examine the effect of medium chain fatty acid (MCA) compared with organic acids (ORA) and fructooligosaccharide (FOS) on eliminating of *S.*Enteritidis colonizing in broiler chickens.

2). to examine the effect of medium chain fatty acid (MCFA), organic acids (ORA) or fructooligosaccharide (FOS) on growth performance, intestinal pH, disaccharidase activities, short chain fatty acids, medium chain fatty acids and ileal digestibility of nutrients.

Conceptual framework of the study



CHAPTER II

BACKGROUND INFORMATION

2.1. Important of *Salmonella* infection

2.1.1 The Organism

The genus *Salmonella* is a member of the bacteria in the family Enterobacteriaceae. *Salmonella* spp. are gram-negative, nonsporing rod (2-4 X 0.5 µm) that lack capsules. They are facultative anaerobic bacteria that can grow well under both aerobic and anaerobic conditions. They ferment glucose but not lactose, reduce nitrates to nitrites and can survive in a wide variety of environmental conditions and nutritional substrates. The optimum temperature to support the growth of *Salmonella* is 37ºC, but some can grow over a range of about 5ºC to 45 ºC. They can grow within pH range of about 4.0 to 9.0, with an optimum pH of about 7.0 (Bangtrakulnonth, 2002).

The *Salmonella* comprises only two species, *S.enteritica* and *S.bongori*; *S.enteritica* is further divided into six subspecies. The majority of zoonotic important *Salmonella* and about 60% of the more than 2,500 known serotypes belong to subspecies/ subgenus I (*S.enterica* subsp. *enterica*) (Bangtrakulnonth, 2004).

The present system of the antigenic classification or serotyping of *Salmonella* is a result of extensive studies of antibody interactions with bacterial surface antigens. Three kinds of surface antigens, somatic O antigens, flagella H antigens, and V_i, determine the reactions of the organisms to specific antisera (Hirsh, 1999). H antigen may occur in either one or both of two forms, called phase 1 and phase 2. The organisms tend to change from one phase to the other. O antigens occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface. V_i antigen is a superficial antigen overlying the O antigen.

2.1.2 Salmonella infection

Salmonella enterica serovar Enteritidis is a major cause of gastrointestinal disease in humans and animal all over the world (Poppe, 2000). In poultry, the sources of *Salmonella* infection are numerous. Chickens may be colonized when they are fed with contaminated feed, water and others which may become cross-contaminated in the breeding houses or during transport and among carcass during processing. Contamination of *Salmonella* in breeder hatcheries is an early critical point for preventing *Salmonella* entry into the poultry operation. *Salmonella* has become established in a primary breeding flock. There are many sources of *Salmonella* for the hens in the breeder farm. *S.* Enteritidis could be transmitted either vertically and/or horizontally. The main route of infection before hatching is thought to be vertical transmission of the *Salmonella* from parent to progeny (Jacob et al., 1978; Padron et al., 1990). Primary breeding flocks are composed of one or more generations that are maintained for the purpose of establishing, continuing, or improving parent lines. As a result of this breeding system to produce pullets for layer flock, one pair of the primary breeding flock may have an offspring numbering hundreds of thousands of birds. Thus, if one or a few of the hens in the primary flock were infected with *S.* Enteritidis and transmitted the infection by transovarian transmission, many of the offspring could be infected (Calnek, 1997). The eggs are contaminated either from the ovary tissue or on their passage through the cloaca (Nakamura et al., 1993). *Salmonella* colonized and proliferated in the cloaca, then ascended in the vagina, which resulted in an increased production of *Salmonella* contaminated eggs (Miyamoto et al., 1997, 1998). Therefore, infected hens can deposit this pathogen into the edible content of developing eggs (Gast et al., 1998). However, contamination of egg via ovaries is not the only source of infection, since contamination of eggshell is involved in the infection of chicken. Eggshells can become contaminated with *Salmonella* by faecal contamination of the surface of the egg. When the egg passes through the cloaca, *Salmonella* in the faeces may penetrate the eggshell (Snoeyenbos et al., 1979). Thus, the hens can be infected with *Salmonella* in the hatchery, because contaminated eggs before lay. However, contamination of the egg after lay (horizontal transmission) is also a concern. The

horizontal transmission of *S.* Enteritidis may be caused by nest box, farm cold room, hatchery truck, or hatchery environment which may lead to contaminated eggs (Nakamura, 1999). The hatchery can be an important point in poultry production. Also, hatcheries can serve as reservoirs of infection, and source of cross contamination.

After hatching, most chickens have a very limited microflora in the gut and are far more susceptible to *Salmonella* colonization than the older ones. In addition, the infected broiler chicks can shed *Salmonella* before leaving the hatchery and being exposed to other known sources of *Salmonella* in the environment (Cox, 1990,). Bailey et al. (1992) reported that a single *Salmonella* contaminated egg could substantially contaminate other eggs and chicks in the hatching cabinet. Cason et al. (1994) demonstrated that airborne *Salmonella* can be transferred from contaminated eggs or chicks in one hatching tray to chicks in adjacent trays. Cox et al. (1990) isolated *Salmonella* from over 75% of the samples taken from several commercial hatcheries. This cross contamination in the hatchery resulted in newly hatched chicks that which highly susceptible to infection by *Salmonella,* so horizontal transmission is especially likely to occur shortly after hatching (Gast et al., 1989).

Feed was often considered as a key route for *Salmonella* infections into poultry flocks because of the use of *Salmonella* contaminated raw materials from rendered plant. Therefore, the final feed may be contaminated because of an insufficient heating process and/or recontamination in the feed mill during transport or storage at the farm. Furthermore, *Salmonella* may multiply in wet feed. This particularly is true for chickens, they can be infected by feed containing < 1 *Salmonella* per gram (Hinton, 1998). The *Salmonella* contamination risk in mash poultry feeds was 2%, whereas pelleted feed of only 1.4% (Veldman et al., 1995). Mccapes et al. (1989) showed that pelleted feed subjected to a heat treatment (60-80°C) could reduce the *Salmonella* contamination rate of the feed. *Salmonella* can be transmitted to chicken from housing, sewage, rodents, water and litter. Chicks that were colonized in a hatchery can subsequently spread the infection to other chicks in the hatchery and during grow out. Moreover, the horizontal transmission among chicks occurs during transport to the farm and the *Salmonella* positive chicks delivered to the growing house provide a ready source of *Salmonella* for colonization of other chicks in the flock. During transportation, chickens are stored in the

open crates that placed on top of each other; thus, the contamination can occur from dropping faeces of an upper crate to a lower crate. There was evidence that the stress of transportation may amplify *Salmonella* shedding and cross contamination can occur at processing (Heyndrickx et al., 2002). On the other hand, *Salmonella* cross contamination takes place during leaving the farm to the processing plant and slaughtered. *Salmonella* contaminated carcasses, thus leading to an introduction of the organism into human food chain. Cycle of *Salmonella* infection is shown in Figure 1.

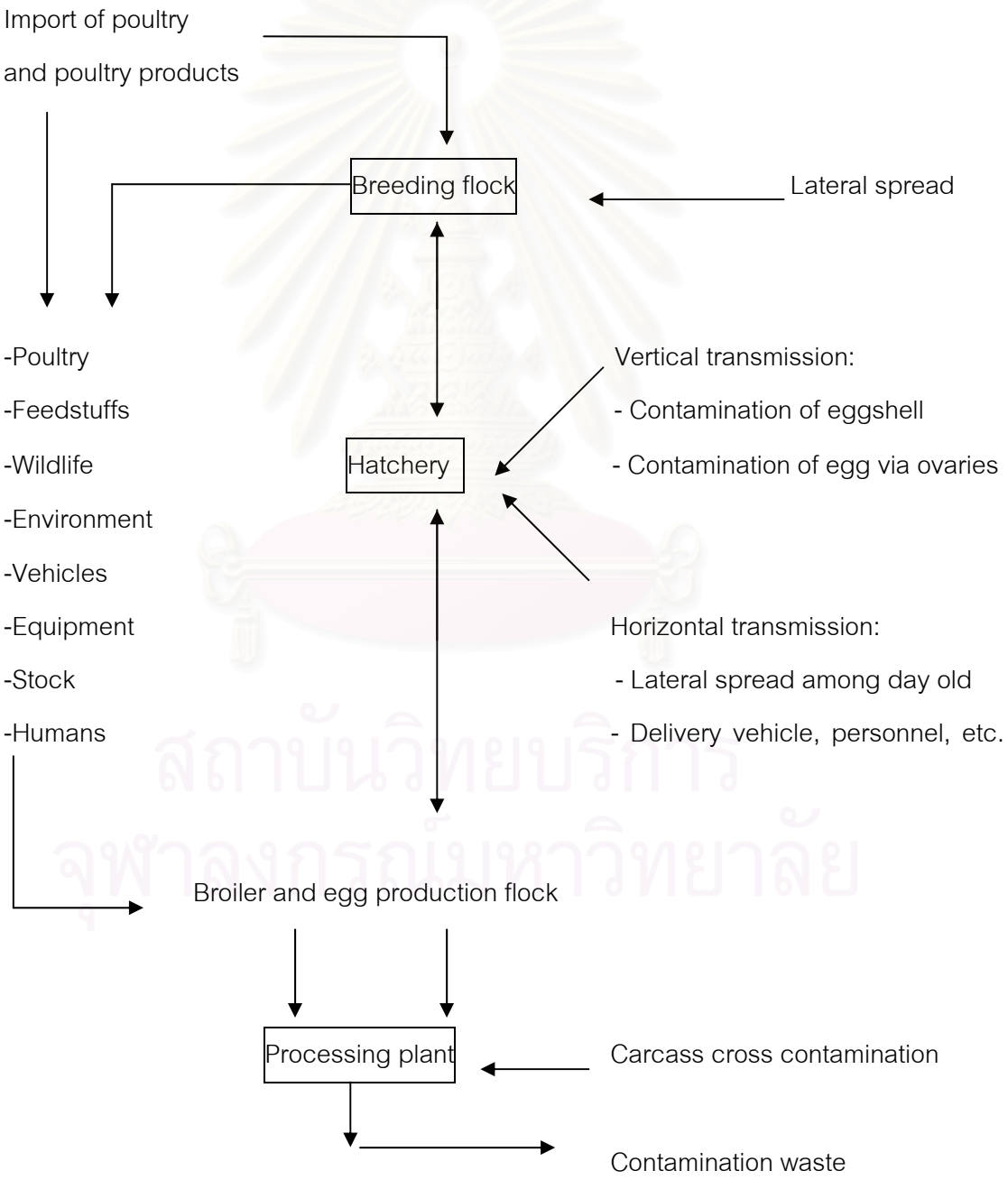


Figure1. Cycle of salmonella infection

2.1.3 Pathogenesis of *Salmonella* infections

The pathogenesis of *S.* Enteritidis in the chicken starts with colonization of the alimentary tract before the induction of systemic disease. The main sites for colonization in the intestinal tract of chickens are the cecum and the crop. Moreover, the organisms are able to enter the epithelium of gut regions.

It is generally recognized that the majority of *Salmonella* infections in chickens are going to be established following ingestion and the crop is a potential site for infection by *Salmonella* (Nurmi and Rantala, 1973; Impey and Mead, 1989). Few types of bacteria are present in the crop, with *Lactobacilli* being the predominant microbes (Barnes et al., 1980). The pH value of crop is around 4 to 5. This prevents extensive growth of *Salmonella* but will allow the development of increased resistance to acidity, which will enhance survival as the *Salmonella* pass through the gizzard and proventriculus. In the proventriculus hydrochloric acid and pepsin are secreted. In the gizzard, where feed is mixed and ground, the pH is about 2 to 3 (Heres et al., 2003). Although, acidity increases in gizzard, it does not entirely kill *Salmonella*.

The surviving *Salmonella* subsequently reach the intestinal tract, which contains bactericidal compounds, such as bile salts. However, *Salmonella* are well adapted to cope with these stress conditions. They can cross the intestinal epithelium after attachment to receptor substance in mucus and this is considered as an initiating step in colonization of bacteria within the intestinal mucosa. Penetration of the intestinal mucosa is a prerequisite for the pathogenesis of systemic infection, this is also thought to be essential for the induction of diarrhea. Invasion occurs specifically via the apical surface, where within a critical distance from cells, *Salmonella* induce disruption and elongation of microvilli, which precedes endocytosis. Invasion is believed to occur by receptor mediated endocytosis, though receptors have not been identified. The ceca are the site within the intestinal tract of infected chickens that most commonly contain *Salmonella* (Fanelli et al., 1988). The ceca was identified as an ideal organ for *Salmonella* isolation during acute and chronic infection, compared with other organ, and

it poses a potential source for environmental contamination (Dhillon et al., 2001). Bacteria are the predominant microbes, specifically obligate anaerobes which are found in the lumen. This has been ascribed to the physical attachment of *Salmonella* organisms to the cecal epithelium (Soerjadi et al., 1982). This adhesion is mainly observed in epithelium near the cecal lumen (Desmidt et.al., 1998), and it is likely that the uptake of bacteria occurs in mature enterocytes at the villus tips (Amin et.al., 1991). After that they penetrate the lamina propria where they replicate, or proceed to deeper tissues. van Immerseel et.al. (2002) indicated that *Salmonella* were first detected 3 hours post-infection in the caecal lumen and 9 hours post-infection in the cecal lamina propria, *Salmonella* colonized in Peyer's Patches and invade to M cell. M cell reside throughout the gut as follicular-associated epithelium that overlays lymphoid follicle, e.g., Payer's patches. M cell have been considered to be the most effective cells for the transport of specific antigens from the intestinal lumen into the underlying lymphoid tissue (Charles et.al.1992). From the infected intestinal tissues the pathogens are drained to the regional lymph nodes, where macrophages that line the lymphatic sinuses form the first effective barrier to prevent further spread. If this host defense mechanism successfully limits bacterial expansion, the infection remains localized to the intestine and the gut- associated lymphoid tissues (GALT). However, if the macrophages located in the draining lymph nodes are unable to limit spread, *Salmonella* can cause a systemic disease. The pathogens spread from the GALT via the afferent lymphatic and the thoracic duct into the vena cava, resulting in the systemic infection the capillary systems of liver and spleen constitute an efficient filtering system, which focuses infection to liver and spleen, and these organs are usually enlarged during systemic infection (Andreas et al., 2000). *S.* Enteritidis colonizes in both spleen and liver after an infection of 1 day old chicken. The organs found more frequently positive in the birds not only the spleen, liver and kidneys but also the less frequent from lungs and heart (Brown et al., 1975) (Fig. 2). Three general categories of toxin have been reported to play role in the pathogenicity of paratyphoid *Salmonella* (Gast, 2000). Endotoxin is associated with the lipid portion of *Salmonella* cell wall lipopolysaccharide (LPS). It will be released into the bloodstream of an infected animal when bacterial cells are lysed, and producing fever (Gast, 2000). LPS also attributes to the resistance of the

bacterial cell wall to attack and digestion by host phagocytes. Loss of the ability to synthesize complete LPS has been impaired ability of *S.typhimurium* to colonize the ceca and invade to the spleen in broiler chicks (Craven, 1994). In the same way, *S.* Enteritidis endotoxin administered intravenously produced liver and spleen lesion in 2-week old birds (Turnbull and Snoeyenbos, 1974). However, enterotoxin activity by *Salmonella* induces secretory response of epithelial cells that results in fluid accumulation in the intestinal lumen (Koupal et al, 1975). A heat labile enterotoxin of *Salmonella* cause structural damage to intestinal epithelial cells, perhaps by inhibiting protein synthesis (Koo et al., 1984).

2.1.4 Immunity

The immune response of chicken to *Salmonella* is divided into non-specific and specific immune mechanisms. Non-specific immune mechanisms include the innate or inherent ways in which the chicken resists disease. Specific immune mechanisms (acquired system) is divided into cell mediated (lymphocytes and phagocytic cells) and humoral mediated in immunity (antibody).

Non-specific barriers to microbial invasion are gastric secretions, lysozymes, bile salts, microflora, and endogenous cationic peptides (Lillehoj and Okamura, 2003). One of the primary cells in the innate immune response to early bacterial invasion by *Salmonella* is the heterophil (Well et al., 1998). Polymorphonuclear leukocytes (PMNs) or heterophils are phagocytic cells involved in the first line of defense against invading microbial organisms (Holt, 2000). However, the chickens could be able to overcome the infection and did not show many clinical signs (Lax et al., 1995).

The non - cellular (humoral) component includes immunoglobulins (antibody) and the producing cells. The antibody produced by B lymphocytes provides the active effectors function for humoral immunity. Antibodies are specific for the foreign material (antigen) to which they attach (Michetti et al., 1992). *Salmonella* can elicit strong antibody response from infected poultry. For example, experimental infection of chicks

with *S.* typhimurium induced strong IgG, IgA and IgM response in serum, intestinal contents and bile could be detected by antigens composed of whole bacterial cells, LPS, flagella and outer-membrane proteins (Hassan et al., 1991). Humoral antibodies (IgA, IgG and IgM) from serum and intestinal mucosal samples of chickens challenged with *S.* typhimurium significantly increased one week post challenge (Brito et al., 1993). *S* .Enteritidis were detected at 18 hr and at 5 days post inoculation (Desmidt et al., 1997). Once *Salmonella* invade the system, protection from infection by humoral mechanisms may not provide adequate protection against infection. They may aid in mucosal immunity, however, through the macrophage-mediated opsonization and destruction of the bacteria (Lillehoj and Okamura, 2003). Because *Salmonella* is a facultative intracellular bacteria, protection from infection by humoral mechanisms alone is unlikely. The cell mediated immunity (CMI) response has been shown to play an important role in host protection against many intracellular pathogens in chickens (Schat, 1994). CMI plays a major role in the controlling *Salmonella* infection. Cellular immunity is mediated by T lymphocytes and these cells can serve either a direct effector function (cytolytic T lymphocytes) or a regulatory function (helper (Th) and suppressor (T_S) T cells) by modifying the activation of B cells or other T cells. Concept of CMI was related between infection with intracellular pathogens of macrophages and induction of the host response that was mediated by activation of macrophages by T cells and their secreted products. T cells mediate immunity against intracellular bacteria by a number of different mechanism, including 1) induction of cytotoxic activity, 2) recognition of target antigens in conjunction with major histocompatibility complex class II antigens, and 3) production of lymphokiness such as IFN- and IL-2 which may directly antagonize bacteria within the host cell and/or stimulate other immune cells to do so (Megruder et al., 1993). However, the elimination of *S.* Enteritidis depends on the humoral immunity, whereby the local response in the gut appeared more effective than the systemic response (Desmidt et al., 1998).

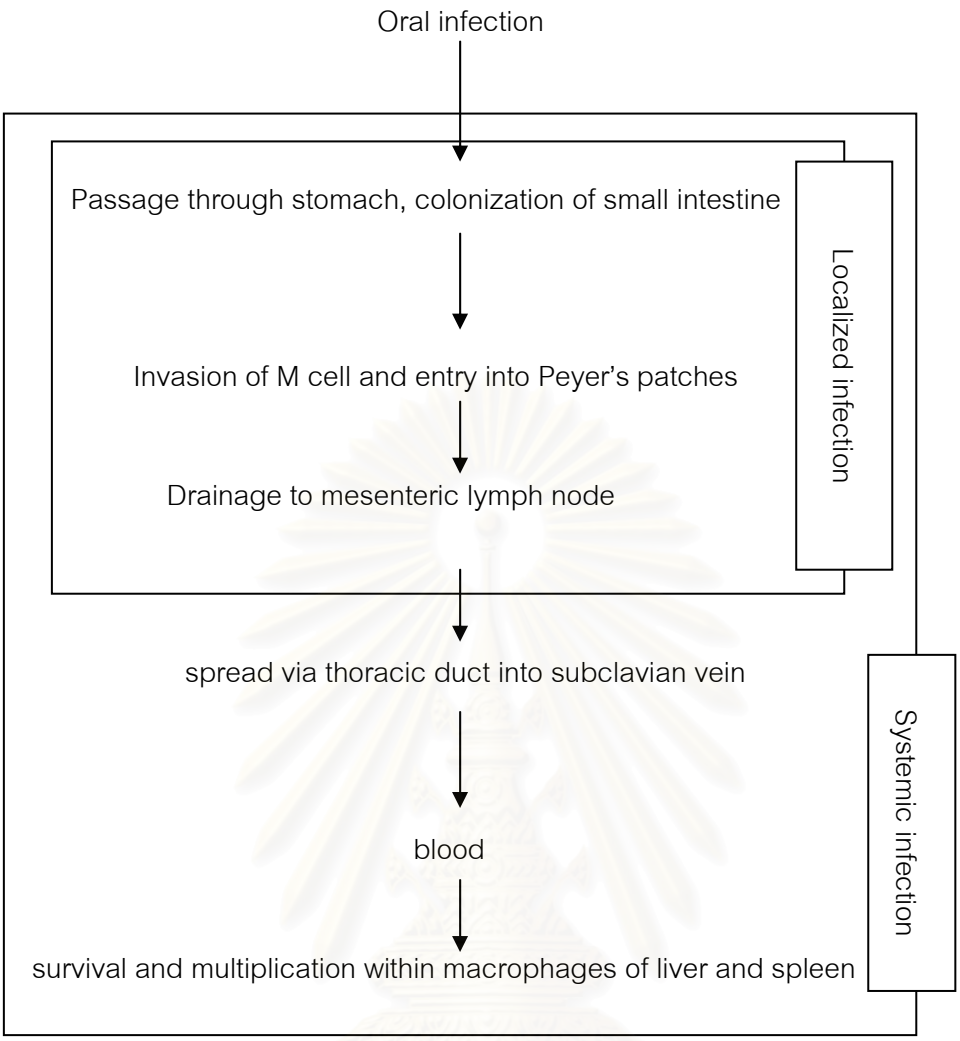


Figure2. Course of *Salmonella* infection

2.2 Methods in controlling *Salmonella* infection

2.2.1 Antibiotic

Antibiotics have been used for many years as growth promoting agents for the chicken industry. Most antibiotic growth promoters act by modifying the intestinal microflora, especially targeting gram positive bacteria, which are associated with poorer health and performance of the animals (Bedford, 2000). The antibiotics that commonly used in livestock production as dietary additives are bacitracins, lincomycin, penicillin, streptomycin, tetracycline, tiamulin, tylosin and virginiamycin. However, the use of antibiotics for feed additives has lead to drug resistant bacteria and the possible presence of antibiotic residues in poultry products. Then, if the resistant bacteria remain in the poultry product, people eating it may become sick from the bacteria. Recently, the antibiotics as growth promoters will be banned from use in the EU in 2006 (David, 2005). The antibiotic free chicken for consumer is enormous concern in poultry production. It has been considered to be the alternatives for the use of antibiotics in poultry industry.

2.2.2 Probiotic

Probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). The most commonly used strains are members of the heterogeneous group of lactic acid bacteria; such as *Lactobacilli*; *Enterococci* and *Bifidobacteria*. There have been numerous studies on probiotics in human and animal nutrition. An effective probiotic should 1) exert a beneficial on the host, 2) be nonpathogenic and nontoxic, 3) contain a large number of viable cells, 4) be capable of surviving and metabolizing in the gut, 5) remain viable during storage and use, 6) have good sensory properties, 7) be isolated from the same species as its intended host (Collins et al., 1999). Mechanisms of probiotic response involve the production of directly inhibitory compounds, reduction of luminal pH through short chain fatty acids production, competition for adhesion site on the gut wall and nutrients and modulation of immune response (Gibson and Fuller,2000 ;

Rolfe,2000). Losada et al. (2002) have shown nutritional and therapeutic benefits effect of using probiotic in Figure 3.

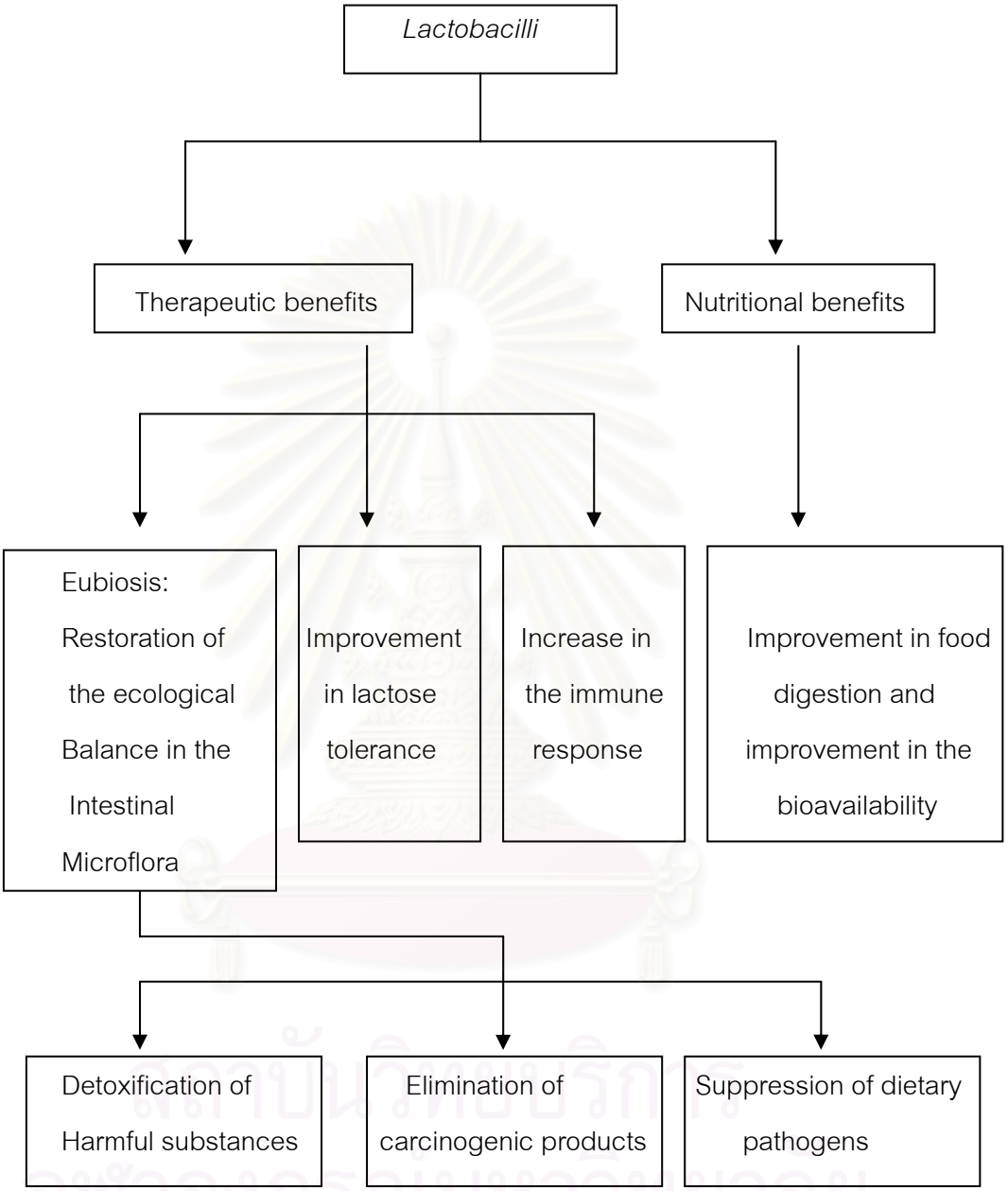


Figure 3. Nutritional and therapeutic benefits of probiotics

However, selection criteria for probiotic much debate and should be taken into account when defining appropriate strains. It is also likely the survival of the probiotic was compromised in the supplement product before ingestion, and even more so in the host after ingestion. The bacteria are confronted by many physiochemical effects that may adversely influence culture viability. These include gastric acid and secretions of the small intestine such as bile salts and pancreatic enzymes. Moreover, in the large intestine, the bacteria must compete effectively with a complex and metabolically active indigenous flora (Collins et al., 1999).

2.2.3 Prebiotic

A prebiotic has been defined as “a nondigestible food ingredient that beneficially affected the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus attempt to improve host health” (Gibson and Roberfroid, 1995). For a food ingredient to be classified as a prebiotic, it must 1) not be hydrolyzed or absorbed in the upper part of the gastrointestinal tract; 2) be a selective substrate for one or a limited number of potentially or beneficial commensal bacteria in the colon, either stimulating the bacteria to grow, become metabolically activated, or both; and 3) be able as a consequence to alter the colonic microflora toward a more healthier composition (Gibson et al, 1999). Thus, survival of food borne pathogens within the upper gastrointestinal tract can potentially be modulated by dietary prebiotics. The beneficial effects of prebiotic are shown in Table 1.

Prebiotics of proven efficacy that are commercially available are fructooligosaccharides (FOS), inulin, lactulose and mananooligosaccharides. FOS are the oligosaccharides most be studied in chickens with respect to their prebiotics effect and their activity against *Salmonella* (Fukata et al., 1999). FOS are composed of fructose units bound by ß (1-2) linkages attached to a terminal glucose unit. FOS are predominantly found in cereals, including barley and wheat. The number of degrees of polymerization (DP) between 2 to 7 for FOS. In vitro fermentation experiment revealed that molecules with a degree of polymerization (DP) >10 are fermented, on average, half

as quickly as molecules with a DP of < 10 (Roberfroid et al, 1998). Therefore, the possible FOS rapid fermentation. The molecular structure of the FOS is shown in Figure 4.

Table 1 Beneficial effect of prebiotics

Increase production of VFA
Lower serum cholesterol, triglycerides
Improve mineral absorption
Increase B vitamin synthesis
Prevent cancer
Increase immune function

Roberfroid, (2000); Davidson , (1998) ; Patterson et al. (2003)

Fructooligosaccharides are neither hydrolyzed by digestive enzymes nor absorbed in the upper gastrointestinal tract. They are transferred intact to the large intestine, where they are available for microbial action. They are rapidly fermented by the microflora residing in the caecum and colon (Andersson, 1999). As a result, stimulation of the endogenous microflora by dietary prebiotic may inhibit colonization of intestinal pathogens by production of organic acids such as bactericidal organic acids (lactic acid and short chain fatty acids) and inhibitory compounds e.g. hydrogen peroxide and bacteriocins. All of these compounds lead to a drop in the pH of the distal small intestine and large intestine. This effect is beneficial for rapid fermentation of FOS by the intestinal microflora which leads to high luminal concentrations of organic acids (Bruggencate et al., 2004). Campbell et al. (1997) reported that supplementing oligofructose, fructooligasaccharide and xylooligosaccharide in rat diet increased the intestinal weight and short chain fatty acids concentration compared with crystalline cellulose. It is reported that FOS can be substituted for subtherapeutic levels of antibiotics to enhance the growth and production efficiency of broilers (Ammerman et al., 1988 a, b, 1989). FOS are mainly known for their ability to increase the endogenous

growth of intestinal *Lactobacilli* and *Bifidobacteria* in humans and animals (Campbell et al, 1997; Gibson and Roberfroid, 1995). Supplementation of FOS have been shown to enhance the growth of *Bifidobacterium* and *Lactobacillus* but inhibit *Eschericha coli* and *Salmonella* in the large intestine (Bailey et al., 1991; Bunce et al.,1995 ; Roberfroid et al., 1998; Xu et al., 2003). In addition, supplementation of 4 g/kg FOS improved the activities of amylase and protease (Xu et al., 2003). In the same way, supplementation of FOS increased ileal villus height, jejunal and ileal microvillus height, and villus height to crypt depth ratio in the jejunum and ileum (Xu et al., 2003). Roberfroid et al. (1998) suggested that a minimum daily dose of 4 g/day of inulin or FOS would be needed to observe an increase in gut *Bifidobacteria*. Prebiotic doses higher than 20g/day might induce some side effect, such as increased flatulence or abdominal bloating (Kieran et al., 2003).

Although FOS intake seems beneficial, its effect was studied only for short periods of ingestion never exceeding 3-4 weeks. Several authors have suggested that gut adaptation could modify the gastrointestinal effects of non digestible carbohydrates (Rao et al., 1994; Weaver et al., 1996).

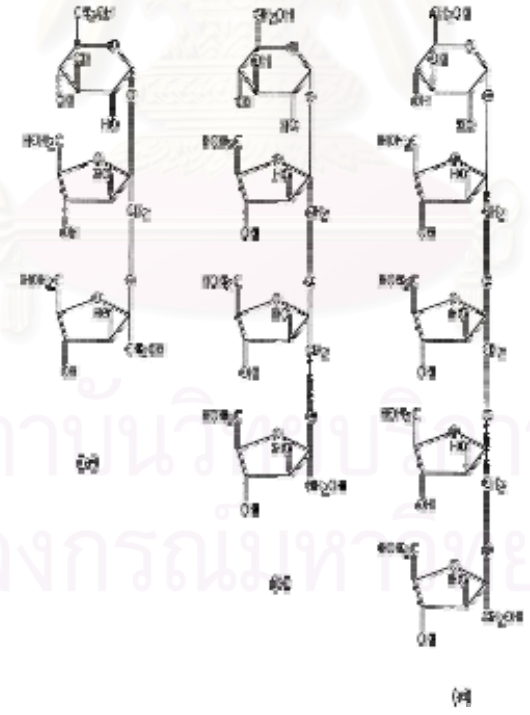


Figure 4. Molecular structures of the FOS. (a) 1-kestose (1-Kestotriose, GF2); (b) nystose (1,1-kestotetraose, GF3); (c) 1-ß-fructofuranosylnystose (1, 1, 1-kestopentose, GF4). (Hogarth et al., 2000)

2.2.4. Organic acids

Organic acids are a group of compounds primarily include the saturated straight-chain monocarboxylic acids and their respective derivatives (unsaturated, hydroxylic, phenolic, and multicarboxylic versions) and are often genetically referred to as fatty acids, volatile fatty acids, or weak or carboxylic acids (Cherrington et al., 1991).

Organic acids have long been used as feed additives and preservatives for preventing food deterioration and increasing the preservation effect of fermented feed. At present, organic acids have been used to prevent infection of pathogenic bacteria, particularly *Salmonella* spp.(Cox et al.,1972 ;Hume et al.,1993 ;Waldroup et al.,1995 ;Matho et al.,1997). The antibacterial activity of organic acids is related to the reduction of pH, because organic acids are lipid soluble in the undissociated form. Molecules of these organic acids can easily penetrate the lipid membrane of the bacterial cell and once internalized into the neutral pH of the cell cytoplasm dissociate into anions and protons (Hsiao et al., 1999). Inside the cell, organic acids inhibit bacterial growth by breaking oxidative-phosphorylation and inhibiting the exchange of adenosine triphosphate (ATP) (Byrne et al., 1979). Export of excess protons requires consumption of cellular adenosine triphosphate (ATP) and may result in depletion of cellular energy (Ricke, 2003). It is demonstrated that sufficient concentrations of volatile fatty acids inhibited growth of *E.coli* and immediately slowed the rates of RNA, DNA, protein, lipid and cell wall synthesis *in vitro* (Cherrington et al., 1990). Waldroup et al. (1995) showed that the use of a formic/ propionic acid blend in the diet of hens may reduce cecal pH and increased the proportion of undissociated acids. Similarly, the use of 0.5% acetic acid in the drinking water reduced crop pH and decreased the recovery of salmonella from crop samples (Byrd et al., 2001). There are some mechanisms that can explain the effect of organic acids against colonization of infected chickens. The first one is the acidifying effect of the organic acids in the anterior part of the gastrointestinal tract since the pH of organic acids is lower than the pH generally observed in the crop (Cox et al., 1972), a potential site for infection by *Salmonella*. The addition of organic acids may result in the lower pH in the crop contents. In addition,

inclusion of formic and propionic acids in the form of Bio-Add™ to the food of hens increased concentrations of acids in the contents of the crop and gizzard (Thompson and Hinton, 1997). However, the effect of the use of organic acids on the control of colonization and infection by *Salmonella* spp. in chickens seems to depend on the type of organic acids, on the dose administered, on the route of administration, and on the inoculum of *Salmonella* spp. (Corrier et al., 1990). Moreover, organic acids are not effective against *Salmonella* spp. when added to dry feed. The antimicrobial effect only happens when the chicken ingests the feed and this is moisturized immediately (Hinton et al., 1988). The effectiveness of organic acids in poultry may also depend on the composition of the diet and its buffering capacity.

2.2.5. Medium chain fatty acids

Medium chain fatty acids (MCFA) containing six to twelve carbon atoms are used in clinical nutrition for dietary treatment of malabsorption syndromes. Molecules of MCFA are smaller, high solubility in water and can be absorbed more rapidly than long chain fatty acids (Seaton et al., 1986). MCFA is often found in natural products, for example, in coconut oil, palm oil and milk.

MCFAs are broken down almost immediately by enzymes in the saliva and gastric juices so that pancreatic fat – digesting enzymes are not even essential. They can be absorbed in the stomach without hydrolysis. They are absorbed more quickly into the intestinal cells without micellar solubilization in intestinal lumen. As a result, MCFA pass directly from the mucosal cell into the portal vein and transported to the liver attached to albumin (Papamandiaria et al., 1998). Minor fractions of MCFA bypass the liver and are distributed to peripheral tissue via the general circulation. In addition, MCFA are independent of carnitine for entry into the mitochondria of all tissues. Medium chain fatty acids seem to be such an energy source, with high digestion and oxidation rates (Chiang et al., 1990). Galluser et al (1993) and Jenkin et al (1993) indicated that medium chain triglycerides (MCTAGs) and MCFA improved in intestinal morphology and function, through their positive effects on crypt cell renewal in the case of hypotrophic

villus. In addition, MCFA have positive effects on epithelial cell membrane bound enzyme activities (Takase and Goda, 1990). The MCFA is not only a source of nutrients but also a source of antiviral and antibacterial activity. Sprong et al (2001) tested the bactericidal activity of fatty acids *in vitro* , using food borne pathogens, i.e. *C. jejuni*, *S.* Enteritidis, *E. coli* O157:H7 all gram negatives including pathogens such as *Pseudomonas*, *Campylobacter*, *Vibrio cholerae*, *Salmonella Typhi*, *Shigella sonnei*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Helicobacter pylori, E. coli, Candida albicans, Chlamydia trachomatis, Neisseria gonorrhoeae* (Isaacs et al., 1992; Petchow, 1996,1998; Bergsson et al.,1998, 1999, 2001a, 2001b). In the same way, the addition of caproic acid to the feed of chicks led to a significant decrease in the level of colonization of ceca and internal organs by *S.* Enteritidis (van Immersell et al., 2004). Therefore, MCTAGs have been shown to be good alternatives for nutritional antibiotics in piglets, due to the high antibacterial activity of the MCFA (Dierick et al., 2002).

The mode of action of MCFA against pathogenic bacteria is unknown, but it has been shown that fatty acids and monoglycerides affect the bacterial membrane (Bergsson et al., 1998, 2001). MCFA with a lower molecular weight and undissociated form can freely penetrate through the semipermeable peptidoglycan/ phospholipids membrane of the microorganisms into the cytoplasm by passive diffusion (Dierick et al., 2002). Once inside the cell, MCFA dissociated into proton and anion due to the alkaline pH in the cytoplasm and this can will lower the pH, suppress cytoplasmatic enzymes and nutrient transport systems and uncouple ATP-driven pumps, leading to death (Hsiao and Siebert, 1999).

CHAPER III

MATERIALS AND METHODS

3.1 Animals and Diets

Six hundred, day-old, straight sex, Cobb 500 broilers were obtained from *S.* Enteritidis-free farm. Before the start of the experiment, cloacal swabs were randomly performed on sixty chicks (one sample/chick) and all sample chicks were tested negative for *Salmonella* spp. Chicks were allocated into four treatment groups as stated in experimental procedure. The average initial body weights of each group were similar. All chicks were inoculated with 0.3 ml *S.*Enteritidis culture (10^6 cfu/ml) at day 3 and 1 ml of 10^8cfu/ml at day 13 post-hatching. They were vaccinated with Newcastle and Infectious Bronchitis diseases at days 10 of age and Gumboro disease at days 14 of age.

The chicks were fed on high-energy starter (1 to 21 d), grower (22 to 35 d), and finisher (36 to 45 d) diets composed of corn and soybean meal as major ingredients. The diets were 1) commercial basal diet (Control group, Organic acid group and MCFA group), 2) commercial basal diet supplemented with 4 g FOS/kg feed (FOS group). At days 22 to 25 and days 45 to 48, Celite, a source of acid–insoluble ash (AIA), was added to all diets (20 g/kg feed) as an indigestible marker. Feed and water were provided *ad libitum* throughout the experiment. Drinking water was changed everyday. The chicks were raised on litter floor pens (the size of pen was 1 x 2 m per 30 birds). The Control group was placed in a room isolated from groups receiving FOS, Organic acid and Medium chain fatty acid. The experiment was conducted in a closed concrete-floor-pen house in an environmentally regulated room. Continuous lighting was maintained throughout the experiment. The average max/min temperature and relative humidity in broilers house were 32/28 °C and 71.5/84.6%, respectively. The total experimental period was 48 days.

Table 2 Composition of diet from proximate analysis

Item	Starter diet	Finisher diet
	(%)	
Moisture	10.25	9.14
Protein	21.11	19.05
Fat	5.02	5.23
Fiber	3.73	3.55

3.2 Experimental procedure

At day old, six hundred chicks were divided into 4 groups of 150 chicks (5 replicates of 30 chicks each).

Chicks in group1 were received the basal diet and given tap water (CON group). Group 2, chicks were received the basal diet supplemented with 4 g FOS/kg feed and given tap water until the end of the experiment (FOS group). Group 3, chicks were received the basal diet and given tap water supplemented with mixed organic acids (lactic acid, citric acid, ascorbic acid and propionic acid) at 1:1000 until the end of the experiment (ORA group). Group 4 chicks were received basal diet and given tap water mixed medium chain fatty acids at 1:1,000 continuously from start until 35 days and 1:2,000 until the end of the experiment (MCA group). The protocol of the experiment is shown in Figure 5.

3.2.1. Protocol of experiment

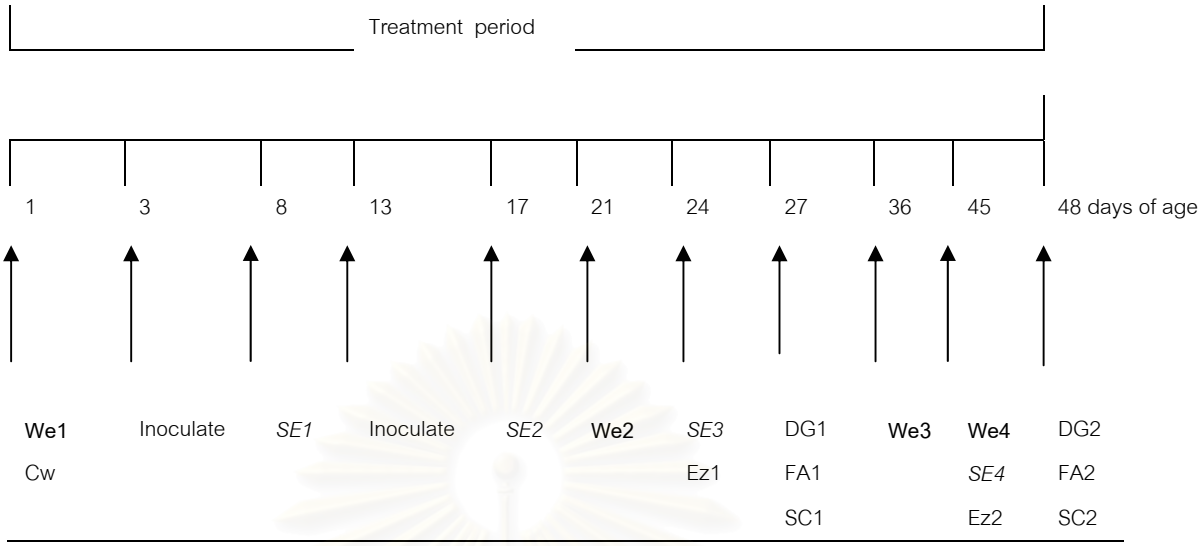


Figure 5 Diagram showing the whole period of experiment

We =Weighing (body weight and feed)

CW = Cloacal swabs

SE = sampling for *S.* Enteritidis determination

Ez = sampling for enzymes activities

DG = sampling for enzymes digestibility

FA = sampling for medium chain fatty acids determination

SC = sampling for short chain fatty acids determination

3.2.2 Sample collection and tissue preparation

The chickens were weighed at days 1, 21, 35, and 45. The feed intake was recorded during days 0 to 21, days 22 to 35 and days 36 to 45. Mortality was recorded daily. At day 3 post-hatching, each chick was inoculated 0.3 ml with tryptone soya broth (TSB) 18 hr. culture of *S.* Enteritidis (nal^r) containing 10^6 colony forming units (cfu) by oral route using esophageal tube. At day 13 of age, 2^{nd} inoculation was made to all chicks with one ml of 10^8 cfu of the same *Salmonella* culture. Twenty chickens from each treatment group were randomly selected and sacrificed at days 8, 17, 24 and 45 of age. The ceca and cecal contents were removed and placed in a plastic bag for

bacteriological study. They were sacrificed with intracardiac injection of pentobarbital sodium (120 mg/kg body weight) using 21G, 2.5 inch needle. The abdomen was exposed and the whole intestine from duodenum to 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 ileocaecal junction. The pH of the intestinal contents were immediately measured *in situ* 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 aluminium foil and stored at -70ºC until analysis. Jejunal mucosal scrapings were analyzed for disaccharidase activity (Dahlquist, 1968).

In addition, ten chickens from each treatment group at 22 and 45 days old were randomly selected. These chickens were fed on diets containing Celite as an indigestible marker. They were sacrificed with intracardiac injection of pentobarbital sodium (120 mg/kg BW) using 21G, 2.5 inch needle. The abdomen was exposed. The ileum and cecum were removed. The contents in the ileum were collected by gentle squeezing with thumb and fingered 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 -20ºC until analysis of nutrient digestibility. The cecal contents were wrapped with aluminium foil and stored at -70ºC until analysis of short-chain fatty acids.

3.2.3. Bacteriological examination

Nalidixic acid resistant, field strain (nal^r) of *S.* Enteritidis, isolated from the chicken obtained from the department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University was kept in stock agar at room temperature before use. It was transferred and streaked on Tryptic Soy Agar (TSA) plate and incubated at 37°C for 18-20 h for examination of purity.

Cloacal swab samples using sterile cotton were placed into buffer peptone water (BPW) as pre-enrichment media and tetrathionate brilliant green broth as enrichment media. Both media were incubated at 37°C for 18-20 h. The 0.1 ml of BPW was then dropped on to MSRV (Modified Semi-Rappaport Vassiliadis) and spread along the edge of the agar plate and incubated at 42°C for 24-48 h. The cultures from TTB and the suspected colonies from MSRV were transferred and incubated at 37°C for 24 h. The pink colonies with black spot on the XLT_4 agar plate was suspected of *S.*Enteritidis. They were tested for biochemical assays including glucose fermentation, hydrogen sulfide gas production from TSI (Triple sugar iron) agar, motility test, lysine decarboxylation and indole production were detected from MIL (Motility indole lysine medium). Serogrouping of *S.*Enteritidis (serogroup D) was confirmed by a slide agglutination test using antiserum of *Salmonella* O polyvalent group A to 67, and specific serogroup D.

3.2.4 Qualitative and quantitative examination of *S.* Enteritidis

Cecal sample was aseptically removed from each chick. The ceca was placed in separate bag, weighed, chopped and put into BPW 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 XLT_4 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 as mentioned in 3.2.3. The number of colony-forming units of *Salmonella* was expressed as $\log_{10}$ *Salmonella* per gram of cecal contents.

The cecal sample of 1:10 dilution was also incubated at 37ºC 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 XLT_4 agar plates were identified as mentioned in 3.2.3.

3.2.5. Determination of mucosal protein concentrations

3.2.5.1 Tissue preparation

Total protein concentrations in jejunal mucosa were determined by using Lowry method (Lowry, 1951). Jejunal mucosal scrapings were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with four parts of distilled water, weight by volume (w/v). Then the homogenate samples were diluted 40 times with distilled water, volume by volume (v/v).

3.2.5.2 Assay procedure

The test tube containing 100 µl of the homogenate samples were added with 3.0 ml of fresh reagent (consisted of 50 ml of 2% Na_2CO_3 in 0.1 M NaOH, 0.5 ml 1% $CuSO_4 \cdot 5H_2O$ and 0.5 ml of 2% Na-tartrate). The test tubes were allowed to settle at room temperature for 10 min. Folin reagent (300 µl) was added into the solution, for 30 min at room temperature. The optical density was read at the wavelength 650 nm by using UV-VIS spectrophotometer (Shimudsu UV 1201) (1 cm light path).

3.2.5.3 Standard solution

The standard curve was plot between the bovine serum albumin (BSA) at 0, 20, 40, 60, 80 and 100 mg%. The slope of the curve was calculated for the concentrations of protein in jejunal mucosa.

3.2.6. Determination of mucosal disaccharidase activities in jejunum

3.2.6.1 Tissue preparation

Mucosal scraping samples from the jejunum were used as sources of enzyme. Mucosal jejunal scrapings were homogenized (Homogenizer, GKH, GT MOTOR CONTROL, GLAS-COL®) with four parts of distilled water, (w/v). The suspensions were well chilled in crushed ice for at least 5 min before and during homogenization. The samples were centrifuged at 3,000 rpm (Centrifuge, GLC-2B, SORVALL), for 10 min and the supernatant was separated for disaccharidase determination.

3.2.6.2 Assay procedure

An aliquot of sample was diluted with distilled water (maltase 1:1,000, sucrase 1:100). The test tube containing 100 µl of the diluted enzyme solution was placed in a water bath for 5 min. Then 10 µl of the substrate buffer solution were added (maltose or sucrose). After incubation at 37°C for 60 min, 2,000 µl of enzyme reagent (Glucose liquicolor, Human Gesallschaft fur Biochemica and Diagnostica mbH, Germany) were added and mixed. These test tubes were placed in the water bath at 37°C for 5 min. The optical density was read at wavelength 500 nm, using UV-VIS spectrophotometer (Shimadzu UV 1201; 1 cm light path).

3.2.6.3 Standard solution

The standard curve of glucose at 0, 10, 20, 30, 40 and 50 mg% was plotted. The slope of the curve was calculated for the concentration of glucose.

Results were expressed as specific activity (unit per milligram mucosal scraping protein). One unit (U) was defined as disaccharidase activity that hydrolyzed 1 micromole (µmole) of the substrate per minute under the experimental condition. The disaccharidase activity was obtained by the following formula:

$$\frac{a \times d}{n \times 1{,}080} \quad \text{units/ml}$$

where

a = µg glucose liberated in 60 minutes

d = dilution factor for enzyme solution

n = number of glucose molecules per molecule of disaccharide (for maltose, n=2 and sucrase n=1)

3.2.7. Determination of nutrients digestibility

3.2.7.1 Determination of acid-insoluble ash

Celite was added as a marker for the determination of ileal digestibility of nutrients. Acid-insoluble ash in Celite was measured as described by Choct and Annison (1992). Two gram of dried diet and 1 g of dried digesta samples from grouding were weighed into sintered glass crucibles (Pyrex®, England). These sintered glass crucibles were then dried at 105 °C for 24 h, and placed into an oven for ashing at 550° for 8 h. After ashing, the crucibles were cooled, and boiled slowly in 4 N HCl for 30 min on a hot plate in fume hood. The ash in crucibles was washed with distilled water using suction pump, and dried at 105° C for 6 h. The ash residue in crucibles was ashed and boiled in the same way. Finally, the ash in crucibles was dried at 105°C for 6 h, the crucibles were cooled in a desiccator and weighed while containing the ash. Percentage of acid-insoluble ash was calculated using the following equation:

$$AIA\ (\%) = \frac{Wf - We}{Ws} \times 100$$

Where Wf = weight of crucible with ash

We = weight of empty crucible

Ws = weight of sample (dry matter)

The percentage of ileal digestibility (ID) of nutrients eg. (protein and fat) was calculated using the following equation:

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

3.2.8. Short-chain Fatty Acids (SCFAs) Determination

Cecal short-chain fatty acid concentrations were analyzed using the modified method from Erwin (1961). Frozen intestinal contents were thawed at room temperature. They were weighed and diluted with the equal volume of distilled water (eg 5 g contents diluted with 5 ml water). The solutions were centrifuged at 9,000 rpm for 10 min. The supernatant was separated for SCFAs determination. Mixture of four standard SCFAs solutions which were 70 mM acetic acid, 30 mM propionic acid, 10 mM butyric acid and 2 mM valeric acid. The internal standard used was isocaproic acid. Distilled water was used as a blank. 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. In case of the small volume of some samples, the same proportion of sample: working internal standard solution at 7:4 was applied. The solutions were centrifuged again at 9,000 rpm for 5 min, the supernatant aliquots were removed. The aliquots were analyzed for SCFAs concentration using a gas chromatography equipped with a hydrogen flame ionization detector. The column (GL Sciences Inc) treated with 1% (wt/wt) H_3PO_4 (length 2.1 m, ID 4 mm, OD 7 mm) packed with 10 % FFAP (80-100 mesh) was used for analyzing. The concentration of individual SCFA was expressed as μmole/g cecal content.

3.2.9 Medium-chain Fatty Acids (MCFAs) Determination

Medium chain fatty acid concentration in plasma and cecal content were analyzed using the modified method from Mingrone et al, (1995). Frozen intestinal contents and plasma were thawed at room temperature. Intestinal content were weighed and diluted with the equal volume of distilled water (eg 5 g contents diluted with 5 ml water). The solutions were centrifuged at 9,000 rpm for 10 min. The supernatant was separated for MCFAs determination.

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 residue was washed twice with ethanol and recentrifuged. The solutions were reduced to 0.5-1 ml, of which 1 µl were directly injected into a Gas Liquid Chromatograph (GLC) Hewlett-Packard mod equipped with a flame ionization detector (FID). MCFA were separated on a 25-m fused silica capillary column of crosslinked methyl siloxane HP-1, 0.32 mm, film thickness 0.17 µm. The concentration of individual MCFA was expressed as µg/ml plasma.

3.2.10. Calculation of the growth performance

The chickens were weighed at 0, 21 and 45 days old. The feed intake was recorded during days 0 to 21, days 22 to 35 and days 36 to 45. Number and body weight of dead chickens were recorded for calculation of mortality and feed conversion ratio (FCR), respectively.

Body weight gain (g/b) = Final body weight – Initial body weight

Average daily gain (ADG, g/b/d) = Body weight gain / Days

Feed intake (g/b) = Total feed intake / Final chick numbers

Daily feed intake (DFI, g/b/d) = Feed intake / Days

Mortality rate (%) = (No. of dead chicks / Total chick numbers) X 100

Feed conversion ratio (FCR, kg feed/kg body weight gain) $= \frac{\text{Total pen feed}}{\text{Final body weight - Initial body weight}}$

3.2.11. Statistical analysis

All data were presented as Mean ± SE. The effects of treatment were analyzed using One-Way Analysis of Variance (ANOVA). If there were any significant effect, Duncan's New Multiple Range Test was used to compare the individual means. Data, which were not complied with the equal variance and homogeneity test, were analyzed using non-parametric methods (Kruskral Wallis and ANOVA). Data on the qualitative *Salmonella* test (positive-negative) before and after treatment were analyzed by Chi-square analysis. The significant level was set as $p < 0.05$.

CHAPTER IV

RESULTS

4.1 Effect on growth performance.

Growth performance of chicks during starter period (days 1-21 of age) is shown in Table 3. After first inoculation of *S.* Enteritidis, It was shown that there were no significant differences in initial weight, average daily gain (ADG), daily feed intake (DFI), among groups of the chicks. However, feed conversion ratio (FCR) of ORA group was significantly better ($p<0.05$) than FOS group but was not significant by different from CON and MCA group. There was no significant difference on mortality rate among groups of chickens.

Growth performance of chicks in grower period (days 22-35 of age) is shown in Table 4. There was no effect of any treatment on the initial weight gain, average daily gain (ADG), daily feed intake (DFI), feed conversion ratio (FCR) and mortality rate among groups of chicks.

At finisher period (days 36-45), growth performance is shown in Table 5. Chicks in ORA and MCA group had significantly higher initial weight and average daily gain (ADG) than CON group but were not significantly different from FOS group. There was no significant difference on initial weight and average daily gain (ADG) between CON and FOS group. There was no effect of any treatment on daily feed intake (DFI), feed conversion ratio (FCR) and mortality rate among groups of chicks.

For the overall period of the trial (days 0-45 of age), growth performance is shown in Table 6. There were no significant difference in final body weight, average daily gain (ADG) between ORA and MCA group. Chicks in ORA and MCA groups had significantly ($p<0.05$) higher final body weight, average daily gain (ADG) than FOS and CON groups. However, CON group had significantly ($p<0.05$) lower final body weight

than FOS group but there was no significant difference in average daily gain (ADG) between CON and FOS groups. There were no significant differences in daily feed intake (DFI) among groups of chicks. In contrast, ORA and MCA group had significantly ($P<0.05$) better feed conversion ratio (FCR) than CON and FOS group. There was no significant difference on mortality rate among groups of chicks.

4.2 Effect of treatments on pH of the small intestines and crop.

The changes in pH of the crop and the intestinal content at the jejunum, ileum and caecum are demonstrated in Table 7.

At days 24, the pH of the jejunum decreased significantly ($p<0.05$) in FOS, ORA and MCA groups, as compared with the CON group. The jejunum pH values in FOS (6.46), ORA (6.37) and MCA (6.35) groups were significantly lower ($p<0.05$) than in CON (6.31) group but there were no differences in the ileal pH and caecum pH among the treatment groups.

At days 45, the pH of the crop decreased significantly ($p<0.05$) in FOS, ORA and MCA groups, as compared with the CON group. Moreover, the pH in MCA group was lower ($p<0.05$) than other groups. The jejunum pH values in FOS, ORA and MCA groups were significantly lower ($p<0.05$) than in CON group but there were no differences in the ileal pH and cecum pH among the treatment groups. It is shown that the pH of ileum in FOS, ORA and MCA groups were lower ($p<0.05$) than CON group and the pH of ileum in MCA and ORA groups were lower ($p<0.05$) than FOS group. Moreover, there was a trend that the pH of the cecum in MCA group was lower ($p>0.05$) than others group but there was no statistically significance.

Table 3 Effect of treatments on growth performance[1] of the broiler chickens (Days 1-21)

Starter period		Treatment[2]			
		CON	FOS	ORA	MCA
Initial weight	(g/b)	41.47 ± 0.90	40.60 ± 1.01	41.67 ± 1.05	41.13 ± 0.80
ADG	(g/b/d)	27.02 ± 0.22	26.88 ± 0.25	27.32 ± 0.46	26.65 ± 0.41
DFI	(g/b/d)	45.29 ± 0.72	46.95 ± 2.08	45.07 ± 1.04	45.37 ± 0.90
FCR		1.68 ± 0.02[ab]	1.75 ± 0.07[b]	1.65 ± 0.05[a]	1.70 ± 0.02[ab]
Mortality	(%)	0.67 ± 1.49	1.33 ± 2.98	0.67 ± 1.49	1.33 ± 2.98

[1] Mean ± SE

[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids.

[a,b]Mean in the same row with different superscripts differed significantly ($p<0.05$).

Table 4 Effect of treatments on growth performance[1] of the broiler chickens (Days 22-35)

Grower period		Treatment[2]							
		CON		FOS		ORA		MCA	
Initial weight	(g/b)	627.48	± 21.73	626.81	± 26.60	639.79	± 18.48	631.60	± 21.67
ADG	(g/b/d)	36.35	± 3.77	37.85	± 1.79	40.77	± 6.87	39.44	± 2.95
DFI	(g/b/d)	72.01	± 1.40	74.73	± 5.59	75.11	± 1.19	72.40	± 4.01
FCR		1.98	± 0.22	1.97	± 0.18	1.84	± 0.17	1.83	± 0.16
Mortality	(%)		0		0		0		0

[1] Mean ± SE

[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids.

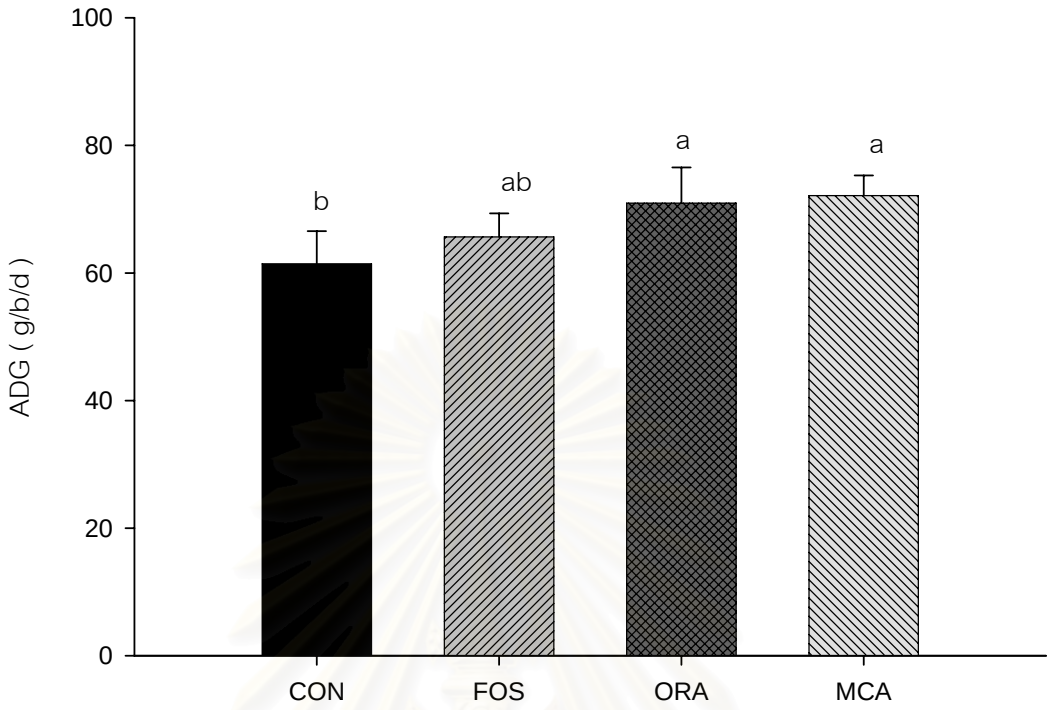
Table 5 Effect of treatments on growth performance[1] of the broiler chickens (Days 36-45)

Finisher period		Treatment[2]							
		CON		FOS		ORA		MCA	
Initial weight	(g/b)	1,169.33	± 24.31[b]	1,216.62	± 62.16[ab]	1,266.48	± 37.29[a]	1,266.86	±45.60[a]
ADG	(g/b/d)	61.44	± 5.12[b]	65.64	± 3.69[ab]	70.94	± 5.60[a]	72.14	± 3.12[a]
DFI	(g/b/d)	130.00	± 5.67	131.62	± 11.81	131.55	± 5.75	134.53	± 5.85
FCR		2.12	± 0.16	2.01	± 0.27	1.86	± 0.15	1.86	± 0.12
Mortality	(%)		0		0		0	1.33	± 2.98

[1] Mean ± SE

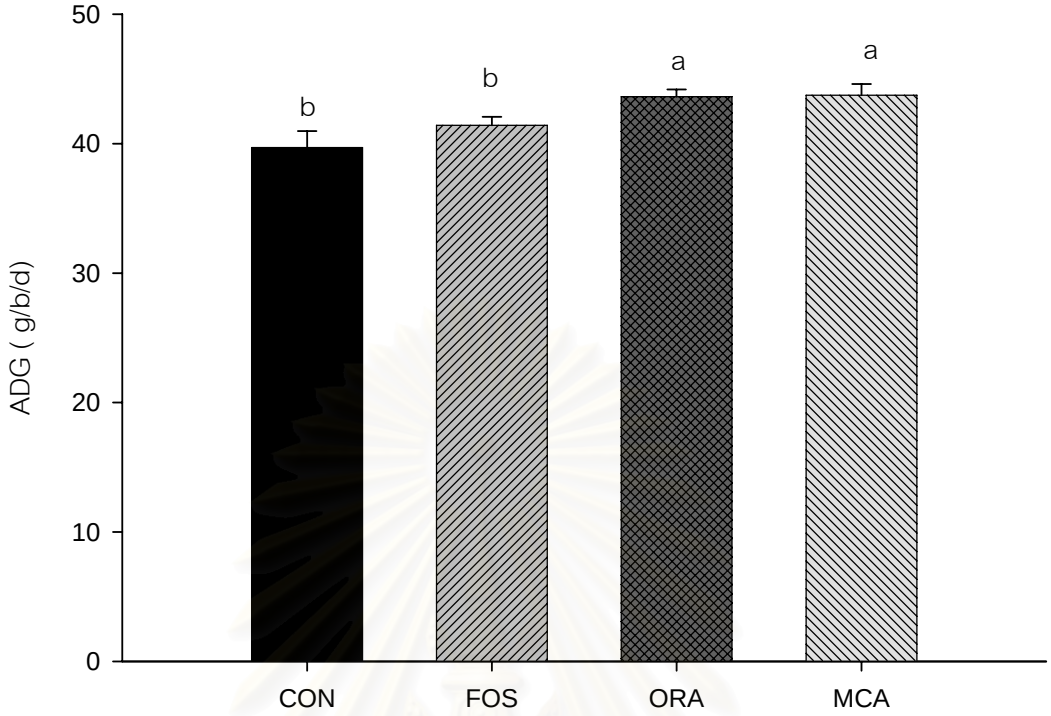
[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids.

[a,b] Mean in the same row with different superscripts differed significantly ($p<0.05$).



[a,b] Different superscripts mean significantly different (P<0.05)

Figure 6 Effect of various treatments on average daily gain (g/b/d) of broiler chickens during 36-45 of age.



[a,b] Different superscripts mean significantly different (P<0.05)

Figure 7 Effect of various treatments on average daily gain (g/b/d) of broiler chickens during 0-45 of age.

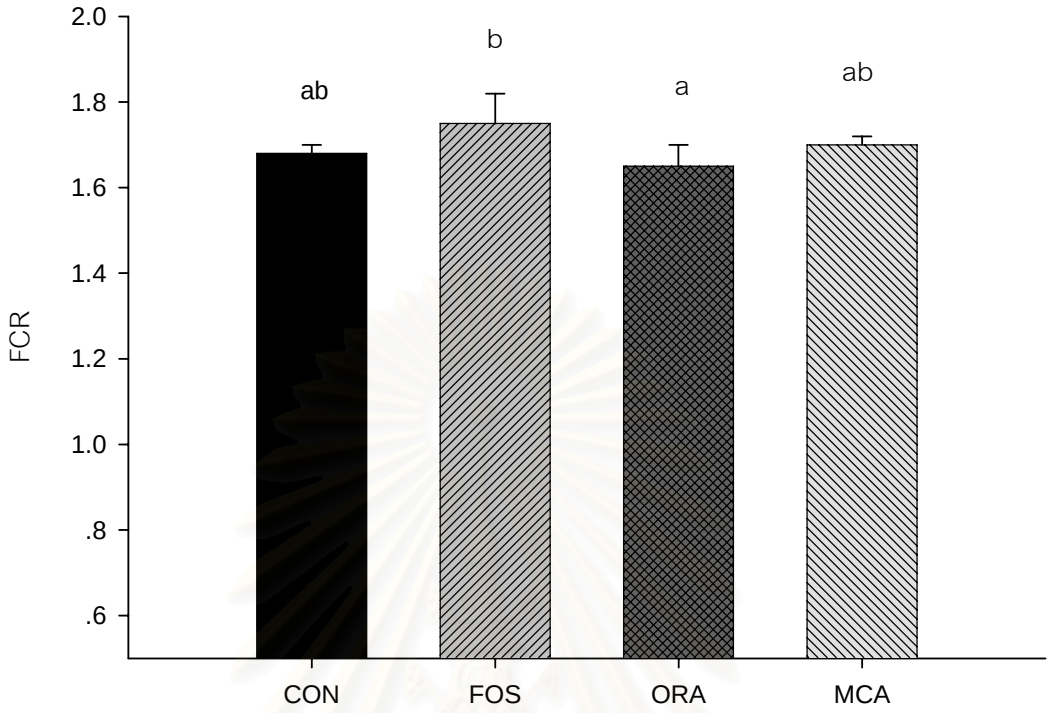
Table 6 Effect of treatments on overall growth performance[1] of the broiler chickens (Days 0-45)

Overall period		Treatment[2]							
		CON		FOS		ORA		MCA	
Initial weight	(g/b)	41.47	± 0.90	40.60	± 1.01	41.67	± 1.05	41.13	± 0.80
Final weight	(g/b)	1,827.69	± 58.22 [c]	1,904.47	± 29.34 [b]	2,004.61	± 29.79 [a]	2,009.23	± 39.34 [a]
ADG	(g/b/d)	39.69	± 1.27 [b]	41.41	± 0.65 [b]	43.62	± 0.56 [a]	43.73	± 0.88 [a]
DFI	(g/b/d)	72.43	± 1.59	74.41	± 5.32	73.50	± 2.03	73.23	± 2.25
FCR		1.82	± 0.04 [b]	1.79	± 0.10 [b]	1.68	± 0.05 [a]	1.67	± 0.03 [a]
Mortality	(%)	0.67	± 1.49	1.33	± 2.98	0.67	± 1.49	2.00	± 4.47

[1] Mean ± SE

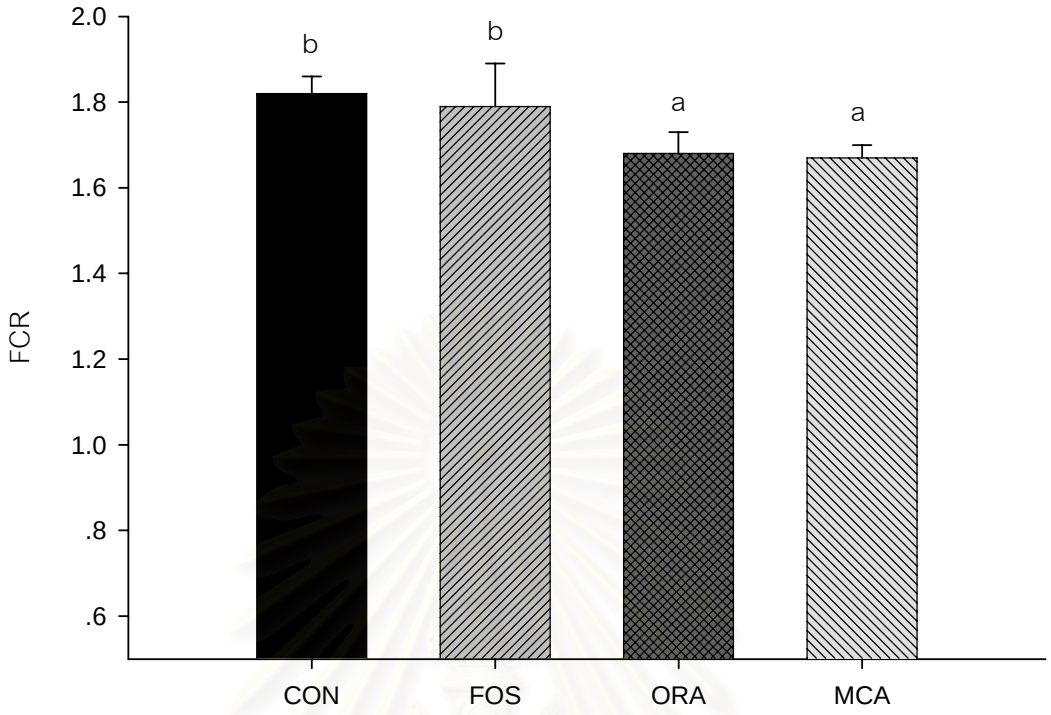
[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids.

[a,b,c] Mean in the same row with different superscripts differed significantly ($p<0.05$).



[a,b] Different superscripts mean significantly different (P<0.05)

Figure 8 Effect of treatment on feed conversion ratio (kg feed/kg gain) of broiler chickens during 1-21 age.



[a,b] Different superscripts mean significantly different (P<0.05)

Figure 9 Effect of various treatments on feed conversion ratio (kg feed/kg gain) of broiler chicken during 0-45 of age

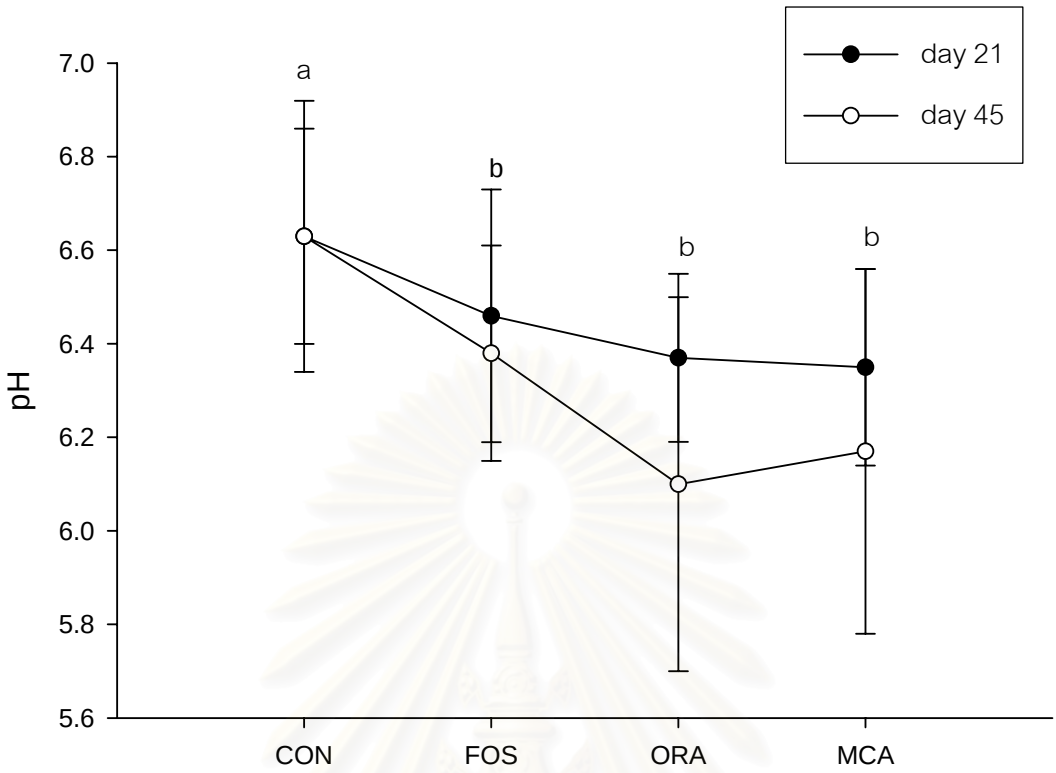
Table 7 Effect of treatments on the intestinal pH[1] of the crop and intestines

Intestinal part	Treatment[2]			
	CON	FOS	ORA	MCA
Crop				
Day 45	5.69 ± 0.03[a]	5.51 ± 0.05[b]	5.17 ± 0.05[c]	4.86 ± 0.04[d]
Jejunum				
Day 24	6.63 ± 0.05[a]	6.46 ± 0.06[b]	6.37 ± 0.04[b]	6.35 ± 0.04[b]
Day 45	6.63 ± 0.06[a]	6.38 ± 0.05[b]	6.10 ± 0.08[b]	6.17 ±0.08[b]
Ileum				
Day 24	7.21 ± 0.04	7.18 ± 0.06	7.19 ± 0.04	7.13 ± 0.05
Day 45	7.10 ± 0.04[a]	6.85 ± 0.11[b]	6.22 ± 0.12[c]	6.18 ± 0.11[c]
Ceca				
Day 24	6.34 ± 0.05	6.37 ± 0.05	6.23 ± 0.03	6.23 ± 0.02
Day 45	6.91 ± 0.08	6.90 ± 0.04	6.76 ± 0.10	6.77 ± 0.06

[1] Mean ± SE; n=20

[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids.

[a,b,c,d] Mean in the same row with different superscripts differed significantly ($p<0.05$).



[a,b] Different superscripts mean significantly different (P<0.05)

Figure 10 The pH of jejunal content of broiler chickens at days 24 and 45 of the experiment.

4.3 Cecal colonization by *S.*Enteritidis

The mean $\log_{10}$ number of *salmonella* 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. No differences in the *Salmonella* numbers in the cecal contents was found between the FOS group and CON group. *Salmonella* numbers were significantly lower in the MCA group compared with CON group on day 24 and 45. Furthermore, It is noted that there was no *Salmonella* Enteritidis found in all chicks in ORA and MCA group on day 45, likewise, *Salmonella* numbers in FOS group trend to be lower compared with CON group.

Table 8 Effect of treatments on *salmonella* colonization[1] in ceca of broiler chicks

Group	Day 17			Day 24			Day 45		
	Detection rate	%[3]	*Salmonella* numbers in ceca of infected chickens (log_{10} cfu/g ± SE)	Detection rate	%[3]	*Salmonella* numbers in ceca of infected chickens (log_{10} cfu/g ± SE)	Detection rate	%[3]	*Salmonella* numbers in ceca of infected chickens (log_{10} cfu/g ± SE)
CON	10/10	100	3.35 ± 0.38[a]	10/10	100[a]	2.63 ± 0.68	7/10	70[a]	ND[4]
FOS	10/10	100	3.26 ± 0.38[a]	8/10	80[ab]	2.23 ± 0.63	2/10	20[b]	ND[4]
ORA	9/10	90	2.00 ± 0.00[b]	9/10	90[ab]	1.36 ± 0.38	0/10	0[c]	ND[4]
MCA	10/10	100	2.23 ± 0.12[b]	6/10	60[b]	1.49 ± 0.49	0/10	0[c]	ND[4]

[1] Mean ± SE

[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids.

[a,b]Mean in the same column with different superscripts differed significantly ($p<0.05$).

[3] Chi-square test of differences among treatments

[4]ND: The values were not detectable.

4.4 Effect of treatments on jejunal disaccharidase activities.

Maltase

The alteration in maltase activity of jejunal mucosa is shown in Table 9 . Maltase activities in FOS and MCA group were significantly higher ($p<0.05$) than CON group and maltase activities of MCA group were increased significantly ($p<0.05$) when compared to ORA group (Figure11). At day 45, there was no significant difference in maltase activities of jejunal mucosa among experimental groups. It was found that maltase activities in MCA group were greater ($p>0.05$) than other groups.

Sucrase

The sucrase activities of jejunal mucosa are shown in Table 9. There was no significance difference in the sucrase activities of each group. The sucrase activities of MCA at day 45 were significant different from FOS group. The sucrase activities of FOS and MCA groups were significantly higher ($p<0.05$) than CON group and MCA group had more ($p<0.05$) sucrase activities than ORA group (Figure12). There was no significant difference in sucrase activities between CON and ORA group.

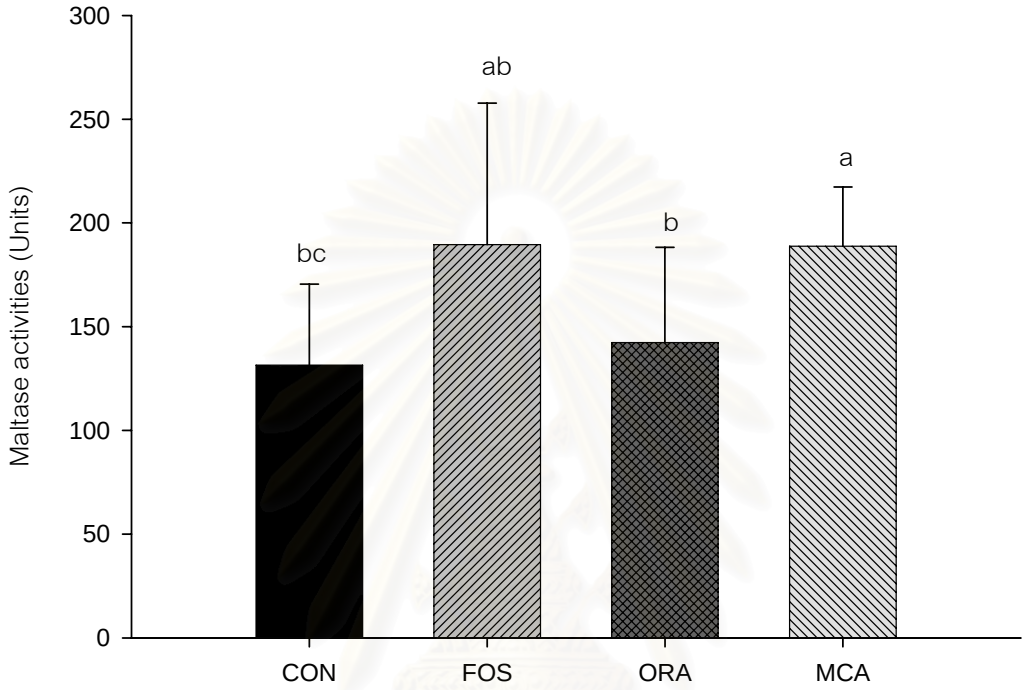
Table 9 Effect of treatments on jejunal disaccharidase activities[1] (units/mg protein) of the broiler chickens

Enzyme	Treatment[2]			
	CON	FOS	ORA	MCA
Maltase (units/mg protein)				
Day 24	131.43 ± 12.35[bc]	189.58 ± 21.58[ab]	142.35 ± 14.51[b]	188.82 ± 9.01[a]
Day 45	95.07 ± 7.68	112.91 ± 13.32	110.84 ± 19.20	141.52 ± 14.05
Sucrase (units/mg protein)				
Day 24	31.32 ± 4.27	35.82 ± 39.40	30.40 ± 2.05	31.52 ± 1.98
Day 45	10.74 ± 1.38[b]	20.68 ± 1.58[a]	13.08 ± 2.14[b]	20.66 ± 2.26[a]

[1] Mean ± SE; n=5

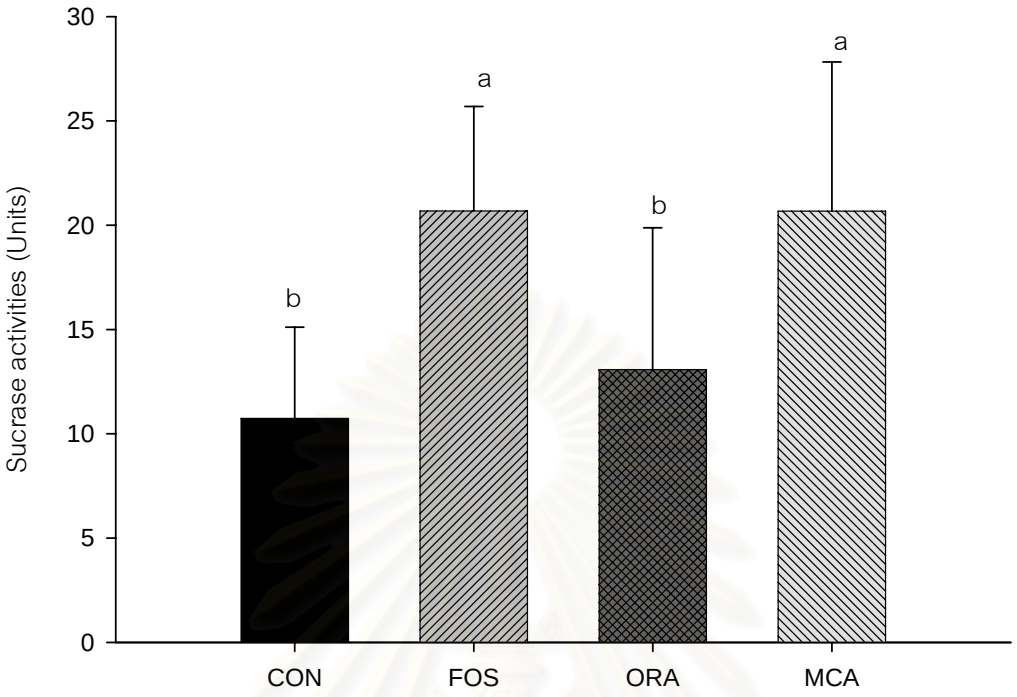
[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids

[a,b] Mean in the same row with different superscripts differed significantly ($p<0.05$).



[a,b,c] Different superscripts mean significantly different (P<0.05)

Figure 11 Effect of various treatments on maltase activities (unit) of jejunal mucosa of the broiler chickens during 1-21 ages.



[a,b,c] Different superscripts mean significantly different (P<0.05)

Figure 12 Effect of treatment on sucrase activities (unit) of jejunal mucosa of the broiler chickens during 36-45 age.

4.5 Effect of treatments on SCFAs concentrations.

Cecal concentrations of each short – chain fatty acids at day 27 of age are shown in Table 10. Cecal acetic acid and valeric acid significantly increased ($p<0.05$) in MCA group, as compared with the CON group (Figure13 and Figure 14). There was no significant difference in propionic acid and butyric acid concentrations. However, the MCA group seems to have the highest propionic acid level. The level of butyric acid concentration in CON group was very low compared to other groups. At finisher period (days 35-45 of age), the concentrations of acetic acid increased significantly ($p<0.05$) in MCA group, as compared to FOS and CON groups. The valeric acid of MCA group was significantly ($p<0.05$) higher than other groups. There was no difference in propionic acid and butyric acid concentrations. Moreover, propionic acid and butyric acid in the MCA group trend to be higher ($p>0.05$) than other groups but there was no statistically significant (Figure15 and Figure 16).

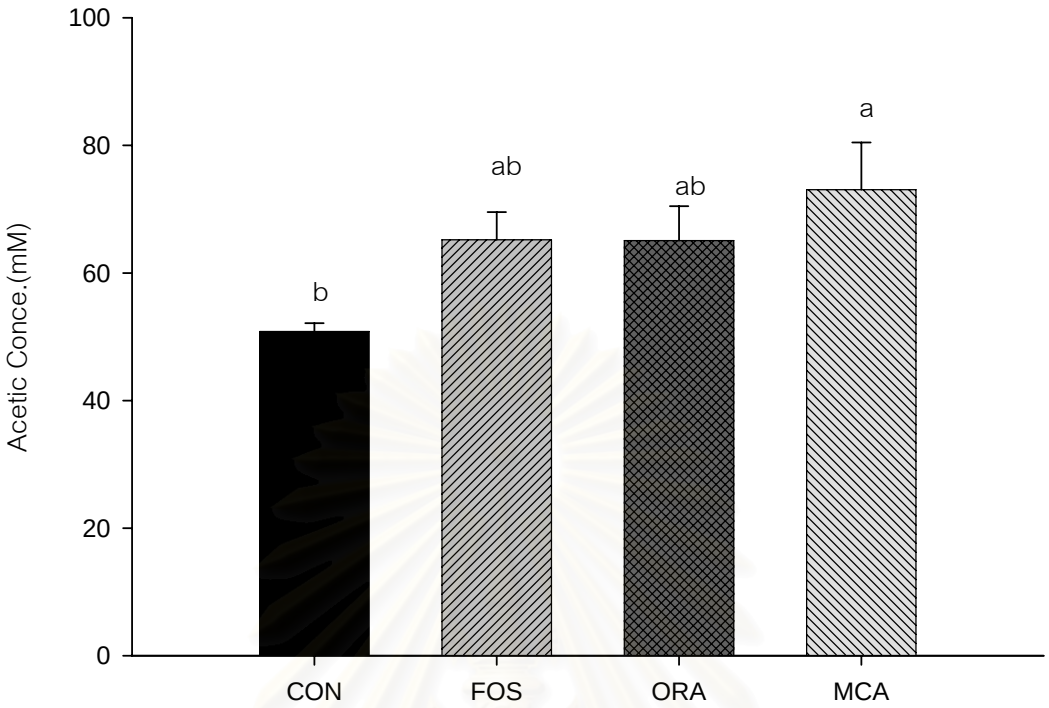
Table 10 Effect of treatments on the short – chain fatty acid (SCFA) concentrations[1] (mmol/ml) in the cecum of broiler

	Short chain fatty acid concentration	Treatment[2]			
		CON	FOS	ORA	MCA
Day 27					
	Acetic acid	50.82 ± 1.31 [b]	65.20 ± 4.34 [ab]	65.07 ± 5.39 [ab]	73.05 ± 7.40 [a]
	Propionic acid	14.68 ± 0.39	15.98 ± 2.06	15.28 ± 0.43	18.49 ±1.89
	Butyric acid	13.75 ± 0.74	19.54 ± 1.89	21.29 ± 3.34	19.40 ± 6.30
	Valeric acid	0.92 ± 0.01 [b]	1.02 ± 0.07 [ab]	1.12 ± 0.06 [ab]	1.31 ± 0.15 [a]
	Total	80.38 ± 1.74	99.51 ± 5.48	102.75 ± 8.18	112.25 ± 11.69
Day 48					
	Acetic acid	49.88 ±10.12 [b]	54.55 ±4.54 [b]	73.22 ±10.08 [ab]	93.71 ± 13.66 [a]
	Propionic acid	16.03 ± 1.79	17.11 ± 0.61	21.73 ± 3.03	25.87 ± 3.33
	Butyric acid	8.11 ± 3.53	9.19 ± 1.74	14.57 ± 3.35	19.26 ± 3.44
	Valeric acid	0.66 ± 0.18 [b]	0.62 ± 0.07 [b]	1.02 ± 0.17 [b]	1.51 ± 0.17 [a]
	Total	57.68 ± 15.23 [b]	81.47 ± 6.11 [b]	110.54 ± 15.73 [ab]	140.35 ± 18.68 [a]

[1] Mean ± SE; n=5

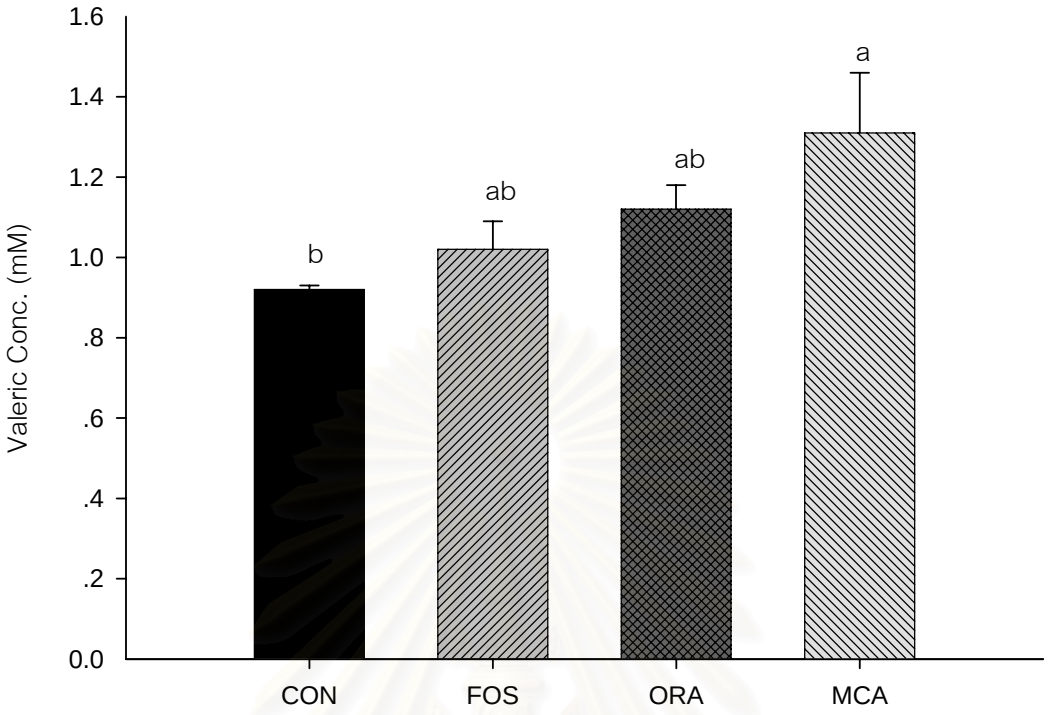
[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids.

[a,b] Mean in the same row with different superscripts differed significantly ($p<0.05$).



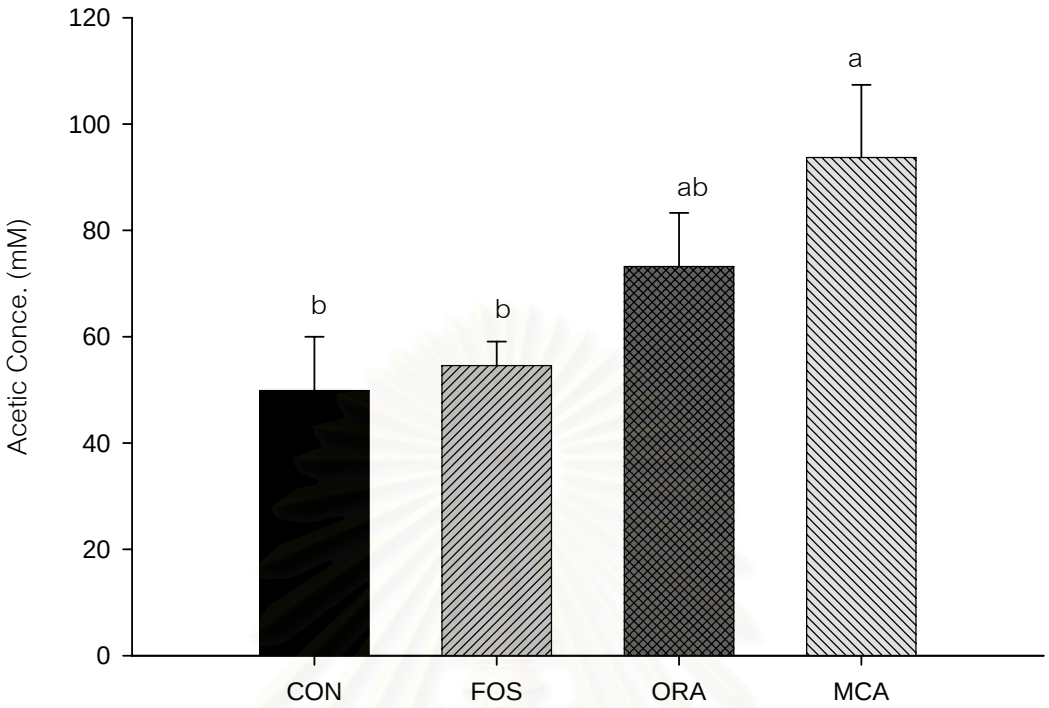
[a,b] Different superscripts mean significantly different (P<0.05)

Figure 13 Effect of treatment on acetic concentration in cecum (1-21)



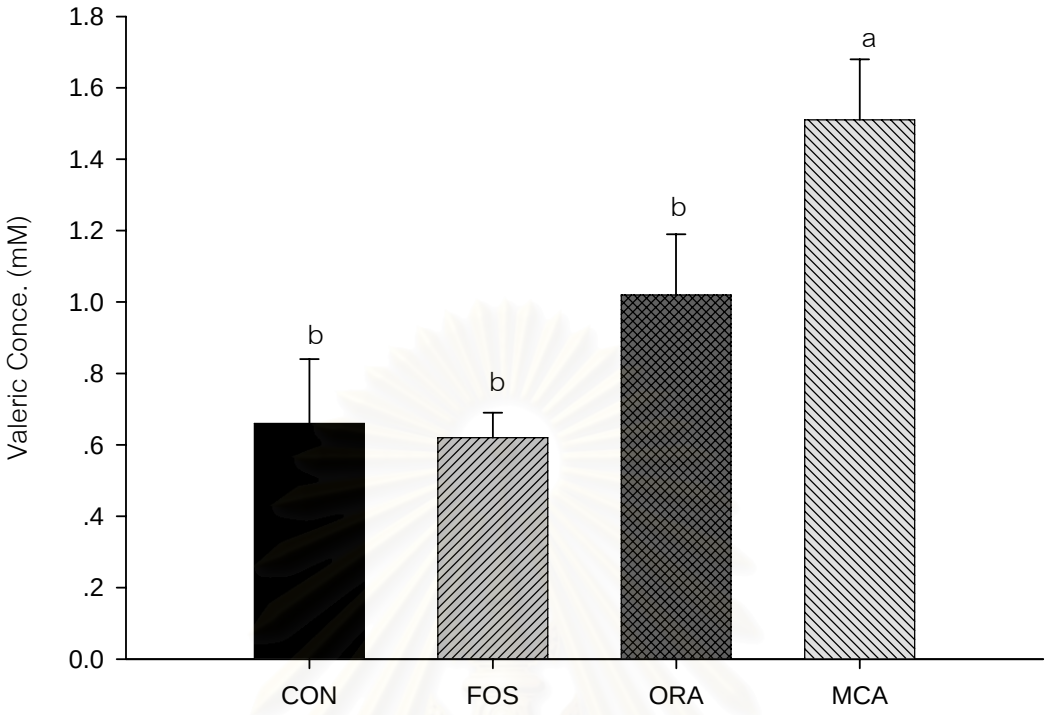
[a,b] Different superscripts mean significantly different (P<0.05)

Figure 14 Effect of treatment on valeric concentration in cecum (1-21)



[a,b] Different superscripts mean significantly different (P<0.05)

Figure 15 Effect of treatment on acetic concentration in cecum (36-45)



[a,b] Different superscripts mean significantly different (P<0.05)

Figure 16 Effect of treatment on valeric concentration in cecum (36-45)

4.6 Effect of treatment on dry matter ileal digestibility of nutrient.

Ileal digestibility of protein

The ileal digestibility of protein of the broiler chickens are demonstrated in Table 11. At day 27, it was found that the ileal digestibility of protein in broiler chickens were no significant difference among each group of broiler chickens.

At day 48, it was found that the ileal digestibility of protein in FOS, ORA and MCA group were significantly ($p<0.05$) higher than CON group. Furthermore, it was showed that the broiler chicken in MCA group had significantly ($p<0.05$) higher ileal digestibility of protein than ORA and FOS groups (Figure 17).

Ileal digestibility of fat

The ileal digestibility of fat of the broiler chickens are demonstrated in Table 11. At day 27, it was found that the ileal digestibility of fat in broiler chickens were no significant difference among each group of broiler chickens.

At day 48, it was found that the ileal digestibility of fat in FOS, ORA and MCA group were significantly ($p<0.05$) higher than CON group. Furthermore, it was showed that the broiler chicken in MCA group had significantly ($p<0.05$) higher ileal digestibility of fat than ORA and FOS groups (Figure 18).

Ileal digestibility of energy

The ileal digestibility of energy of the broiler chickens is demonstrated in Table 11. At day 27, it was found that there was no significant difference in the ileal digestibility of energy in broiler chickens among each group of broiler chickens.

At day 48, it was found that the ileal digestibility of energy in FOS, ORA and MCA group were significantly ($p<0.05$) higher than CON group. Furthermore, it was showed that the broiler chicken in MCA group had significantly ($p<0.05$) higher ileal digestibility of energy than ORA and FOS groups. In addition, the broiler chickens in ORA group had significantly ($p<0.05$) higher ileal digestibility of energy than FOS group. (Figure 19).

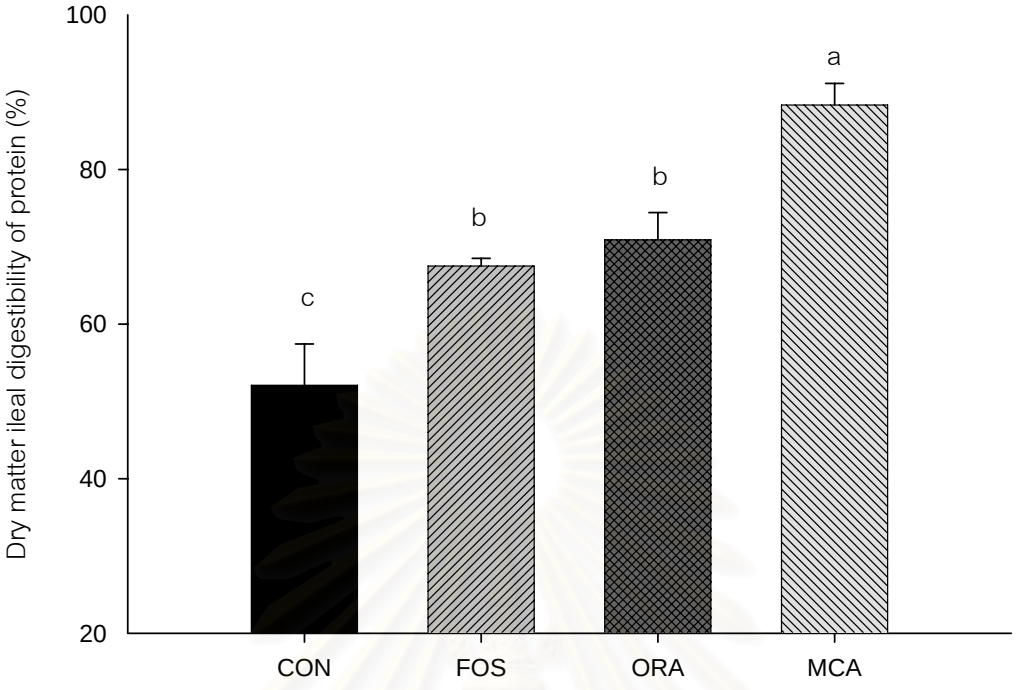
Table 11 Effect of treatments on dry matter ileal digestibility of nutrient[1] (%) of the broiler chickens

Nutrient		Treatment			
		CON	FOS	ORA	MCA
Protein					
	Day 27	77.60 ± 0.82	77.18 ± 3.66	77.31 ± 1.54	73.98 ± 1.48
	Day 48	52.09 ± 2.39^{c}	67.52 ± 0.44^{b}	70.89 ± 1.58^{b}	83.34 ± 1.24^{a}
Fat					
	Day 27	89.48 ± 0.46	89.79 ± 1.00	91.03 ± 1.27	90.60 ± 0.70
	Day 48	24.15 ± 4.05^{c}	46.31 ± 0.85^{b}	54.52 ± 2.43^{b}	74.10 ± 1.93^{a}
Energy					
	Day 27	73.78 ± 1.08	73.69 ± 4.37	73.48 ± 1.67	69.61 ± 1.75
	Day 48	59.85 ± 2.15^{d}	73.87 ± 0.49^{c}	76.97 ± 1.25^{b}	88.46 ± 1.21^{a}

[1] Mean ± SE; n=5

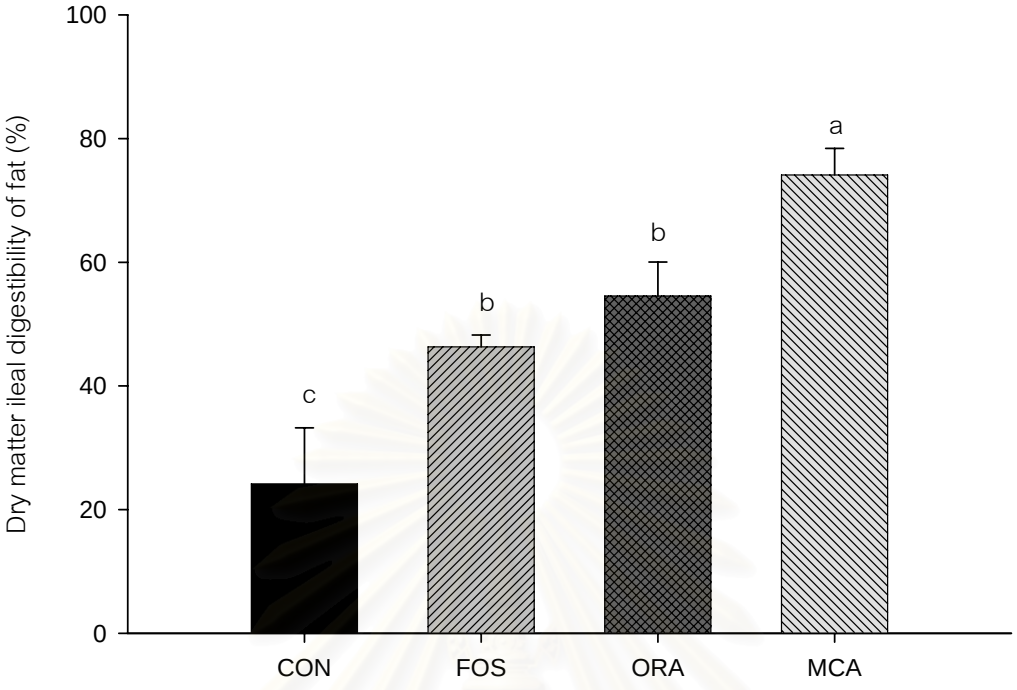
[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids.

[a,b,c,d] Mean in the same row with different superscripts differed significantly ($p<0.05$).



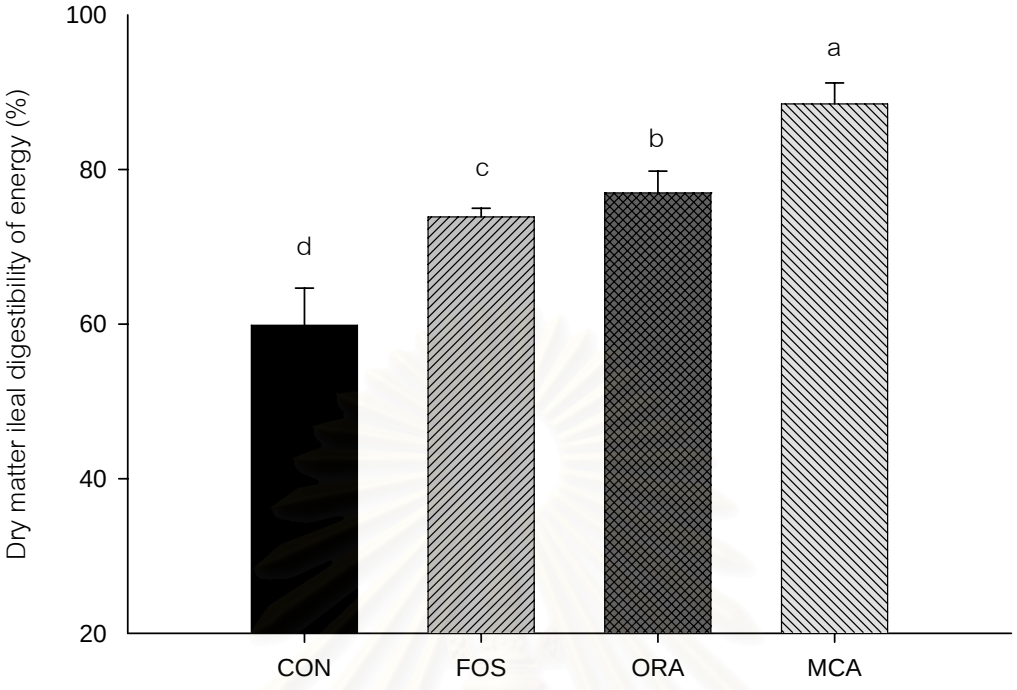
[a,b] Different superscripts mean significantly different (P<0.05)

Figure 17 Effect of treatment on dry matter ileal digestibility of protein (%) of broiler chickens (36-45)



[a,b] Different superscripts mean significantly different (P<0.05)

Figure 18 Effect of treatment on dry matter ileal digestibility of fat (%) of broiler chickens (36-45)



[a,b] Different superscripts mean significantly different (P<0.05)

Figure 19 Effect of treatment on dry matter ileal digestibility of energy (%) of broiler chickens (36-45)

4.7 Effect of treatment on MCFAs concentrations

Plasma concentrations of each medium chain fatty acids at day 21 and 45 of age are shown in Table 12. The caproic (C 6) concentrations were highest in chicks in MCA group. Moreover, the caprylic acid (C 8) concentrations were found only chicks in MCA group. For the total MCFAs concentration, chicks in MCA group had the highest MCFAs concentrations while chicks in CON group did not found MCFAs concentration. However, chicks in all groups were not found MCFAs concentration in cecal content at day 21 and 45 of age.

Table 12 Effect of treatments on the medium – chain fatty acid (MCFA) concentrations[1] (mmol/ml) in the plasma of broiler

Medium chain fatty acid concentration	Treatment[2]			
	CON	FOS	ORA	MCA
Day 27				
Caproic acid (C6)	0^{c}	8.00 ± 8^{b}	6.84 ± 6.84^{b}	40.43 ± 4.98^{a}
Caprylic acid (C8)	0^{b}	0^{b}	0^{b}	27.34 ± 13.02^{a}
Day 48				
Caproic acid (C6)	0^{c}	13.58 ± 8.34^{b}	0^{c}	34.21 ± 0.65^{a}
Caprylic acid (C8)	0^{b}	0^{b}	0^{b}	37.70 ± 4.33^{a}

[1] Mean ± SE; n=5

[2] Treatments were CON: control; FOS: fructooligosaccharide 4g/kg feed; ORA: organic acid; MCA: medium chain fatty acids.

[a,b,c] Mean in the same row with different superscripts differed significantly ($p<0.05$).

CHAPTER V

DISCUSSION

Starter period (1-21days)

It is demonstrated that the number of *Salmonella* colonized in ceca of MCA and ORA groups were slightly lower than CON group. It is possible that this may be due to the antibacterial activity of medium chain fatty acids (MCFAs) and organic acids. They diffuse into the bacterial cell in 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 MCFAs 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 is dependent on both the total concentration of organic acid and pH (Hinton et al., 1990). It is demonstrated that pH of crop and small intestines in MCA, ORA and FOS groups were significantly decreased compared to 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). It is proposed that the antimicrobial activity of organic acids was depended on the pKa of the acid, molecular weight (MW) and lipophilic/ hydrophilic character (Dierick et al., 2002). The pKa of MCFAs was 4.9 (Hsiao and Siebert, 1999) and pKa of SCFAs was < 4.8 and the pH in the crop ranged between 4 and 7 (Soerjadi et al., 1982), thus, most of the MCFAs were in an undissociated form. In addition, it is demonstrated that chicks in MCA group had significantly higher concentrations of acetic acid and valeric acid than other groups. Moreover, concentrations of SCFA similar to these found in the ceca have been shown to inhibit the growth *Salmonella*, this inhibition is increased with the reduction in redox potential of the ceca accompanied by a lower pH of the ceca (McHan and Shotts, 1993). *Salmonella* infection can lead to change in the intestinal mucosa (Suzuki, 1992). Changes in intestinal morphology such as shorter villi and

deeper crypts have been associated with the toxins (Yason et al., 1987). These resulted in the reduction of enzyme production. It is demonstrated that chicks in MCA group had significantly higher maltase activity than CON group. It is possible that MCFA restored the mucosal cell function as seen in the improvement of brush border disaccharidase enzymes. MCFAs 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 MCA group had high MCFA concentration in portal vein. Odle. (1997) showed that medium chain fatty acid had a specialized energy source, and better utilized in the neonatal piglet. Kishi et al. (2002) showed that MCFAs were utilized as immediate energy source in insufficient fat digestion.

This study showed that daily feed intake (DFI) and weight gain of chicks were not significant different among all treatments (Table3). However, feed conversion ratios (FCR) of chicks in FOS group were higher than other groups. It is possible that 4 g/kg FOS was extensive to chicks in this starter period. Fructooligosaccharide (FOS) was a fragment of fructose molecules connected with ß1,2 linkage which was not well utilized by host enzymes. Therefore during the first period of life, young chicks required time to adapt to FOS. Furthermore, chicks in ORA group tended to have lower feed conversion ratio (FCR) than chicks in MCA group. Low intake of water supplemented with MCA than other group was observed and this may be due to the taste of MCA and chicks needed time to adapt to drinking.

Grower and Finisher period (22-45 days)

There were no significant difference in final body weight, DFI, ADG and FCR among all treatments during grower period. Chicks in MCA and FOS groups tended to have better growth performance in grower period than in starter period. Moreover, in the finisher period, it is demonstrated that chicks in MCA and ORA group had significantly higher initial weight and ADG than CON group. It is possible that better growth performance may be due to an antibacterial effect of organic acids. The exact mechanism of these additives in protection of *Salmonella* to colonize in the intestine is still unknown. Several mechanisms have been

suggested: competition for nutrients, competition for receptor sites, immunomodulation, production of antimicrobial substrates, or production of acetate, propionate, and butyrate (van der Wielen et al., 2000). It was found that chicks in MCA group had higher short chain fatty acids in the ceca, especially acetate and valerate, compared to other groups. 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 was found that MCA and ORA demonstrated an antibacterial action against *Salmonella* and decrease pH in the crop and intestinal tract. In addition, van Immerseel. (2002) reported that there was no effect of FOS on pH of 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 crop in chicks fed on FOS was significantly lower than CON group at day 45. It might be possible that FOS was fermented by *Lactobacilli* in 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 FOS group tended to have higher SCFAs in cecal contents, compared to chicks in CON group, but these effects were not significant. It is possible that gut adaptation could modify the gastrointestinal effects of nondigestible carbohydrate (Rao et al., 1994). Brunsgaard et al. (1995) and Weaver et al. (1996) indicated that in rat fed on different types of indigestible polysaccharides, it took 3-12 wk to stabilize cecal SCFA concentrations. Blay et al. (1999) indicated that effect of FOS was studied only for short periods of ingestion never exceeding 3-4 wk.

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 tremendous impact on nutrient supply to the animal. The intestinal villus and crypt morphology in chickens has been associated with intestinal function and chicken growth. In the finisher period, the numbers of *Salmonella* in 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 resulted 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. Chicks in MCA group had more disaccharidases enzyme activity than other group. Gaillot et al. (1993) indicated that the liver is the main site of MCFA utilization and suggests that a substantial proportion of these acids may also be utilized in the intestinal mucosa. The results agree with a previous study that MCFA have positive effect on epithelial cell membrane bound enzyme activities (Takase and Goda, 1990). Furthermore, MCFA improved in intestinal morphology and function, through their positive effects on crypt cell renewal (Jenkins and Thompson, 1993). It is demonstrated that chicks in FOS group had an increased in sucrase activities in the jejunum and had slightly 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 ferment FOS because of the innate secretion of a ß-fructoside enzyme and some other bacteria to produce short chain fatty acids (SCFAs) (Gibson, 2004). Sakata (1987) reported that acetate, propionate and n-butyrate have a dose dependent stimulatory effect on epithelial cell production rates in the jejunum and distal colon. Moreover, SCFAs 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 showed the trophic effects of SCFA on epithelial cell proliferation (Frankel et al., 1994). Golden (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 *Lactibacilli* can synthesize enzymes, these increasing the intestinal digestive enzyme activity (Sissons, 1989). There was no significant difference in maltase activity in chicks fed on FOS. It is possible that variation may occur among mucosal samples tested. It is apparent that the standard variations of maltase activity were high so that it may obscure the difference between FOS and CON groups. 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 MCFA 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 MCFAs (caproic acid and caprylic acid) were found in portal vein of chicks fed on water supplemented with MCFA. It

is proposed that MCFA 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 MCFAs 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 MCFA compared with LCFA. Moreover, it is demonstrated that there was no of MCFAs found in ceca of MCA group. It is possible that MCFAs were be entirely absorbed in the small intestine or may be utilized by colonic mucosa. Jorgensen (2001) indicated that the colonic mucosa can both metabolize and transport MCFAs. Octanoate and decanoate were oxidized to CO_2 as well as butyrate and thus provided energy to the colonic epithelium (Jorgensen, 2002). Thus, energy for digestion in the gastrointestinal tract, reesterification and chylomicron formation and transport is not necessary for MCFA (Guillot et al., 1993). These MCFAs serve 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 FOS group had significantly higher caproic acid (C6) in portal vein, compared to CON group. It is possible that fermentation of FOS can lead to the production of some C6. However, it is noted that only one sample from five samples in both FOS and ORA group was found. Furthermore, it is demonstrated that chicks in MCA group also had higher digestibility of nutrients than other groups. It is possible that MCFA had an increased in brush border enzyme and they are absorbed more quickly into the intestinal lumen (Papamandjaris, 1998). In addition, MCFA 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 supply of energy to this organ (Bach et al., 1982; Decker, 1996). Moreover, Galluser et al. (1993) suggested that a greater solubility of MCFAs would facilitate uptake by the intestinal mucosa and they improved intestinal morphology and functions. Furthermore, this study indicated that chicks in FOS group had higher nutrient digestibility than 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 intestine (Sissons, 1989). It is proposed that increased the enzyme activity would affect the efficiency of nutrient digestibility.

In the overall period, chicks in MCA and ORA groups have better growth performance than other groups. There were no significant differences in FCR in FOS group. In contrast, Xu et al. (2003) showed that 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 d. These results indicated that the effects of FOS on body weight and food intake might be affected by differences in the animal model, animal gender, experimental period, supplemental method, and dose and type of FOS used (Hsu et al., 2004).

In conclusion, the results of this study demonstrated that MCA, ORA and FOS supplemented in chicks were of beneficial in ameliorating *Salmonella* colonization. MCA supplementation was equally effective with organic acids in decreasing the levels of colonization in ceca and improved growth performance. The advantage of MCFA were reducing pH in crop and small intestines and increasing SCFA concentrations in ceca. Moreover, MCFA improved disaccharidase activity and digestibility of nutrients. They were well utilized by intestinal mucosa cells and could be found in portal vein. The growth performance and digestibility of nutrients were improved in chicks in ORA group compared to chicks in CON group. The beneficial effect of FOS in decreasing the levels of *Salmonella* colonization in ceca was inferior to MCA and ORA groups. Chicks in FOS group had an increased in disaccharidase activity and digestibility of nutrients compared chicks in CON group.

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