ผลของเยรูซาเลม อาร์ทิโช้ค (Helianthus tuberosus L.) เป็นสารทดแทนยาปฏิชีวนะ ต่อการเจริญเติบโต การเปลี่ยนแปลงทางกายภาพ และชีวภาพของลำไส้เล็กส่วนปลาย และ ลำไส้ใหญ่ในลูกสุกรหย่านม

นาย พิภพ สดสี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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EFFECTS OF JERUSALEM ARTICHOKE (HELIANTHUS TUBEROSUS L.), A REPLACEMENT OF ANTIBIOTIC, ON GROWTH PERFORMANCE PHYSICAL AND BIOLOGICAL CHANGES OF ILEUM AND LARGE INTESTINE IN WEANING PIGS

Mr. Phiphob Sodsee

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for the Degree of Master of Science in Animal Physiology

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Thesis Title	Effects of Jerusalem artichoke (*Helianthus tuberosus* L.), a replacement of antibiotic, on growth performance, physical and biological changes of ileum and large intestine in weaning pigs
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Field of Study	Animal physiology
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พิภพ สดสี : ผลของเยรูซาเลม อาร์ทิโช้ค (*Helianthus tuberosus* L.) เป็นสารทดแทนยาปฏิชีวนะ ต่อการเจริญเติบโต การเปลี่ยนแปลงทางกายภาพ และชีวภาพของลำไส้เล็กส่วนปลาย และ ลำไส้ใหญ่ในลูกสุกรหย่านม (EFFECTS OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS* L.), A REPLACEMENT OF ANTIBIOTIC, ON GROWTH PERFORMANCE PHYSICAL AND BIOLOGICAL CHANGES OF ILEUM AND LARGE INTESTINE IN WEANING PIGS.) : อาจารย์ที่ปรึกษา : ผศ.น.สพ.ดร. กฤษ อังคนาพร, อาจารย์ที่ปรึกษาร่วม : รศ.สุวรรณา กิจภากรณ์, 64 หน้า, ISBN 974-17-3868-4

การวิจัยในครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการใช้เยรูซาเลม อาร์ทิโช้ค (*Helianthus tuberosus* linn.) ทดแทนยาปฏิชีวนะในอาหารต่อคุณลักษณะการเจริญเติบโต และการเปลี่ยนแปลงทางกายภาพและชีวภาพของลำไส้ของลูกสุกรหย่านม โดยทำการทดลองในลูกสุกรหย่านมเพศผู้ที่ตอนแล้วจำนวน 40 ตัวและเพศเมียจำนวน 40 ตัว น้ำหนักเริ่มต้น 7.08$\pm$0.89 กิโลกรัม ลูกสุกรจะถูกแบ่งแบบสุ่มออกเป็น 5 กลุ่ม ตามอาหารทดลองซึ่งประกอบด้วย อาหารควบคุม อาหารผสม 3% เยรูซาเลมอาร์ทิโช้ค (3%อาร์ทิโช้ค) อาหารผสม 6%เยรูซาเลมอาร์ทิโช้ค (6%อาร์ทิโช้ค) อาหารผสม 1% ฟรุ๊กโตโอลิโกแซคาไรด์ (FOS) อาหารผสมยาปฏิชีวนะ (Antibiotic) ให้กินแบบไม่จำกัดเป็นเวลา 5 สัปดาห์ บันทึกน้ำหนักสุกร และอาหารที่ 2 และ 5 สัปดาห์ ในวันสุดท้ายของการทดลองสุกรจำนวน 1 ตัวต่อซ้ำจะถูกนำมาเก็บตัวอย่างลำไส้โดยแบ่งออกเป็น 5 ส่วนตามกายวิภาค เพื่อบันทึกความยาวและน้ำหนัก เก็บตัวอย่างอาหารในลำไส้และเนื้อเยื่อลำไส้เพื่อตรวจความเป็นกรด-ด่าง กรดไขมันสายสั้น ปริมาณ DNA และ RNA สุกรที่เหลือจะถูกใช้วัดหาอัตราการส่งผ่านอาหารโดยใช้ Cr_2O_3 เป็นตัวบ่งชี้ ผลการทดลองไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่มอาหารทดลองต่อคุณลักษณะการเจริญเติบโต และตัวชี้วัดอื่น ๆ ($P>0.05$) สุกรที่ได้รับอาหารควบคุมมีอัตราการตายสูงสุด และสุกรที่ได้รับอาหาร 3% อาร์ทิโช้ค มีแนวโน้มให้คุณลักษณะการเจริญเติบโตดีที่สุด ($P>0.05$) ปริมาณกรดไขมันสายสั้น และน้ำหนักสัมพัทธ์ของลำไส้ใหญ่ของสุกรที่ได้รับอาหาร 3% และ 6%อาร์ทิโช้คมีแนวโน้มสูงขึ้นไปในทางเดียวกัน สุกรที่ได้รับ FOS มีแนวโน้มทำให้ระยะเวลาการส่งผ่านของอาหารสั้นลง แต่ไม่มีผลต่อระยะเวลาการส่งผ่านอาหารในกลุ่มอื่นๆ ดังนั้นการใช้เยรูซาเลมอาร์ทิโช้คทดแทนยาปฏิชีวนะในอาหารมีแนวโน้มว่าจะส่งผลดีต่อคุณลักษณะการเจริญเติบโตได้ในสุกรหย่านมได้แต่ยังต้องการการศึกษาเพิ่มเติมหรือทำซ้ำอีกในอนาคต

ภาควิชา สรีรวิทยา	ลายมือชื่อนิสิต.....
สาขาวิชา สรีรวิทยาการสัตว์	ลายมือชื่ออาจารย์ที่ปรึกษา.....
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PHIPHOB SODSEE: EFFECTS OF JERUSALEM ARTICHOKE (HELIANTHUS TUBEROSUS L.), A REPLACEMENT OF ANTIBIOTIC, ON GROWTH PERFORMANCE, PHYSICAL AND BIOLOGICAL CHANGES OF ILEUM AND LARGE INTESTINE IN WEANING PIGS. ADVISOR: ASSISTANT PROFESSOR. KRIS ANGKANAPORN, THESIS COADVISOR: ASSOCIATE PROFESSOR. SUWANNA KIJPARKORN, [64] pp. ISBN 974-17-3868-4

The aim of this study was to evaluate the use of Jerusalem artichoke (*Helianthus tuberosus* linn.) as and antibiotic substituted in the diet on growth performance, both physically and biologically gastrointestinal changes in weaning pigs. Forty castrated male and forty female weaned crossbred pigs of 7.08$\pm$0.89 kg initial body weight were used in the experiment. Pigs were randomly allocated and fed according to 5 experimental diets arrangement, treatment diets were control (Control), 3%Jerusalem artichoke (3%artichoke), 6%Jerusalem artichoke (6%artichoke), 1% Fructooligosaccharide (FOS) and Antibiotic. All pigs were fed with corresponded diet in ad libitum basis for five weeks, and growth performances were determined at the 2^{nd} and 5^{th} week. At the end of study, intestines of euthanized pigs were anatomically separated into 5 sections. The length and weight of each intestinal section were measured. Content and tissue were collected for pH, short-chain fatty acids (SCFAs), DNA and RNA determinations. The remaining pigs were use for transit time determination by using Cr_2O_3. There was no significant difference in growth performance and all intestinal function parameters among all arranged diets ($P>0.05$). Pigs fed on the control diet had the highest mortality rate. The 3% artichoke group tended to have better growth performance ($P>0.05$). The higher relative wet weight in 3% and 6%artichoke groups were corresponded with higher total SCFAs concentration in the colon. Dietary supplementation of FOS reduced the transit time of feed in the GI tract while other diets did not have any effect. Incorporation of Jerusalem artichoke powder as an antibiotic substituted in the pig diets may be beneficial in production efficiency. Further studies on the use of Jerusalem artichoke as prebiotic are needed.

Department	Physiology	Student's signature…….
Field of study	Animal physiology	Advisor's signature…….
Academic year	2003	Coadvisor's signature……

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CHAPTER I
INTRODUCTION

The pig industry in Thailand is currently expanded with more pigs raised in industrial extensive farm than the small backyard one. In year 2002, there were approximately 6.7 million pigs and 20,193 pigs which were exported and make the profit about 16.10 million baht per year (Office of agricultural economics, 2003). There are a number of things of concern in raising pig for instants management, nutrition and disease control. At weaning period, piglets are very susceptible to digestive infection because maternal immunity markedly declined (Miller and Stokes, 1994). Moreover, at this time the function of gastrointestinal tract is not fully developed that makes piglets prone to be infected with some pathogenic bacteria and virus. Another period of pig production that is susceptible to digestive stress is piglet at the weight of 20-25 kg, which are subjected of moving into new house and feed. The stress that occurred could cause a serious health problem and could deteriorate the pig production (Wilson and Friendship, 1996).

Normally, piglets in the weaning period (6 weeks of age) have mortality rate less than 1%, but some farms the mortality rate rose up to 10%. In most cases, diarrhea is more frequency problem found. The infected pigs are stunt and exacerbate their feed efficiency. The raising period may be markedly longer than normal. In addition, pigs in this group would have abnormally high mortality caused by many pathogenic bacteria, such as *Escherichia coli*, *Clostridium perfringen* type C, *Serpulina hyodysenteriae* and *Lawsonia intracellularis*, etc (Fahey et al., 1990). To treat these infections, farmers often use antibiotics to overcome the pathogenic bacteria and increased the hygiene of housing system. Antibiotics are usually mixed in feed as the growth promoter. The purpose of using low level antibiotic in feed are to control the number of pathogenic bacteria and promote the growth of beneficial bacteria in the in the intestine. This could prevent or cure animal from mild infection (McNitt et al., 1996).

During this decade, people are concerned about the use of low level of antibiotic in feed. It can develop the drug resistant bacterial strain in animal. Once it happened, these animal bacteria could transfer its resistant gene to human bacteria, and finally some infected disease in human are more difficult to be cured compare to the past. So, scientists try to supplement many new products to substitute antibiotic for controlling pathogenic bacteria in the gastrointestinal tract. For example, probiotic, by using beneficial bacteria in feed that can regenerate in the gastrointestinal tract, could control the pathogenic bacteria in gastrointestinal tract of animal (Sanford, 1996). Another newly concept is prebiotic, for instance short-chain and fermentable dietary fiber, in order to increase the population of beneficial bacteria. These beneficial bacteria can keep the balance of all bacterial population and finally reduce population of pathogenic bacteria and improve the digestibility of feed in the gastrointestinal tract (Mateos and Blas, 1998). These two new concepts seem to be more acceptable than antibiotics that now seem continuously to be limited to be used in animal industries.

Jerusalem artichoke (*Helianthus tuberosus* L.) is one of the tuber plants that its tuber composes of non-digestible but fermentable oligosaccharides, inulin and oligofructose. It is very interesting to use them as feed additive in weaning pig and study its effect on some gastrointestinal functions. So, the hypothesis of the present study is that using of Jerusalem artichoke as feed additive could regulate physical and biological environment in distal small intestine and colon of piglets. The objectives of this experiment were:

1). To evaluate the effect of Jerusalem artichoke as feed additive on growth performance of weaning pigs.

2). To evaluate the effect of Jerusalem artichoke as feed additive on gastrointestinal transit time, short-chain fatty acids (SCFAs) concentration and pH of intestinal content in ileum, caecum and colon of weaning pigs.

3) To evaluate the effect of Jerusalem artichoke as feed additive on intestinal weight and DNA and RNA content of mucosal tissue in ileum, caecum and colon of weaning pigs.

CHAPTER II
BACKGROUND INFORMATION

Functional Food

The traditional role of diet is to provide enough nutrients to meet the requirements of a balance diet, while giving the consumer a feeling of satisfaction and well-being (Robertfroid, 1998). Nowadays, the knowledge in the bioscience supports the hypothesis that diet can control and modulate various functions in the body. In addition, it also contributes to a state of good health and reduces the risk of some diseases, so these bring to the concept of “functional food”. Robertfroid (1996) give the definition of a functional food as “Food which contains (in adequate concentration) one or a combination of components which affects functions in the body”. International food information council foundation (IFIC) gives the glossary term of functional food as “Foods that may provide health benefits beyond basic nutrition”. The abilities of these functional components may reduce risk cancer, aid in digestion, decrease risk of tooth decay or improve various other body functions or reduce disease risk. In the animal production industry, animal nutritionist and scientist also use functional food concept to promote the growth and reduce some certain disease. This reduces the use of chemical and antibiotic agents in livestock. One of the most interesting potentially functional food is prebiotic. Jenkins et al. (1999) have shown some colonic and systemic physiological effect of using non digestible oligosaccharides as prebiotic in table1 with mention of how these physiologic functions relate to disorder in table 2.

Prebiotic

One of the functional food that was extensively studied is “Prebiotic”. Gibson and Robertfroid (1995) gave the definition of prebiotic as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon”. Many of nondigestible fibers have been shown to benefit the hosts by selectively stimulating the activity of the residence bacteria already in

the colon. Prebiotic fermentation should be directed towards potentially health promoting bacteria, with indigenous Lactobacilli and Bifidobacteria being preferred target organisms (Gibson *et al*., 1999).

Currently, there are many desirable properties of prebiotics that have been listed. Gibson *et al*. (1999) have identified some characteristics of them: active at nutritionally feasible dose (it is appropriate that this is as low as possible), lack of side effects, fine control of microflora modulation, persistence throughout the colon and inhibit adhesion of pathogens. At present, food components which seem to exert best prebiotic effect are the nondigestible oligosaccharides.

Table1 Potential effects of nondigestible oligosaccharides: physiological effect*

Local	Systemic
↑ Fecal bulk	↓ , (↑) Cholesterol
↑Selective bacteria	↓TG (↓insulin : ↓Glucose)
↑ SCFAs production	↓ NH_3
↑ Mineral absorption	↓ Urea
↑ vitamin B synthesis	↑ vitamin B
	↑ Immune function
	(↑ Glutamine ?)

* Abbreviation: SCFA, short-chain fatty acid: TG, triglyceride.

Reference: Jenkins et al. (1999)

Table 2 Potential effects of nondigestible oligosaccharides: disease prevention or therapy.

Local	Systemic
Ulcerative colitis	Coronary heart disease-hyperlipidemia
Colon cancer	Uremia
Constipation	Hepatic encephlopathy
	Cancer risk
	Osteoporosis

Reference: Jenkins et al. (1999)

Nondigestible Oligosaccharide

Oligosaccharides are carbohydrate with a low degree of polymerization (DP) and molecular weight (Yun. 1996). They are oligomeric carbohydrates, the osidic bond of which is in a spatial configuration that allows resistance to hydrolytic activities of intestinal digestive enzymes, but are sensitive to metabolic effects of colonic bacteria. These microorganisms can ferment the carbohydrate to produce short-chain carboxylic acids and gases, as well as increase metabolic energy, growth and proliferation (van Loo et al., 1998). Some general categories of oligosaccharides include fructooligosaccharides (FOS), inulo-oligosaccharide, glucooligosaccharide, galactooligosaccharide, isomaltooligosaccharides, polydextrose, soy oligosaccharides and xylooligosaccharides. Some oligosaccharides are prepared naturally from chicory, Jerusalem artichoke and soy (Slavin, 1999). In the case of inulin and FOS, they compose of β-D-fructofuranoses attached by β-D-glucopyranosyl or β-D-fructopyranosyl residues. They constitute a group of oligosaccharides derived from sucrose that are isolated from natural vegetable sources. Generally the product with DP from 2-60 is labeled as inulin (Rafftiline), whereas FOS is define as DP<10 (Robertfroid, 1999). The Molecular structure of the FOS is shown in Figure 1.

Oligosaccharides are readily water-soluble and exhibit some sweetness. Fructooligosaccharide (FOS) have been described as 0.4-0.6 times as sweet as sucrose (Robertfroid, 1993). Sweetness decreases with longer chain-length. Inulin with a DP higher than 20 does not have a sweet taste. Depending up on chain length and composition, oligosaccharides may contribute functional benefits to the diet, such as water binding, gel forming and fat replacement value. Since oligosaccharides are not digested and absorbed in the small intestine, they have no caloric value in the traditional sense. It is thought that oligosaccharides have a caloric contribution of about 1.5 kcal/g due to colonic fermentation, which is similar to that of soluble dietary fiber (Slavin, 1999).

Oligosaccharides have many interesting physiological effects that attracted scientific interest. Since the animal body handles them in a similar manner to dietary fiber, oligosaccharides may share some of their desirable properties. The desirable characteristics of prebiotic with respect to the structure of oligosaccharides are shown in table 3. *In vitro* study, oligofructose and inulin selectively stimulate the growth of

Bifidobacterium spp. and *Lactobacillus spp.,* genus of bacteria considered beneficial to health (Wang and Gibson, 1993). *In vivo* study on human, oligofructose and inulin significantly increased *Bifidobacteria spp.* from 6% to 22% and decreased *Bacteroides spp.*, *Clostidium spp.* and *Fusobactrium spp.* (Gibson et al., 1995). The oligosaccharides also had several effects on the physical and biological changes in the gastrointestinal tract. Campbell et al. (1997) reported the using of oligofructose, fructooligosaccharide and xylooligosaccharide in rat diet increased the intestinal weight and SCFAs concentration compared with crystalline cellulose. Supplementation of FOS in neonatal pigs diet increased the density and labeled mucosal cells in caecum, proximal and distal colon (Howard, 1995). Since these microbial and physiological regulations of intestine and SCFAs concentration-increasing effect of oligosaccharides exert them as one of the best prebiotic.

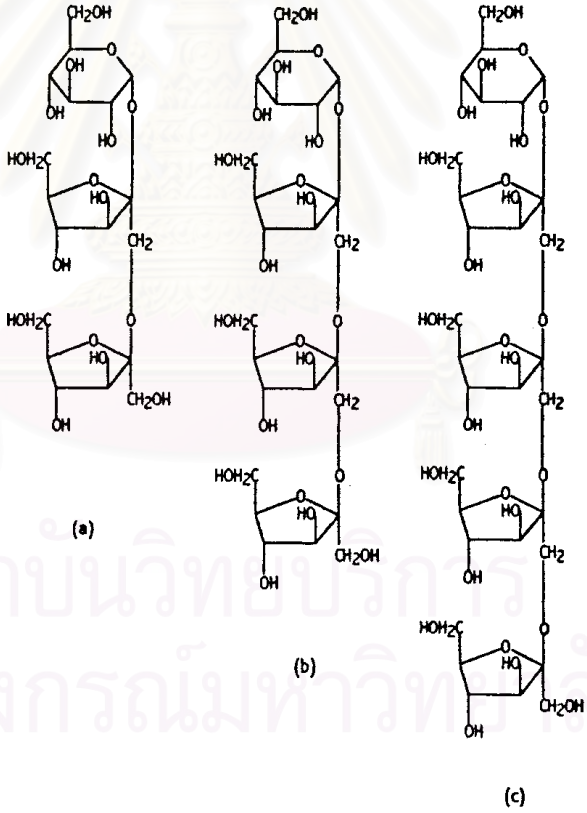

Figure 1. Molecular structures of the FOS. (a) 1-kestose (1-kestotriose, GF2); (b) nystose (1,1- kestotetraose, GF3); (c) 1-β-fructofuranosylnystose (1,1,1-kestopentose, GF4). (Hogarth et al.,2000)

Table 3 Desirable properties of prebiotic oligosaccharides

Desirable attribute	Properties of oligosaccharides
Active at nutritionally feasible dose (it is Appropriate that this is as low as possible)	Selective stimulation and proliferation of "beneficial" bacteria e.g. Bifidobacteria, Lactobacilli, in a complex microbial ecosystem.
Lack of side effects	No stimulation of gas producers, putrefactive microorganisms, pathogens, etc. in a complex microbiota.
Fine control of microflora modulation	Selective fermentation in mixed culture
Persistence throughout the colon, i.e. towards distal areas	High molecular weight, slow fermentation
Varying viscosity	Available in different molecular weights and linkages
Good storage and processing stability	Possess 1-6 linkages and pyranosyl sugar ring
Differing sweetness	Varied monosaccharide composition
Inhibit adhesion of pathogens	Possess receptor sequence

Reference: Gibson *et al*. (1999).

Short-chain Fatty Acids (SCFAs)

Short-chain Fatty Acids (SCFAs), were originally described as "volatile fatty acids (VFA)" because they were measured by steam-distillation, after acidification of intestinal contents. Now steam-distillation has been superseded by gas-liquid chromatography, this terminology has been largely abandoned, and they are usually called "short-chain fatty acids (SCFAs)" (Wrong, 1995). In practice, the term "SCFAs" is usually applied in a restricted sense to acetic, propionic and n-butyric acids, the three organic acids those are most abundant and generated by microbial fermentation within the digestive tract.

SCFAs that are produced by bacterial metabolism of unabsorbed carbohydrates in the mammalian colon provide the predominant anions in the colonic lumen (Charney et al., 1998). The pKa values of some SCFAs are shown in Table 4. The proportional concentration of three majors SCFAs are 60-75% for acetic acid, 15-25% for propionic acid and 10-15% for butyric acid (Charney et al., 1998). The total concentration of SCFAs are different in each species for example, 106 mM in rumen of sheep, 74 mM in caecum of rabbit, 210 mM in colon of pig and 124 mM in colon of human (Bergman, 1990). Their concentrations in pigs have been shown to increase as the proportion of fiber reaching the hindgut increases (Kass et al., 1980). Breves and Stuck (1995) have reported SCFAs concentration in hindgut of some animal species as shown in Table 5.

Table 4 **pKa values of some SCFAs**

SCFAs	Rational formula	pKa
Acetic (Ethanoic)	CH_3COOH	4.56
Propionic (propanoic)	C_2H_5COOH	4.67
n-Butyric (Butanoic)	C_3H_7COOH	4.63
n-Valeric (Pentanoic)	C_4H_9COOH	4.64

Reference: Fukashima, (1995)

SCFAs in gastrointestinal tract showed several beneficial evidences to human and animal. In primary ileal and colonic cell culture, 0.05-0.5 mM SCFAs increased the proliferation and increased the expression of actin and myosin (Le Blay et al., 2000). *In vitro* studies, Ichikawa and Sakata (1998) demonstrated the effect of SCFAs in rat intestine on the increase of colonocyte proliferation rate. *In vivo* study, SCFAs instilled into atrophic and defunctioned rat colon for 14 days can increase colonic wet weight compared with those of the placebo group (Kissmeyer-Nielsen et al., 1995). 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. In addition, SCFAs also demonstrated the effects on transit time. *In vitro* study using isolated rat colon, SCFAs can

inhibit the contractions rate of proximal, mid and distal regions (Squires et al., 1992). Cherbut et al. (1997) suggested that SCFAs may be one of the mediators involves in the lleo-colonic break in the gastrointestinal tract. There were still several unclear biological effects of SCFAs that were not reviewed. So, SCFAs are the interesting fermentative product that needed further study on physiological function both in human and animals.

Table 5 SCFAs concentration in the intestinal tract of dogs, pigs, large ungulates and primates.

Animal	Bowel segment	SCFA concentration (mM)	SCFA proportions (%) (acetate:propionate:butyrate)
Dog	Caecum/colon	140	-
Pig	Caecum	100-140	52:38:10
	Proximal colon	80-130	53:34:10
	Distal colon	20-65	66:24:11
Equine	Caecum	118	85:10:3
	Colon	115	-
Elephant	Caecum	138	63:20:15
	Colon	65-148	72:16:9
Rhinoceros	Caecum	144	79:14:6
	Colon	53-81	70:16:11
Hippopotamus	Colon	28-35	75:16:5
Baboon	Caecum	160	-
	Colon	90-150	-
Sykes monkey	Caecum	160	-
	Colon	130	-

Reference: Breves and stuck (1995)

Production of Short-chain Fatty Acids

The microbial fermentation within the digestive tract occurs when carbohydrate material enters the rumen or colon and they are attacked by hydrolytic microbial enzyme. In the case of insoluble carbohydrates, attack requires the physical attachment of bacteria to the surface of the plant particle, the enzymes themselves being part of the surface coating of the bacteria. Enzymatic action liberates glucose, other monosaccharides, and short-chain polysaccharides into the fluid phase, outside the microbial cell bodies. Although free in solution, these products of microbial enzyme action do not become immediately available to the host animal; rather, they are quickly subjected to further metabolism by the microbial mass. Glucose and other sugars are absorbed into the cell bodies of the microbes. Once into the microbial cells, glucose enters the glycolytic or Embden-Meyerhof pathway. This is the same glycolytic pathway that exists in mammalian cells, and as in mammalian tissues, catabolism of glucose through this pathway yields two molecules of pyruvate for each molecule of glucose. In this process, two molecules of oxidized nicotinamide adenine dinucleotide (NAD) are reduced to NAD hydrogen (NADH), and the molecules of adenosine triphosphate (ATP) are formed from adenosine diphosphate (ADP). The potential energy represented by the ATP formed in this reaction is not directly available to the host animal but is the major source of energy for maintenance and growth of microbes (Herdt, 1997).

If fermentative digestion were to occur under aerobic conditions, which it does not, the pyruvate produced by the glycolytic process would enter the citric acid (Kreb's) cycle and be metabolized to carbondioxide and water, as occur under the aerobic conditions of mammalian cells. Furthermore, in an aerobic system the NADH produced would be oxidized in the cytochrome oxidase system with additional production of ATP and the regeneration of NAD. But fermentative digestion is actually not an aerobic system; on contrary, it proceeds in a reductive, highly anaerobic environment. Therefore, a different mechanism must be provided for the oxidation of NADH and other reduced cofactors such as flavin adenine dinucleotide hydrogen ($FADH_2$). If such a mechanism were not available, all oxidized cofactors present would be reduced and metabolism would come to a halt. Because no atmospheric oxygen is available, some other compound may serve as an electron sink for the oxidation of enzyme cofactors (Herdt, 1997).

In fermentative digestion, pyruvate can act as an electron sink, being further reduced to provide for regeneration of NAD and the general removal of excess electrons, with an additional yield of ATP. In addition, carbon dioxide can be reduced to methane, accepting electrons for the regeneration of NAD and flavin adenine dinucleotide (FAD). The metabolic pathways of these reactions are illustrated in figure 2. These pathways lead to the major end products of the fermentative digestion of carbohydrate, the short-chain fatty acids (SCFAs) (Herdt, 1997).

Short-chain Fatty Acids as a Quantitative Indicator of Microbial Fermentation

Upon measurements of SCFA concentrations in hindgut contents, a quantitative estimate of the rate of microbial fermentation has often been attempted. Although increased SCFA concentrations were found in response to an increased intake of fiber. In a number of experimental studies, quantitative changes in the rate of microbial fermentation do not necessarily result in alterations of SCFA concentrations. This is mainly due to the fact that the determination of the actual SCFA concentration is influenced not only by the production rate, but also by changes in the rate of absorption or the distribution volume, i.e. the fluid volume of hindgut contents. It has been demonstrated in different studies in pigs that fluid volumes within the upper hindgut significantly increase when the percentage of dietary cellulose or crude fiber enhances. Therefore, it must be concluded that the SCFA production rate has to be measured directly in order to quantify the rate of microbial fermentation.

Different experiment techniques have been introduced to measure SCFA production rate quantitatively under in *vivo* conditions and most of these methods have been established in ruminants. In the rumen, both single injection and the continuous infusion technique of either radioactively or ^{13}C-labelled SCFA have been applied to calculate SCFA production rate. These methods have also been applied for measuring SCFA production was used in the large intestine of rabbits, ponies and pigs. With ^{13}C-labelled acetate in pig model, the mean acetate production rate was found to increase from 27.4 mmol/h to 56.2 mmol/h when the dietary crude fiber content was raised from 5.1% to 18.3%. Similar changes were recorded for propionate by the application of

^{13}C-labelled propionate. The major advantage of the continuous technique is that the rate of interconversion between the individual SCFA can be determined (Breves and Stuck, 1995).

Jerusalem Artichoke

Jerusalem artichoke is classified in the family of Asteraceae, Genus of Helianthus L. The scientific name is *Helianthus tuberosus* Linn. (Natural resources conservation service-USDA, 1998). Jerusalem artichoke is in common with its close relative, the sunflower. This plant is a native species of North America. Jerusalem artichoke have been grown in small areas for many years in the USA and Europe to produce tubers, sold as a root vegetable (Biological for non food product, 2002). It was cultivated pre-Columbian times in the north-eastern United States up to the middle of the 18th century when it was superseded by the potato. The plant is an annual herb with stems 1-3m tall, with vegetation similar to sunflower but different by stem tubers. The upper stems are multi branched and slender. Leaves are opposite, ovate and often coarsely toothed, prominently veined with broad winged stalks. The flower heads (when produced) are much smaller than sunflower, being only 4-8cm in diameter with yellow disc, and ray florets, and are carried individually on branch stems. The whitish/yellow tubers, formed late in the season are numerous, up to 12 x 6cm long, very irregular and knobbly in unselected forms but almost smooth in others, and with crisp flesh (Interactive European Network for Industrial Crops and their Applications, 2002).

The tubers contain a high proportion of the carbohydrate inulin, a polymer of fructose; the high-fructose syrups derived from the tubers may be used in the food industry and also for the production of ethanol and other industrial raw materials. Duke (1997) have reported that Jerusalem artichokes contain about 80% water, the remainder of the dry matter basis made up of about 15% protein, 1% fat, 75% nitrogen-free extract with 60% inulin, 4% fiber and 5% ash. Interactive European Network for Industrial Crops and their Applications (2002) report that the tubers contain 13-18% carbohydrates, of which nearly 80% are the carbohydrate inulin, a natural polymer of fructose which cannot be digested by the gut. From its composition, some researchers conducted the experiment

on using Jerusalem artichoke as prebiotic in feed. Farnworth et al. (1995) reported the incorporation of 3% Jerusalem artichoke in pigs feed could significantly increase fecal SCFAs concentration. Moreover, the smell of fresh manure from pigs fed 3% or 6% Jerusalem artichoke was significantly sweeter, less sharp and pungent, and had less skatole smell than the manure from pigs fed on the control diet (Farnworth et al., 1995). So, Jerusalem artichoke might potentially be developed for using as prebiotic in feed of the animals.

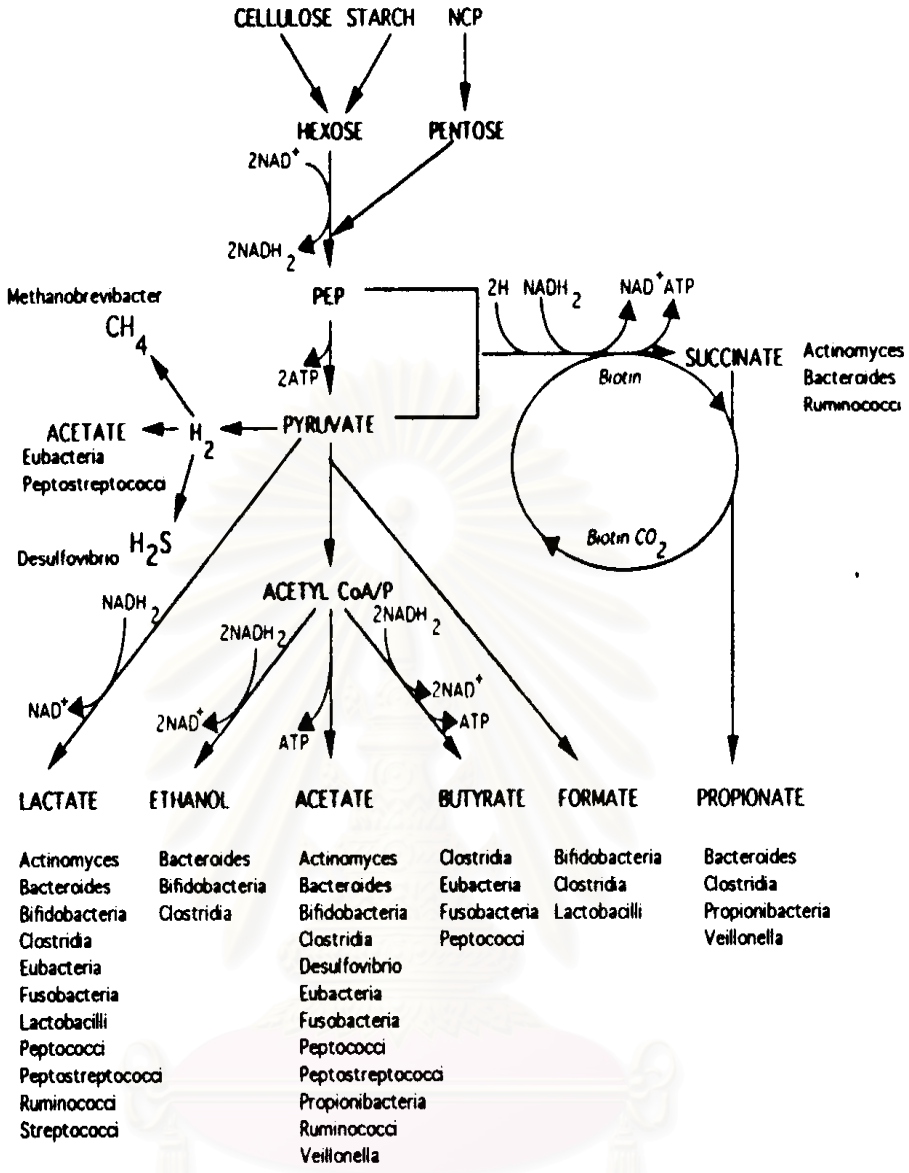

Figure 2. Overview of major pathways of carbohydrate metabolism in the large intestine and the principal fermentation products formed by individual groups of anaerobic bacteria. NCP (non-cellulolytic polysaccharides) include pectins and hemicelluloses. Propionate is shown as being produced by the succinate pathway, since few species in the human colon form this metabolite using the acrylate pathway. (Macfarlane and Gibsin, 1995)

CHAPTER III
MATERIALS AND METHODS

Animals

Forty castrated male and forty female weaned crossbred pigs (Yorkshire X Landrace X Duroc) of 7.08 $\pm$ 0.89 kg initial body weight were used in the experiment. Following an acclimated period of 7 days, all animals were randomly allocated into 5 treatment diets which comprised of 4 replications of 4 pigs each. Each treatment was composed of 2 replicates of male and 2 replicates of female as diagram shown in Figure3.

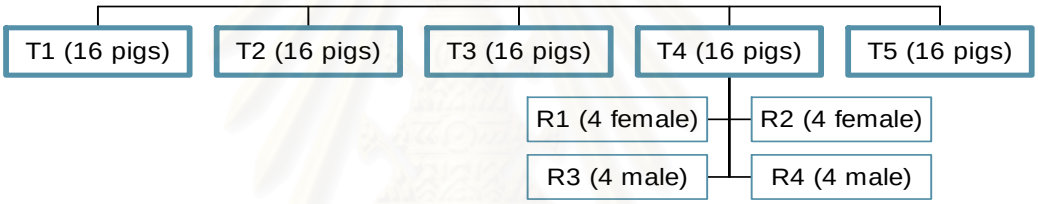

Figure 3 Diagram of the experimental design

Jerusalem Artichoke Flour Preparation

The fresh tuber of Jerusalem artichoke from the same harvest season was kept in stored room at the temperature of 2-5 °C until drying process. The drying process composed of several steps. Firstly, the fresh tuber were cleaned and chopped by the slicing machine. After that, the tuber in the small pieces was brought to the oven at the temperature of 60 °C for 36 hours. Next, the dry tuber was grounded using the cutting mill at the sieve diameter of 2 millimeter. Finally, the dry flour of Jerusalem artichoke was mixed again in the feed mixer and kept at –20 °C until use.

Feed and Feeding

There were five treatment diets in this experiment (Table 6). The control diet (T1), was the basal diet with the ingredient shown in Table 7. Jerusalem artichoke diet (T2 and T3), contained jerusalem artichoke powder at 3% of diet (3%artichoke) for T2 and 6% of diet (6%articoke) for T3 (substitute for the equal percentage of broken rice that was withdrawn). Fructooligosaccharide diet (FOS; Raftilose® P95) (T4), contained FOS at 1% of the diet. Antibiotic diet (T5), contained chlortetracycline (feed grade) at 110 ppm of the diet. Diet ingredients and chemical composition of the experimental diets are shown in Table7 and 8. All treatment diets were calculated to reach the nutrient requirement of NRC (1994) and were isocaloric and isonitrogenous. The pigs were fed their respective diets for a period of 5 weeks. Diets and water were given ad libitum.

Table 6 The description of the treatments in the experiment

Treatment	Description
T1	Control diet
T2	3% Jerusalem artichoke (3%articoke)
T3	6% Jerusalem artichoke (6%articoke)
T4	1% Fructooligosaccharide (FOS)
T5	Antibiotic diet

Data Collection

Live body weight and feed intake were recorded at the 2nd and the 5th week of experimental period. Number of sick and death pigs, cadaver weight, temperature, relative humidity and any remarked environmental conditions were recorded on daily basis. The diagram of experimental procedure is shown in Figure 4.

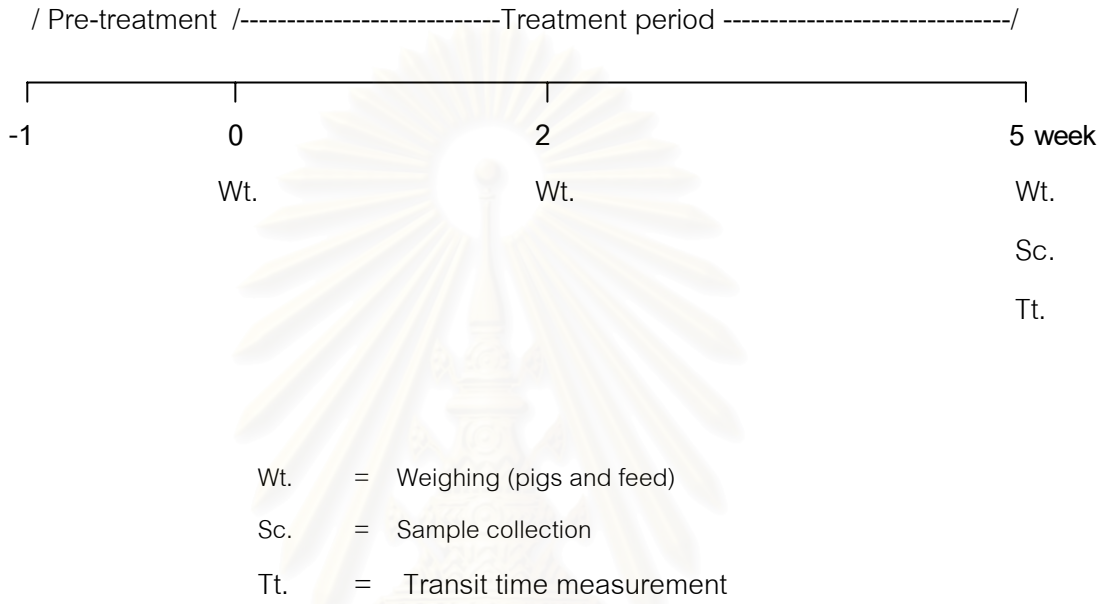

Figure 4 Experimental period diagram

Sample Collection and Tissue Preparation

At the 5th week of experimental period, one pig in each replicate of all treatment groups was randomly selected and euthanized by overdose intravenous injection of pentobarbital sodium. Immediately after slaughter, the whole intestine was removed and separated by ligature into 5 sections. These comprised the distal jejunum (30 cm upper from ileum), the ileum (from ileocecal junction to 2 cm anterior of ileocecal ligament attachment), the cecum, the two sections of colon (one is descending and the other is ascending colon). After ligation, all separated sections of intestine were removed from each other. All sections except jejunum were weighed and the length of each intestinal section were measured. Digesta samples from all intestinal sections were collected into containers by gently squeezing with thumb and fingers for pH measurement using a

semisolid glass electrode probe pH meter. Digesta samples were kept frozen and stored at –20 °C until analysis. After digesta drained and perfused with tap water, the emptied intestines were weighed again. Subsequently, the intestinal sections were longitudinally opened and fixed on foam plate. The tissue was collected in equal area for each sample by pressing a circular metal block (1 cm in diameter) on the tissue, then the tissue weight and tissue weight without fat were determined. The mucosal samples were scraped using glass slide and kept in folded foil paper and stored at –70 °C until the analysis for DNA and RNA content were performed.

The Calculation of Growth Performance

The feed intake and individual live body weight data collected at the 2nd and 5th week of the experiment were used to calculated the average daily gain (ADG), average feed intake (AFI) and feed/gain (FCR) in each replicate. The formula is shown below.

$$\text{Average daily gain (ADG, kg/day)} = \frac{\text{Final BW} - \text{Initial BW}}{\text{Days}}$$

$$\text{Average feed intake (AFI, kg/day)} = \frac{\text{Feed intake}}{\text{Days}}$$

$$\text{Feed/Gain (FCR)} = \frac{\text{AFI}}{\text{ADG}}$$

Short-chain Fatty Acids (SCFAs) Determination

Caecal short-chain fatty acid concentrations were analyzed using the method modified 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 removed for the SCFAs determination. Standard SCFAs solution was prepared and there were four SCFAs, 70 rnM 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 and the supernatant aliquots were removed. The aliquots were analyzed for the concentration of SCFAs using a gas chromatograph equipped with a hydrogen flame ionization detector. The column used for analysis (GL Sciences Inc) was treated with 1% (wt/wt) H_3PO_4 (length 2.1 m, ID 4 mm, OD 7 mm) and packed with 10% FFAP (80-100 mesh). The concentration of individual SCFA was expressed as μ mole/g caecal content.

The Determination of Ribonucleic Acid (RNA) and Deoxyribonucleic Acid (DNA) Contents

Frozen mucosal scrapings were homogenized with 0.2 N perchloric acid (PCA) and all homogenized samples were centrifuged at 500g for 10 minutes. The precipitate of both RNA and DNA fractions were washed with cold 0.2N PCA and recentrifuged. The precipitation was solubilized for RNA in 3.0 ml of 0.3N potassium hydrochloride (KOH) and placed at 37 °C for 90 minutes. Two milliliter of 10%PCA were added into the tube and placed on ice for 10 minutes and centrifuged as described above (Berseth et al, 1983 ; Simmen et al, 1990). The supernatant was separated for the RNA determination using ultraviolet absorption measurement (Flek and Begg, 1954) by two wavelengths (λ) of 260 and 232 nm (wavelengths of maximal and minimal absorption of RNA). The content of pigs intestinal mucosal RNA was calculated from the following formula:

$$C_{RNA} = 3.40\ A_{260nm} - 1.44A_{232nm}$$

DNA fraction in the intestinal pellet was solubilized in 10% PCA and heated to 70 °C for 20 minutes. The DNA content of the samples was determinded by the Burton procedure as modified by Giles and Myers (1965). Two milliliter of 4% diphenylamine in glacial acetic acid was added to 2 ml of the DNA solution followed by 0.1 ml of aqueous 1.6 mg/ml of acetaldehyde. After incubation at 30 °C overnight, the optical density difference at λ 595-700 nm were read against blank in the without DNA solution.

Transit time Determination

At the end of the experiment, each treatment group (2 pigs/replicate) received the same diet supplemented with 10 g/kg Cr_2O_3 as an indigestible marker. These Cr_2O_3 containing diets were given to pigs once in the afternoon. After Cr_2O_3 diet feeding, fecal color of pigs was observed every 30 minutes by two observers. The transit time was measured by the length of time from the beginning of Cr_2O_3 diet feeding until the green color of Cr_2O_3 in the feces was observed.

Feed Chemical Analytical Procedure

All feed samples were analyzed in duplicate. Dry matter content in diets was determined at 103°C. Nitrogen content was determined by the Kjeldahl method and Crude protein was calculated as Kjeldahl N x 6.25. Crude Fat was determined by Ether extract procedure (AOAC, 1995).

Tissue Weight and Defatted Tissue Weight

Tissues removed from all 5 intestinal sections (area approximately 0.78 cm^2) were weighed again. Then they were dried in an oven at 80 °C until the weight was constant. Next, fat in dried intestine samples was extracted by ether for 48 hours. After all fat was extracted, tissues were put in the oven again. Finally, no-fat-dried weight of intestinal tissue was recorded.

Statistical Analysis.

The data was analyzed by the Analysis of Variance procedure for complete randomized design (CRD) and the different means were compared using Duncan's new multiple range test with the significant level at $P < 0.05$ (Steel and Torrie, 1960).

CHAPTER IV
RESULTS

Growth Performance

The average temperature and relative humidity of the entire experimental period were 26.38 - 32.15 °C and 59 - 87 %, respectively. At the end of the 2nd week of the experiment, pigs received antibiotic diet had slightly better growth performance than other diets (P>0.05). The pigs in the control diet and 3% artichoke diet had higher mortality rate (18.75±23.94 and 6.25±12.50, respectively) than other diets. At the 5th week of the experiment, pig fed on 3% artichoke diet had better FCR and ADG. Finally, for the overall 5 weeks of experiment, the 3% artichoke diet had the best growth performance but it was not significant compared to the other diets. The data are demonstrated in Tables 9 -11. For the transit time, there was no significant difference among all treatment diets (Figure 5).

Physical Changes of the Intestine

All treatment diets did not affect the pH of intestinal content in all intestinal part compared to those in control diet (Table 12). For relative wet intestinal weight with content to body weight and relative emptied intestinal wet weight to body weight, 3% artichoke diet tended to be heavier than those of other diets (P>0.05) (Figure 6 and Figure 7). The effect is more prominent in the proximal colon. In addition, there was no significant difference on the length and defatted-dry weight of the intestine (Figure 8 and Table 13).

Table7 Composition of the experimental diet

Ingredient	Composition, Kg
Broken rice	513.10
Palm oil	30.00
Soybean meal (44%)	251.00
Full fat soybean	84.00
Fish meal (61%)	40.00
Whey powder	50.00
Calcium carbonate	9.00
Mono-dicalcium phosphate	11.000
Salt	3.00
DL-Methionine	0.50
L-Lysine	0.05
Threonine	0.08
Premix*	8.27
Total Batch	**1000.00**

* Premix / Kg feed contained A 12,000 IU,D3 2,400 IU,E 18 mg, K3 3 mg, B1 1.2 mg, B2 3.6 mg, B6 1.8 mg, B12 0.018 mg, Nicotinic acid 24 mg, D-Calcium pantothenate 16 mg, Folic acid 0.6 mg, Biotin 0.1 mg, Choline chloride 300 mg, Mn 42 mg, Zn 120 mg, Fe 100 mg, Cu 1500 mg, I 1.5 mg, Co 0.84 mg and Se 0.2 mg

Table 8 Chemical feed analysis, Fed basis

Nutrients	Artichoke	Control	3%Artichoke	6%Artichoke	FOS	Antibiotic
Moisture, %	3.80	7.20	7.04	7.03	7.12	7.19
Crude protein, %	7.79	20.42	20.58	20.49	20.44	20.51
Crude fat, %	0.27	4.10	4.17	4.39	4.47	4.51
Crude fiber %	3.10	0.99	1.10	1.13	1.20	1.21
Ash %	4.47	6.18	6.10	6.17	6.15	6.22
Calcium %	0.11	0.83	0.85	0.84	0.88	0.82
Phosphorus %	0.2	0.98	0.93	0.93	0.92	0.95

Table 9 Growth performance of pigs at the 0 - 2nd week of the experiment (Mean ± SD)

Parameter	Treatment				
	Control	3% Artichoke	6% Artichoke	FOS	Antibiotic
Av.starting weight (kg)	6.75±0.79	7.25±1.27	6.99±0.76	7.20±0.99	7.18±1.20
Av.final weight (kg)	10.22±1.21	9.96±1.12	10.08±1.78	9.96±2.07	10.56±1.30
ADG (kg/pig/day)	0.25±0.06	0.24±0.04	0.23±0.08	0.20±0.08	0.24±0.04
Av. Feed intake (kg/pig/day)	0.35±0.05	0.35±0.09	0.33±0.06	0.28±0.10	0.32±0.05
FCR	1.41±0.17	1.47±0.14	1.48±0.36	1.47±0.09	1.36±0.17
Mortality (%)	18.75±23.94	6.25±12.50	0	0	0

Means within a row without superscript does not differ (P>0.05)

Table 10 Growth performance of pigs at the 2nd – 5th week of the experiment (Mean±SD)

Parameter	Treatment				
	Control	3% Artichoke	6% Artichoke	FOS	Antibiotic
Av.starting weight (kg)	10.23±1.21	9.96±1.12	10.08±1.79	9.96±2.07	10.56±1.30
Av.final weight (kg)	18.73±2.62	20.25±3.79	19.95±1.45	17.86±3.50	20.43±1.65
ADG (kg/pig/day)	0.41±0.07	0.45±0.09	0.44±0.03	0.38±0.08	0.43±0.03
Av. Feed intake (kg/pig/day)	0.73±0.04	0.68±0.17	0.72±0.06	0.62±0.12	0.69±0.01
FCR	1.85±0.31	1.50±0.14	1.65±0.12	1.64±0.06	1.61±0.05
Mortality (%)	0	0	0	0	6.25±12.50

Means within a row without superscript does not differ (P>0.05)

Table 11 Growth performance of pigs at the 0 – 5th week of the experiment (Mean±SD)

	Treatment				
Parameter	Control	3% Artichoke	6% Artichoke	FOS	Antibiotic
Av.started weight (kg)	6.75±0.79	7.25±1.27	6.99±0.76	7.20±0.99	7.18±1.20
Av.final weight (kg)	18.73±2.62	20.25±3.79	19.95±1.45	17.86±3.50	20.43±1.65
ADG (kg/pig/day)	0.34±0.06	0.37±0.08	0.37±0.03	0.30±0.07	0.38±0.03
Av. Feed intake (kg/pig/day)	0.58±0.04	0.55±0.14	0.57±0.06	0.48±0.11	0.57±0.03
FCR	1.72±0.26	1.47±0.09	1.52±0.10	1.59±0.04	1.50±0.07
Mortality (%)	18.75±23.94	6.25±12.50	0	0	6.25±12.50

Means within a row without superscript does not differ (P>0.05)

Table 12. The effect of treatments on pH of the intestinal content (Mean ± SD)

	Treatment				
Intestinal part	Control	3% Artichoke	6% Artichoke	FOS	Antibiotic
Jejunum	5.66±0.45	6.33±0.27	5.87±0.63	6.13±0.87	6.22±0.29
Ileum	6.33±0.60	6.71±0.33	5.98±0.59	6.37±0.90	6.50±0.32
Caecum	5.69±0.22	5.68±0.31	5.41±0.21	5.78±0.28	5.69±0.10
Proximal Colon	5.79±0.40	5.98±0.41	6.15±0.47	6.47±0.27	6.14±0.29
Distal Colon	6.36±0.49	6.36±0.40	6.30±0.41	6.23±0.19	6.52±0.17

Means within a row without superscript does not differ (P>0.05)

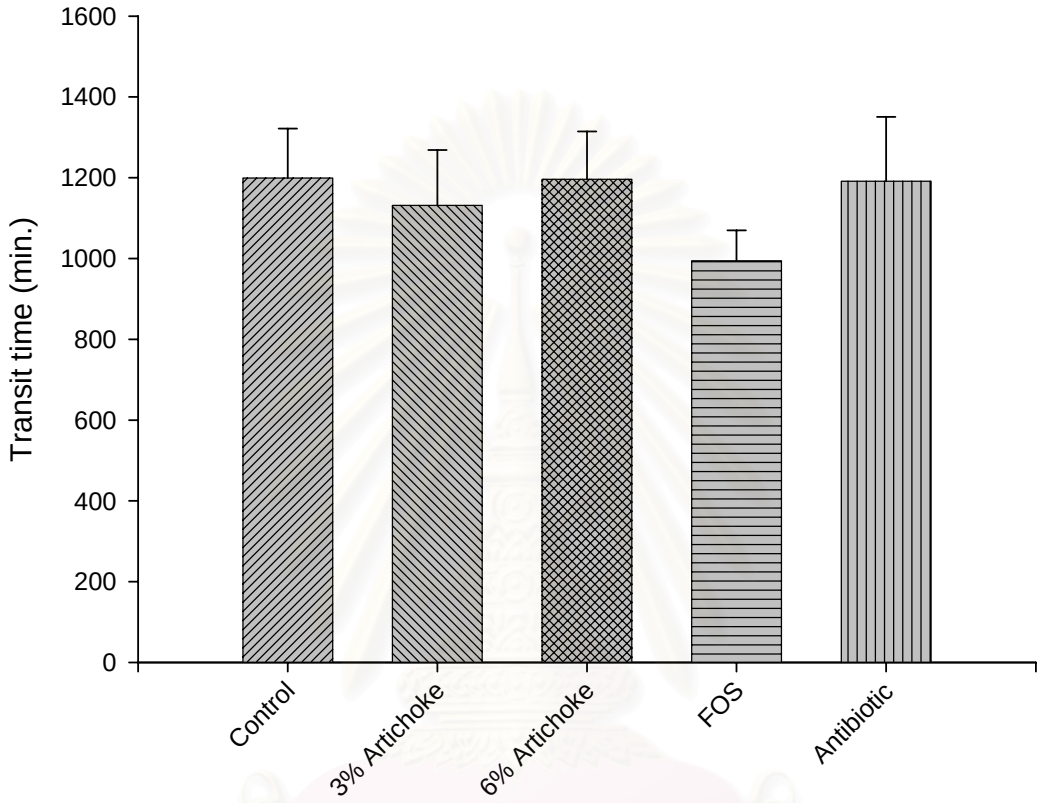

Figure 5 The effect of treatments on transit time at 5 weeks of the experimental period using Cr_2O_3 as an indigestible marker

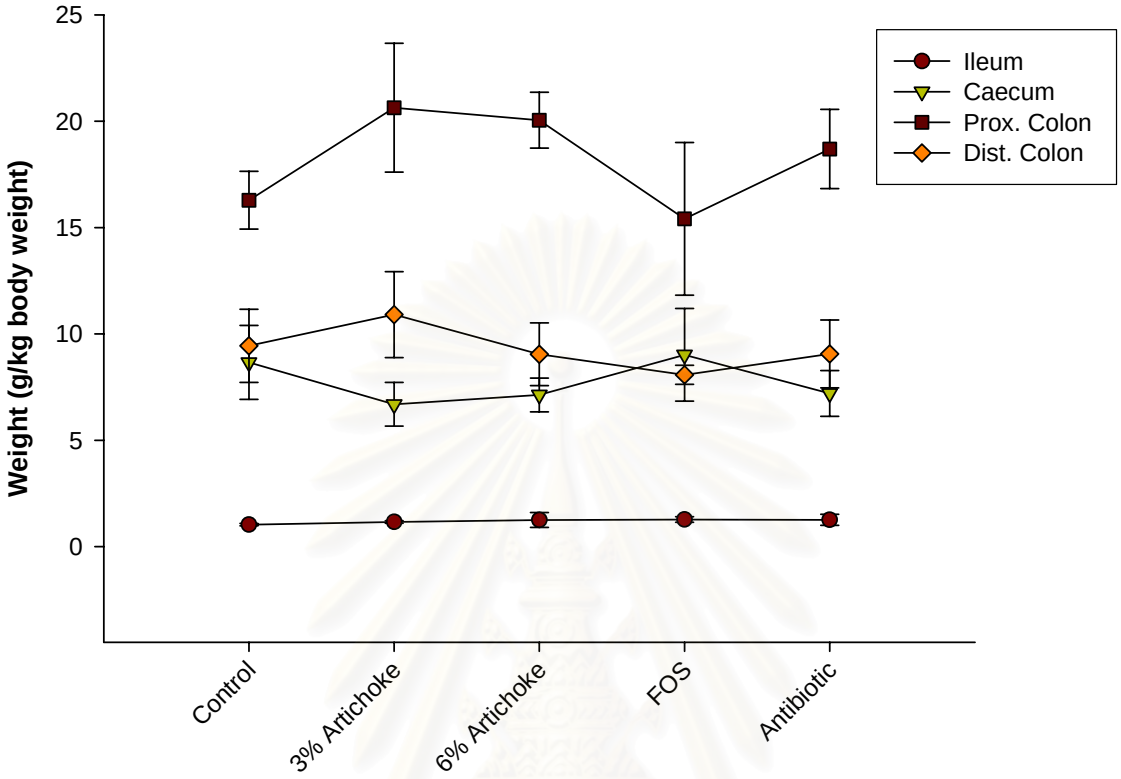

Figure 6. The effect of treatments on relative intestinal wet weight with content to body weight at 5 weeks of the experimental period

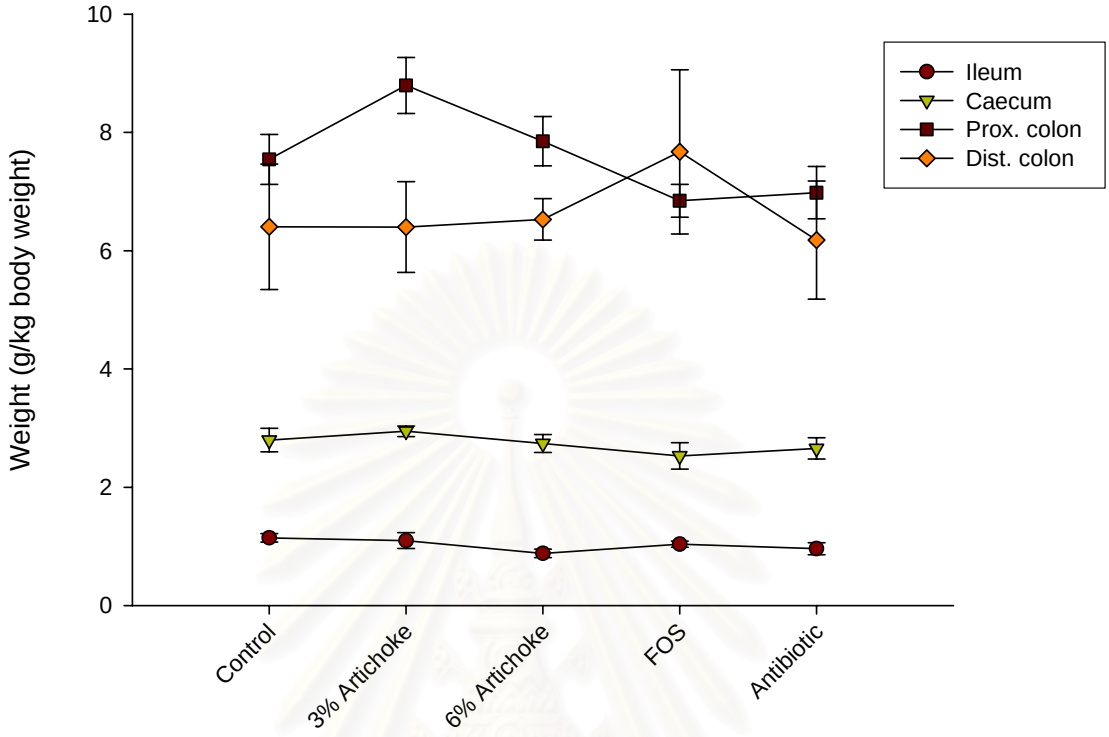

Figure 7. The effect of treatments on relative intestinal emptied wet weight to body weight at 5 weeks of the experimental period

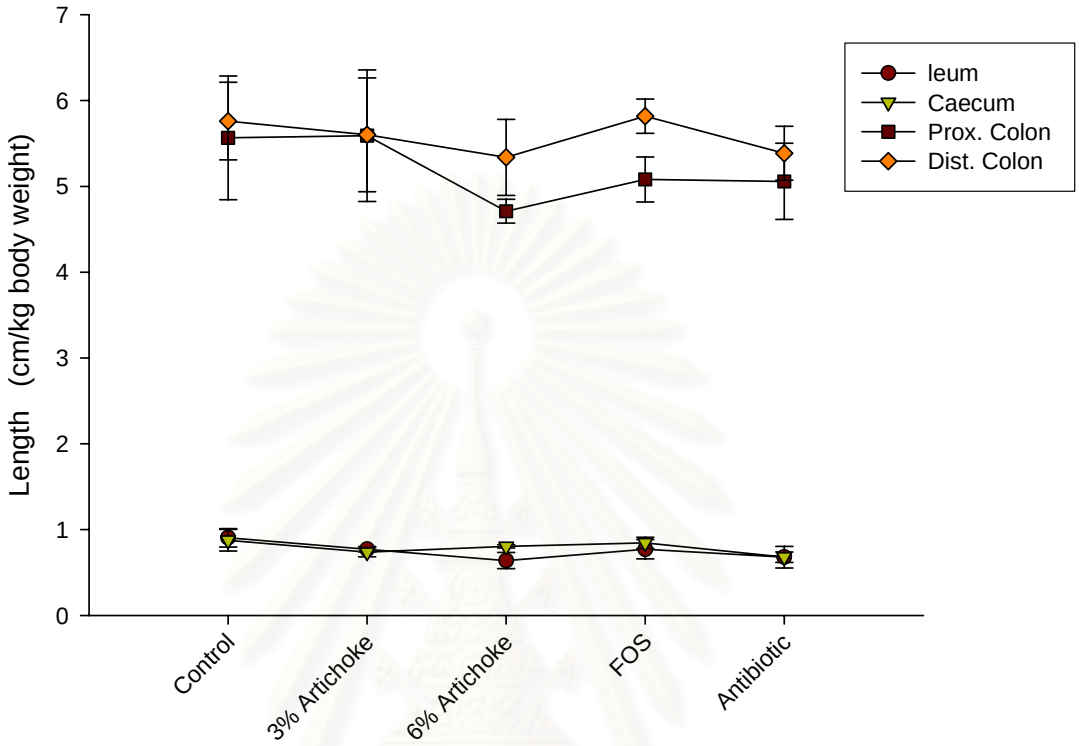

Figure 8 The effect of treatments on the intestinal length at 5 weeks of the experimental period

Table 13 The effect of treatments on defatted-dry weight (g) of intestinal tissue(Mean±SD)

	Treatment				
Intestinal part	Control	3% Artichoke	6% Artichoke	FOS	Antibiotic
Jejunum	0.023±0.006	0.020±0.006	0.021±0.003	0.022±0.004	0.024±0.004
Ileum	0.030±0.002	0.024±0.006	0.027±0.002	0.021±0.006	0.026±0.006
Caecum	0.020±0.004	0.017±0.003	0.024±0.004	0.016±0.004	0.018±0.007
Proximal Colon	0.017±0.004	0.018±0.006	0.015±0.003	0.019±0.006	0.017±0.006
Distal Colon	0.021±0.002	0.014±0.002	0.019±0.002	0.016±0.005	0.014±0.003

Means within a row without superscript does not differ (P>0.05)

SCFAs Concentration

The jejunal acetate and butyrate concentrations were highest in pigs fed on antibiotic diet while propionate concentration were highest in pig received 3%artichoke diet (Figure 9). For the total SCFAs concentration, pig fed on antibiotic diet had the highest SCFAs concentrations (23.42±12.70 mM) while the FOS diet had the lowest SCFAs concentration (13.82±5.78 mM). In ileum (Figure 10), pig received antibiotic diet had the highest total SCFAs (21.74±4.98 mM), and 3% artichoke diet had the lowest total SCFAs concentrations (12.00±6.96 mM). For individual SCFA, the antibiotic diet showed the highest acetate: the 6% artichoke diet had the highest propionate and butyrate.

In Caecum (Figure 11), there was no significant difference in total SCFAs concentration, however the control diet seem to have the highest acetate and propionate while 6% artichoke diet has the highest butyrate concentration. In proximal and distal colon, the highest total SCFAs concentration was found in 3% and 6% artichoke diet while the others had similar total SCFAs concentration (Figure 12 and Figure 13). The proportional percentage of SCFAs in all diet was shown in Table 14. The detail of SCFAs concentration is shown in appendix.

Table 14 The proportional percentage of SCFAs in each part of intestine (Acetate:Propionate:Butyrate:Valerate)

	Treatment				
Intestinal part	Control	3% Artichoke	6% Artichoke	FOS	Antibiotic
Jejunum	53:39:8:0	55:41:4:0	53:37:7:0	61:36:3:0	67:22:11:0
Ileum	57:39:3:0	52:44:4:0	57:34:9:0	54:40:5:0	69:23:7:0
Caecum	53:29:16:2	55:30:14:2	50:27:21:3	55:30:14:2	57:30:12:1
Proximal Colon	58:27:13:2	60:26:13:2	56:26:15:2	61:27:11:2	59:26:13:2
Distal Colon	58:26:14:2	61:24:13:2	57:26:15:2	62:26:10:2	59:25:14:2

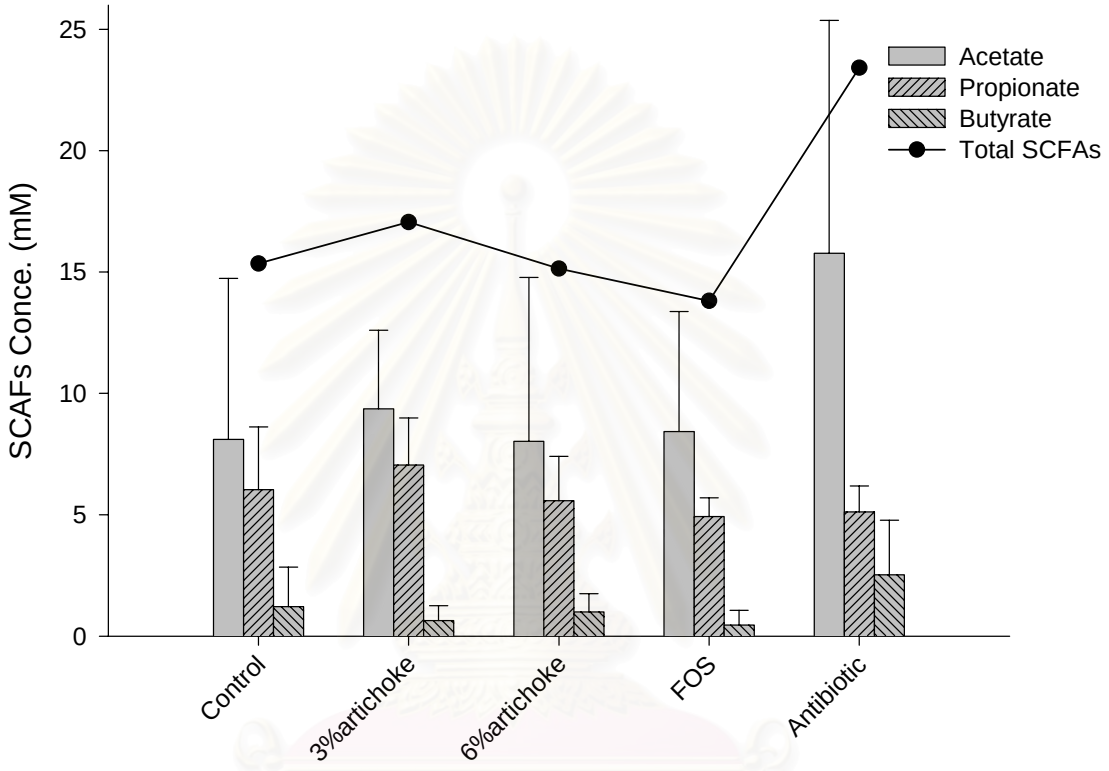

Figure 9 The effect of treatments on SCFAs concentration in the jejunum at 5 weeks of the experimental period

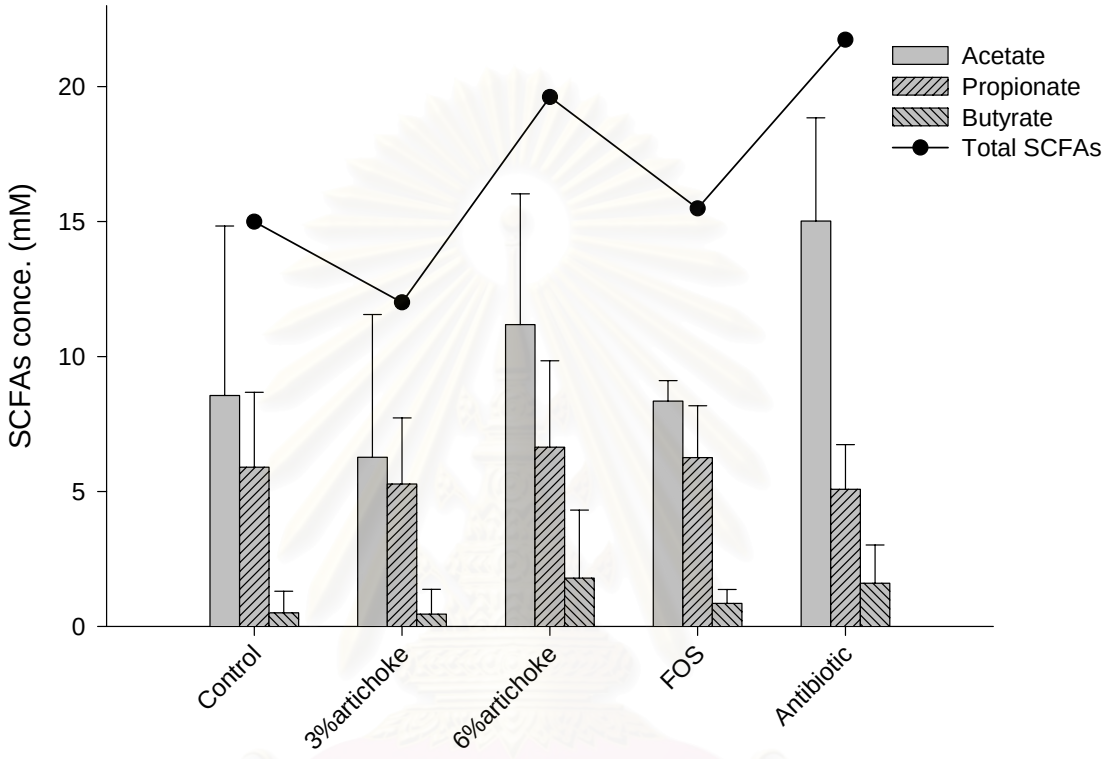

Figure 10 The effect of treatments on SCFAs concentration in the ileum at 5 weeks of the experimental period

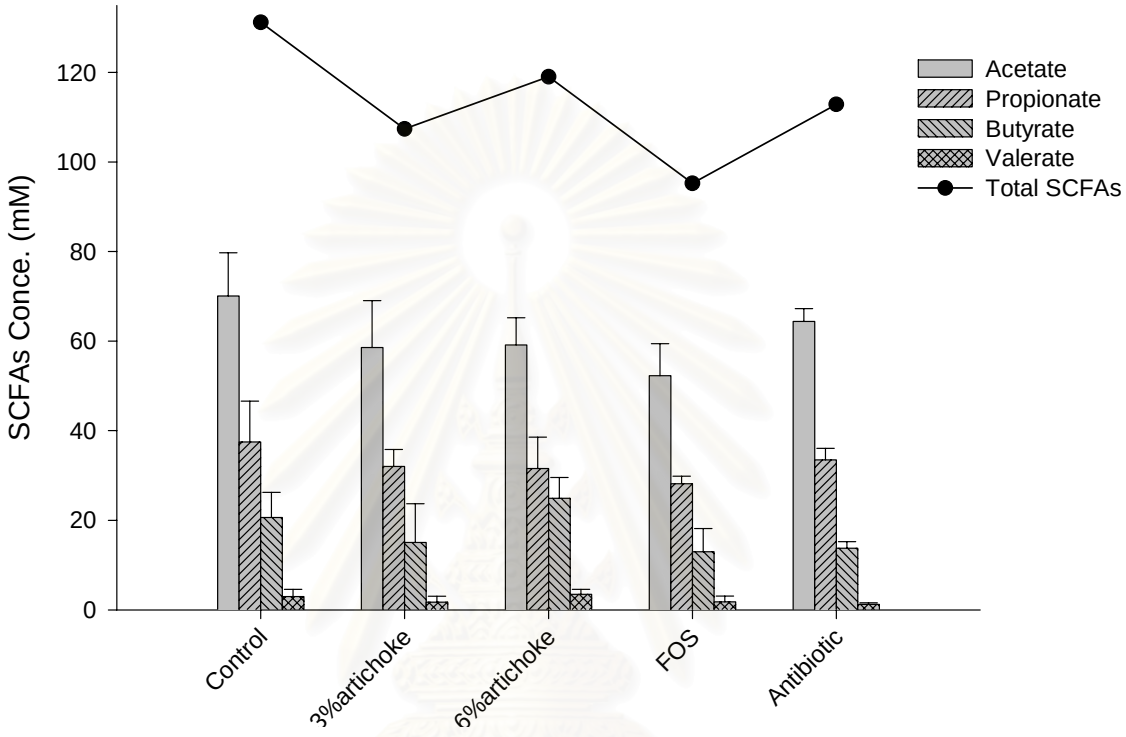

Figure 11 The effect of treatments on SCFAs concentration in the caecum at 5 weeks of the experimental period

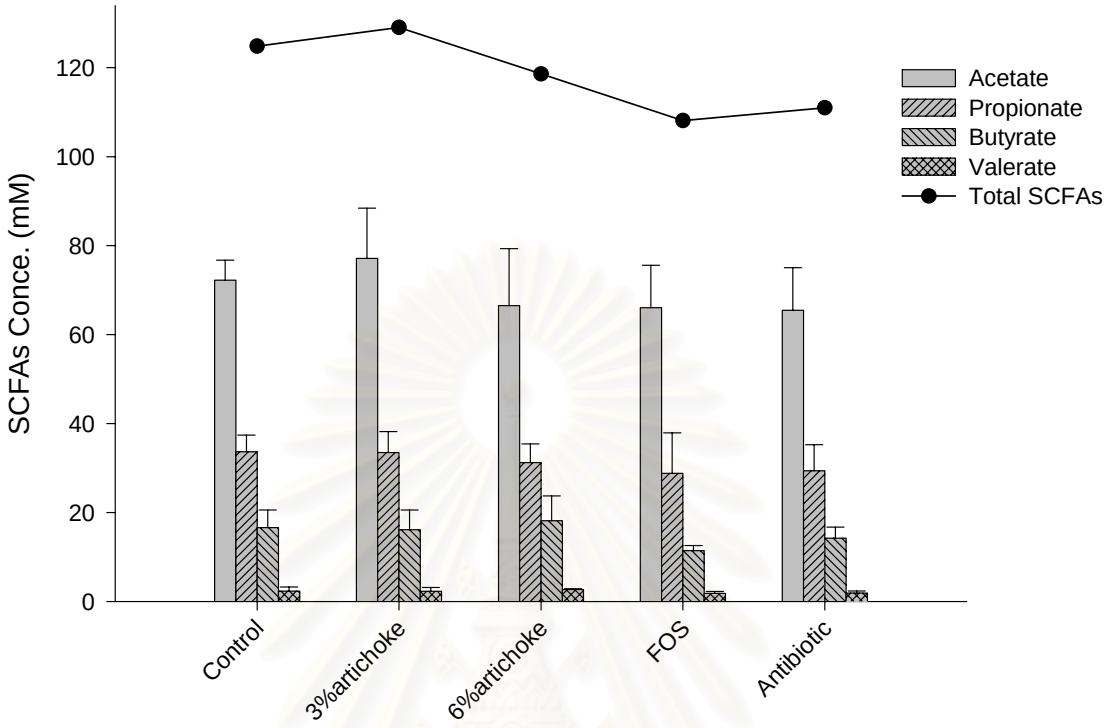

Figure 12 The effect of treatments on SCFAs concentration in the proximal colon at 5 weeks of the experimental period

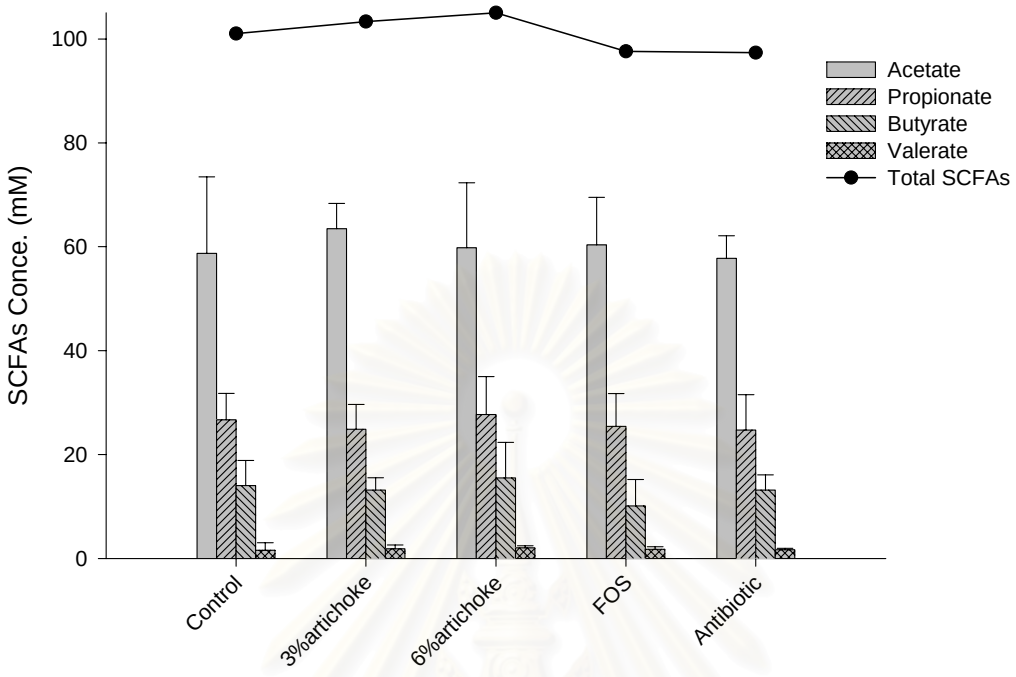

Figure 13 The effect of treatments on SCFAs concentration in the distal colon at 5 weeks of experimental period

DNA and RNA Content of Intestinal Mucosa

There was no significant difference in DNA content of the mucosal scraping from pigs' intestines at 5 weeks of the experimental period. The average DNA content of control, 3% artichoke, 6% artichoke, FOS, and antibiotic diets were 3.36±0.35, 3.02±0.19, 3.14±0.52, 3.07±0.61 and 3.06±0.67 mg/g of tissue wet weight, respectively. For the RNA content measured in the same samples, 6% artichoke diet and antibiotic diet had significantly higher RNA content than those of the control and FOS diets at ileum ($P<0.05$). The average RNA content of control, 3% artichoke, 6% artichoke, FOS, and antibiotic diets were 0.402±0.033, 0.382±0.031, 0.403±0.043, 0.370±0.049 and 0.358±0.050 mg/g tissue wet weight, respectively. The detail is shown in Table 15 and Table 16.

Table 15 The effect of treatments on DNA content at 5 weeks of the experimental period (Mean ± SD)

	Treatment				
Intestinal part	Control	3% Artichoke	6% Artichoke	FOS	Antibiotic
Jejunum	3.68±0.63	3.41±0.48	3.59±0.73	3.86±0.66	3.31±0.84
Ileum	3.54±0.96	3.45±0.86	4.17±0.81	3.58±0.83	3.76±0.72
Caecum	3.26±0.69	2.68±1.06	2.86±0.36	2.88±0.59	2.17±1.20
Proximal Colon	2.83±1.07	2.48±0.74	2.14±0.74	2.35±0.41	3.22±1.25
Distal Colon	3.50±0.75	3.08±1.04	2.96±0.74	2.66±0.86	2.86±0.77

Means within a row without superscript does not differ (P>0.05)

Table 16 The effect of treatments on RNA content at 5 weeks of the experimental period (Mean ± SD)

	Treatment				
	Control	3% Artichoke	6% Artichoke	FOS	Antibiotic
Jejunum	0.303±0.011	0.368±0.044	0.338±0.023	0.366±0.020	0.304±0.083
Ileum	0.320±0.074^{a}	0.358±0.048^{ab}	0.444±0.036^{b}	0.361±0.062^{a}	0.444±0.026^{b}
Caecum	0.472±0.0047	0.421±0.111	0.477±0.081	0.423±0.043	0.363±0.027
Proximal Colon	0.430±0.07	0.384±0.097	0.367±0.100	0.343±0.119	0.446±0.064
Distal Colon	0.451±0.058	0.378±0.097	0.390±0.051	0.359±0.053	0.358±0.050

a, b Means in the same row with different superscripts differed significantly (P<0.05)

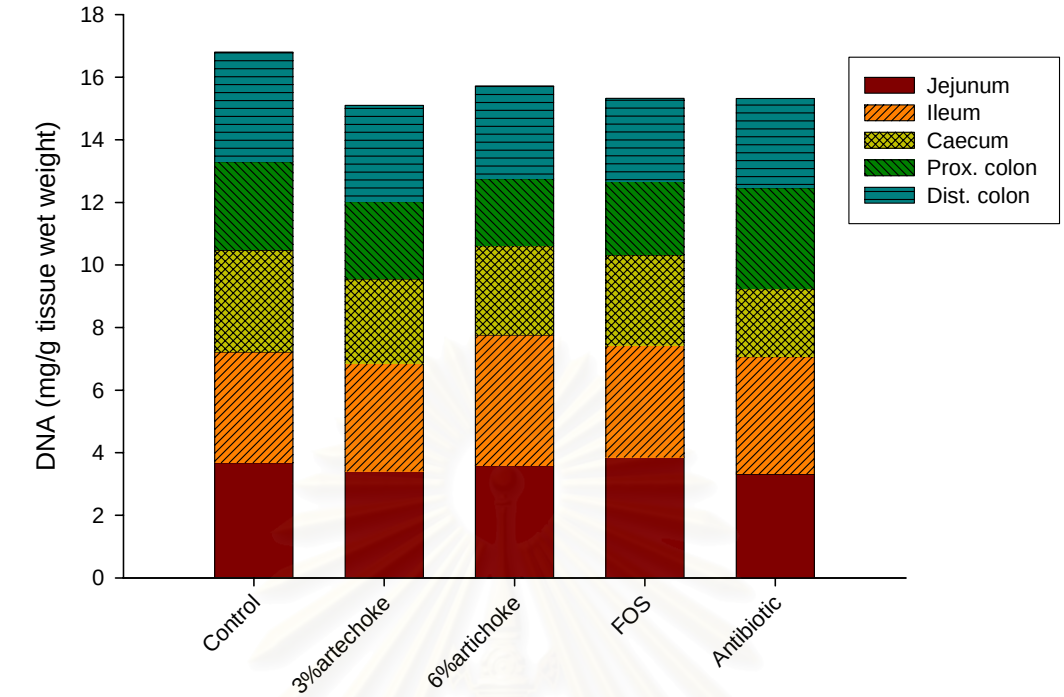

Figure 14 The effect of treatments on DNA content at 5 weeks of the experimental period.

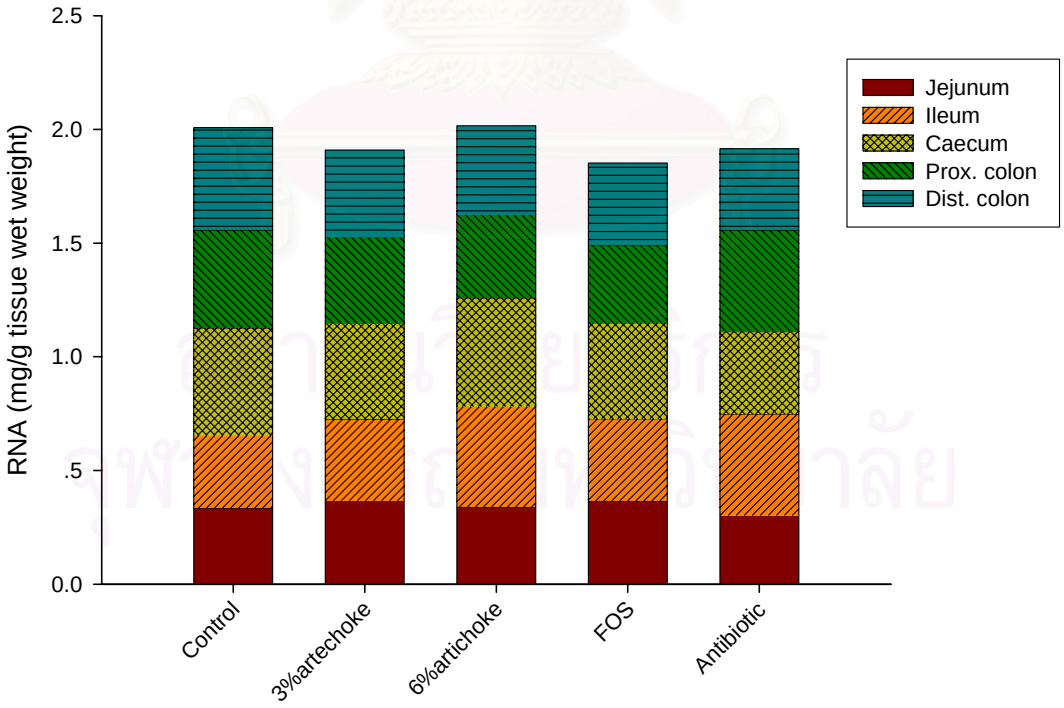

Figure 15. The effect of treatments on RNA content at 5 weeks of the experimental period.

CHAPTER V
DISCUSSION

Growth Performance

There was no significant difference on growth performance of pigs among treatment diets. This finding was similar to a previous report, the inclusion of 1.5% Jerusalem artichoke flour to weaning pig diet did not affect the feed intake, body weight gain, or feed efficiency between groups (Farnworth et al., 1991). In contrast, the inclusion of 6% Jerusalem artichoke in the diet reduced total feed intake of pigs compared with 0, 1% and 3% Jerusalem artichoke diet (Farnworth et al., 1995). In the present study, pigs received 3% artichoke tended to have the most preferable growth performance at 5 weeks of the experiment.

The highest mortality rate was found in the control group without feed additive. All infected pigs showed the sign of anorexia, emaciation and diarrhea. After death, carcasses were sent for necropsy examination at the animal hospital, Faculty of Veterinary Science, Chulalongkorn University. Results from death pigs indicated bacterial infection and septicemia. There are several factors affecting the mortality rate of weaning piglets. The colostral antibodies absorbed into the blood stream of the piglet during the first 24 hours of life are declining rapidly in the weaning period (Miller and Stokes, 1994). The piglet is eventually deprived of protective antibodies from milk. In addition, the stress from many activities affected the pigs in the experiment, for example transportation of experimental pigs from the farm to the experimental unit, new environment, new social hierarchy and new feed. These stress factors could suppress the piglet immune system (Fahey et al., 1990). In the present experiment, piglets under stress had less mortality when feed additive such as Jerusalem artichoke, FOS and antibiotic were used, though, the difference was not statistically significance.

Short-chain Fatty Acids

Bergman (1990) reported that caecum is the site of the highest production of SCFAs in almost all of hindgut-fermenting animal. Breves and Stuck (1995) reported that the total SCFAs concentration were in between 100 and 140 mM in caecum, 80 and 130 mM in proximal colon and 20 and 65 mM in distal colon. In the present experiment, the average SCFAs concentrations in disregard of treatment diet were 113.15±18.56 mM in caecum, 118.31±16.77 mM in proximal colon and 100.88±17.08 mM in distal colon (Appendix). The concentrations were in the range that has been reported except in distal colon that the concentrations were higher. This higher concentration might be due to the difference in the sampling site. The samples of distal colon content in all treatment were collected from the proximal end of distal colon, so this could make the SCFAs concentration higher than that of the previous report.

The proportional percentage of each SCFA was 52-69% for acetate, 22-44% for propionate, 3-21% for butyrate and 0-3% for valerate (Table 14). Fleming et al. (1989) showed proportional percentage of each SCFA, they found that 65% was present as acetate, 25% as propionate and 10% as butyrate in hindgut of pigs. The proportion of propionate and butyrate in the present experiment were higher than that of the previous report. The concentration and molar proportions of individual SCFAs could be varied in response to many dietary factors, such as level and source of neutral detergent fiber (NDF), crude fiber contents and the ratio of enzymatically degradable carbohydrates to crude fiber (Bach et al., 1991).

Interestingly, propionate tended to be in the highest proportion in jejunum, ileum and caecum in 3%artichoke group. For butyrate, 6%artichoke group tended to be in the highest proportion in ileum, caecum, proximal colon and distal colon. Pigs fed on diet with antibiotic had the highest proportion of acetate in jejunum, ileum and caecum, but for FOS group, the highest concentration of acetate was in the colon. For the characteristics of SCFAs proportion, Bergman (1990) and Herdt (1997) reported that the typical proportion of SCFAs depended on the type of diet. Animal that ate greater amount of fiber tended to have the higher proportion of acetate while animal fed greater amount of starch tended to have the higher proportion of propionate (Herdt, 1997). In addition, animal fed greater

amount of nonstarch polysaccharide (NSP; sugar beet pulp, for example) showed an increase in the proportion of propionate compared with animal fed with high fiber diet (Christine et al., 2000). Therefore pigs fed on 3%artichoke and 6%artichoke diet having the higher level of oligosaccharides should have SCFAs concentration pattern similar to those of the animals fed with high NSP diet. Moreover, a slightly higher fiber content in pigs fed on diet with antibiotic and FOS (Table 2) had the higher acetate proportion compared with other groups. It should be noted that the change in proportion did not affect the absolute amount of total SCFAs.

Physical Changes of the Intestine

According to the result, all treatment diets did not show any effect on the length of intestine and defatted-dry weight of the intestinal tissue. For intestinal weight, the control group, 3%artichoke group and 6%artichoke group tended to have higher wet intestinal weight with content and relative emptied wet intestinal weight than those of the other groups ($P>0.05$) in the proximal colon. These results corresponded with the higher amount of total SCFAs found in these 3 groups, especially in the proximal colon, the major fermentation site. Numerous studies reported that SCFAs were trophic to colonic mucosa. *In vitro* studies showed an increase in colonocyte proliferation rate under SCFA stimulation (Sakata and Engelhardt, 1983; Ichikawa and Sakata, 1998). *In vivo* study by Kissmeyer-Nielsen et al. (1995) showed that SCFAs instilled into atrophic and defunctioned rat colon for 14 days exhibited the higher colonic wet weight compared with those of the placebo group. 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. However, the mechanism by which SCFAs were trophic to the intestinal mucosa remained unclear (Mortensen and Nielsen., 1995). Stimulation of the microcirculation in the intestinal wall could, at least in part, explain the trophic effect of SCFAs in the large intestine. Mortensen et al. (1990) reported that the sodium salt of SCFAs, both separately and in mixture, had concentration dependent relaxant effects on colonic resistance arteries *in vitro*. These findings were in agreement with a study of autoperfused denervated dog colon preparation showing that SCFAs after instillation in the lumen

increased colonic blood flow (Kvietys and Granger, 1981). There was also another explanation of SCFAs on having trophic effect on intestine. It could be an increasing of the mucosa metabolism since SCFAs are the preferred oxidative fuel for the colonic mucosa. Roediger (1980) identified luminal n-butyrate as the major respiratory fuel of the colonocytes, accounting for 70% of oxygen consumption. Colonocyte must, therefore, obtain most of their energy from luminal SCFAs produced by colonic fermentation of dietary carbohydrates (Rabassa and Roger, 1992).

DNA and RNA Content of the Mucosa

In the present experiment, DNA content of intestinal mucosa was in the range of 2.14 and 3.86 mg/g tissue wet weight while RNA content is in the range of 0.03 and 0.47 mg/g tissue wet weight. Previous report in newborn pigs showed that the average of DNA and RNA content of intestinal mucosa were 4.96 and 2.5 mg/g tissue wet weight, respectively (Simmen et al., 1990). Although hardly comparable between two experiments, in this report, DNA content was slightly lower and RNA content was much lower. The variation could be due to several possible factors, usually, the difference in the age of animal, laboratory facilities and laboratory techniques. The present experiment did not show any changes on DNA and RNA contents among treatment diets. Additionally, the relationship between SCFAs concentrations and DNA or RNA content of mucosa was not found. *In vitro* studies using isolated, viable colonic epithelial cell culture for 24-h in the absence of butyrate did not exhibit reduction in the rate of energy consuming process such as DNA, protein synthesis and the total DNA content (Gibson et al., 1991). In contrast, Kripke et al. (1989) found that continuous infusion of either butyric acid (20-150 mM) or a mixture of SCFAs (acetate, propionate and n-butyrate: 70, 35 and 20 mM, respectively) into the colon increased the mucosal DNA content of the jejunum and proximal colon.

Transit Time

The average transit time of all treatment groups was 1137.71±134.71 minute (approximately 19 hours). This was very close to the study of Steven and Hume (1995) who used Cr-EDTA as a marker. The marker can be detected at the terminal colon of pigs within 16 hours after an oral intake. The rate of food passage through the digestive tract may be influenced by several factors such as the amount of energy derived from diet, management and environmental factor, genetic background, excitement, amount of feed intake and pelleting ration for example (Mateos et al., 1980). In the present experiment, control group, 6%artichoke group and antibiotic group had similar transit time (1199.00±122.51, 1196.00±118.53 and 1191.00±159.34 minutes, respectively). Pigs received diet with 3% artichoke had slightly shorter transit time (1131.50±1363.66 minutes) and the shortest transit time was found in FOS group (994.00±75.51 minutes). The difference between average longest and shortest transit time is about 205 minutes. Although there was no statistically difference which might be due to the small sample size (n = 4 per treatment), it is obviously a long time for nutrient digestion and fermentation in the gastrointestinal tract of pigs. Consequently, this might affect the nutrient digestibility, SCFAs production and growth performance of pigs. The causes of shortest transit time in FOS group may be in part by the increasing of osmotic force from FOS which is the purified soluble fiber (Clausen et al., 1998). Administration of FOS in healthy human subject caused the increasing of fecal, and exhibited the laxative effect (Clausen et al., 1998).

There were several articles on the effect of SCFAs on transit time. Cuche et al. (2000) demonstrated that ileal SCFAs can inhibit gastric motility by humoral pathway involving the release of an inhibiting factor, which is likely to be peptideYY. SCFAs may be one of the mediators involves in the “Ileo-colonic break”, i.e. the inhibition of gastric emptying by the presence of nutrients in the distal ileum and proximal colon (Cherbut et al., 1997). In colon, an *in vitro* study using isolated colon demonstrated that infusion of SCFA inhibit the rate of contractions in the proximal, mid and distal regions (Squires et al., 1992). All these researches might explain, in part, that the lowest SCFAs in almost intestinal part of FOS group could be accounted for the shortest transit time.

Conclusion

In present study, using of Jerusalem artichoke as feed additive did not show any significant difference on growth performance, the physical and biological changes of ileum and large intestine in weaning pigs. However, 3% Jerusalem artichoke supplemented in diet tended to improve the growth performance of weaning pigs. The possible reasons might, in part, due to higher SCFAs concentration in their colon. Since there were several reports supported that SCFAs could have a trophic effect to colon and delay the gastrointestinal transit time, so pigs fed 3% artichoke diet tended to have higher colon wet weight and longer transit time. Pigs fed on 1% fructooligosaccharide diet did not promote SCFAs production in the hindgut and by itself caused the shortest gastrointestinal transit time, but it tended to show better growth performance and showed the less mortality rate when compared with those of the control diet. In conclusion, using of prebiotic as feed additive could benefit the weaning pig production in comparison to antibiotic. Further studies on the use of Jerusalem artichoke as prebiotic on the improvement of nutrient digestibility, microbial change and local immune systemic in large intestine of pigs are needed.

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APPENDIX

SCFAs CONCENTRATION

Table 1 The effect of treatments on SCFAs concentration (mM) in Jejunum (Mean ± SD)

	Treatment				
	Control diet	Artichoke 3%	Artichoke 6%	FOS 1%	Antibiotic
Acetate	8.11±6.64	9.36±3.24	8.03±6.75	8.43±4.94	15.77±9.60
Propionate	6.03±2.59	7.05±1.94	5.58±1.83	4.93±0.77	5.12±1.07
Butyrate	1.22±1.63	0.64±0.61	1.00±0.75	0.46±0.61	2.53±2.25
Valerate	0	0	0	0	0
Total	15.35±8.81	17.06±4.19	15.14±9.39	13.82±5.78	23.42±12.70
C2:C3:C4:C5	53:39:8:0	55:41:4:0	53:37:7:0	61:36:3:0	67:22:11:0

Table 2 The effect of treatments on SCFAs concentration (mM) in ileum (Mean ± SD)

	Treatment				
	Control diet	Artichoke 3%	Artichoke 6%	FOS 1%	Antibiotic
Acetate	8.56±6.28	6.27±5.29	11.18±4.84	8.35±0.75	15.02±3.82
Propionate	5.90±2.78	5.27±2.45	6.64±3.20	6.25±1.93	5.09±1.65
Butyrate	0.50±0.80	0.46±0.92	1.79±2.52	0.85±0.52	1.60±1.42
Valerate	0	0	0	0	0
Total	15.00±7.77	12.00±6.96	19.61±6.88	15.49±0.91	21.74±4.98
C2:C3:C4:C5	57:39:3:0	52:44:4:0	57:34:9:0	54:40:5:0	69:23:7:0

Table 3 The effect of treatments on SCFAs concentration (mM) in caecum (Mean±SD)

	Treatment				
	Control diet	Artichoke 3%	Artichoke 6%	FOS 1%	Antibiotic
Acetate	70.11±9.64	58.56±10.51	59.14±6.11	52.29±7.17	64.40±2.88
Propionate	37.52±9.08	32.05±3.79	31.57±6.99	28.19±1.64	33.48±2.60
Butyrate	20.61±5.63	15.08±8.62	24.89±4.66	12.98±5.15	13.76±1.45
Valerate	2.96±1.61	1.73±1.28	3.44±1.13	1.80±1.25	1.23±0.32
Total	131.19±23.17	107.41±22.39	119.05±11.66	95.26±7.10	112.87±2.40
C2:C3:C4:C5	53:29:16:2	55:30:14:2	50:27:21:3	55:30:14:2	57:30:12:1

Table 4 The effect of treatments on SCFAs concentration (mM) in proximal colon (Mean±SD)

	Treatment				
	Control diet	Artichoke 3%	Artichoke 6%	FOS 1%	Antibiotic
Acetate	72.23±4.52	77.12±11.32	66.51±12.80	66.03±9.52	65.45±9.58
Propionate	33.68±3.72	33.49±4.69	31.21±4.23	28.81±9.11	29.38±5.86
Butyrate	16.61±3.98	16.13±4.43	18.15±5.58	11.45±1.15	14.23±2.48
Valerate	2.33±0.93	2.30±0.85	2.70±0.17	1.83±0.40	1.91±0.44
Total	124.84±7.27	129.03±17.26	118.57±18.49	108.11±19.13	110.97±17.37
C2:C3:C4:C5	58:27:13:2	60:26:13:2	56:26:15:2	61:27:11:2	59:26:13:2

Table 5 The effect of treatments on SCFAs concentration (mM) in distal colon (Mean ± SD)

	Treatment				
	Control diet	Artichoke 3%	Artichoke 6%	FOS 1%	Antibiotic
Acetate	58.73±14.72	63.47±4.88	59.79±12.54	60.36±9.15	57.77±4.33
Propionate	26.70±5.07	24.86±4.78	27.71±7.31	25.44±6.29	24.71±6.82
Butyrate	14.04±4.83	13.17±2.36	15.52±6.84	10.09±5.12	13.17±2.91
Valerate	1.57±1.45	1.86±0.76	2.03±0.41	1.74±0.53	1.68±0.24
Total	101.05±23.21	103.36±11.52	105.05±26.02	97.63±15.90	97.34±13.55
C2:C3:C4:C5	58:26:14:2	61:24:13:2	57:26:15:2	62:26:10:2	59:25:14:2

Table 6 The average SCFAs concentration (mM) [from all treatment groups (n=20)] of intestinal content (Mean ± SD)

	Short-chain fatty acid (mM)				
Intestinal part	Acetate	Propionate	Butyrate	Valerate	Total SCFA
Jejunum	9.94±6.59	5.74±1.75	1.17±1.41	0.11±0.48	16.96±8.47
Ileum	9.87±8.75	5.83±2.27	1.04±1.39	0.02±0.05	16.77±11.29
Caecum	60.90±9.24	32.56±5.84	17.46±6.83	2.23±1.36	113.15±18.56
Proximal Colon	69.47±9.99	31.31±5.60	15.31±4.16	2.21±0.65	118.31±16.77
Distal Colon	60.03±9.11	25.88±5.58	13.20±4.55	1.78±0.73	100.88±17.08

BIOGRAPHY

Mister Phiphob Sodsee was born on January 7th, 1976 in Nakornsawan, Thailand. He graduated from Faculty of Veterinary Science, Chulalongkorn University as a Doctor of Veterinary Medicine in 1998. Currently he is working as instructor at Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University.